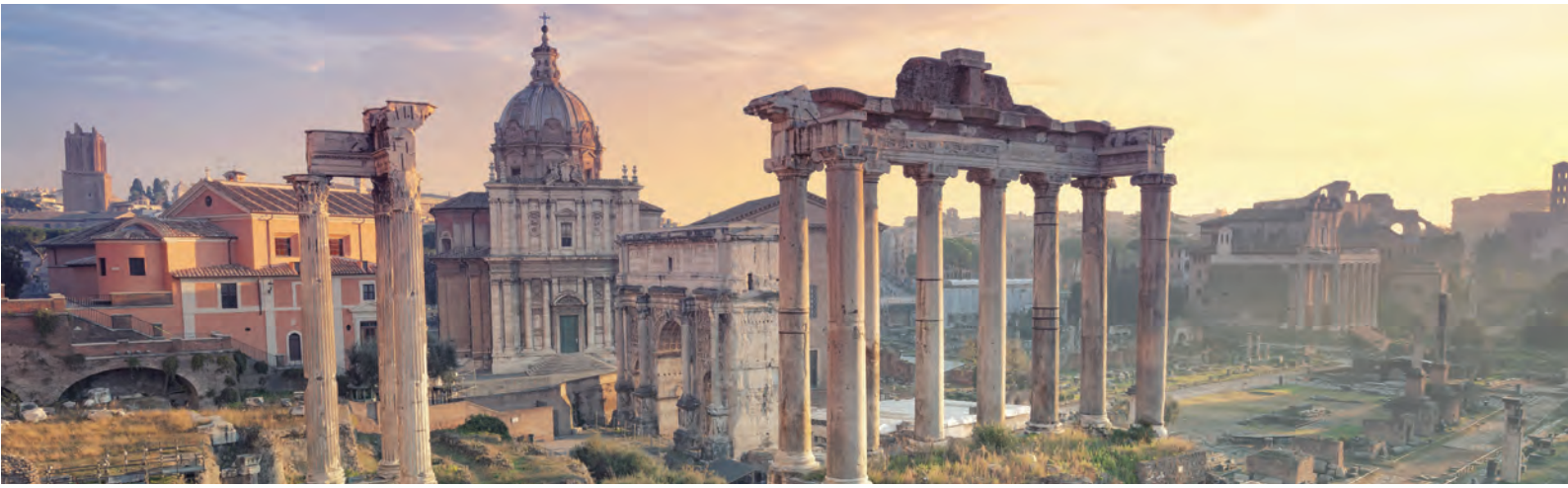
ABSTRACT BOOK

The 9th International Conference on

Legionella

Rome, 26th - 30th September 2017



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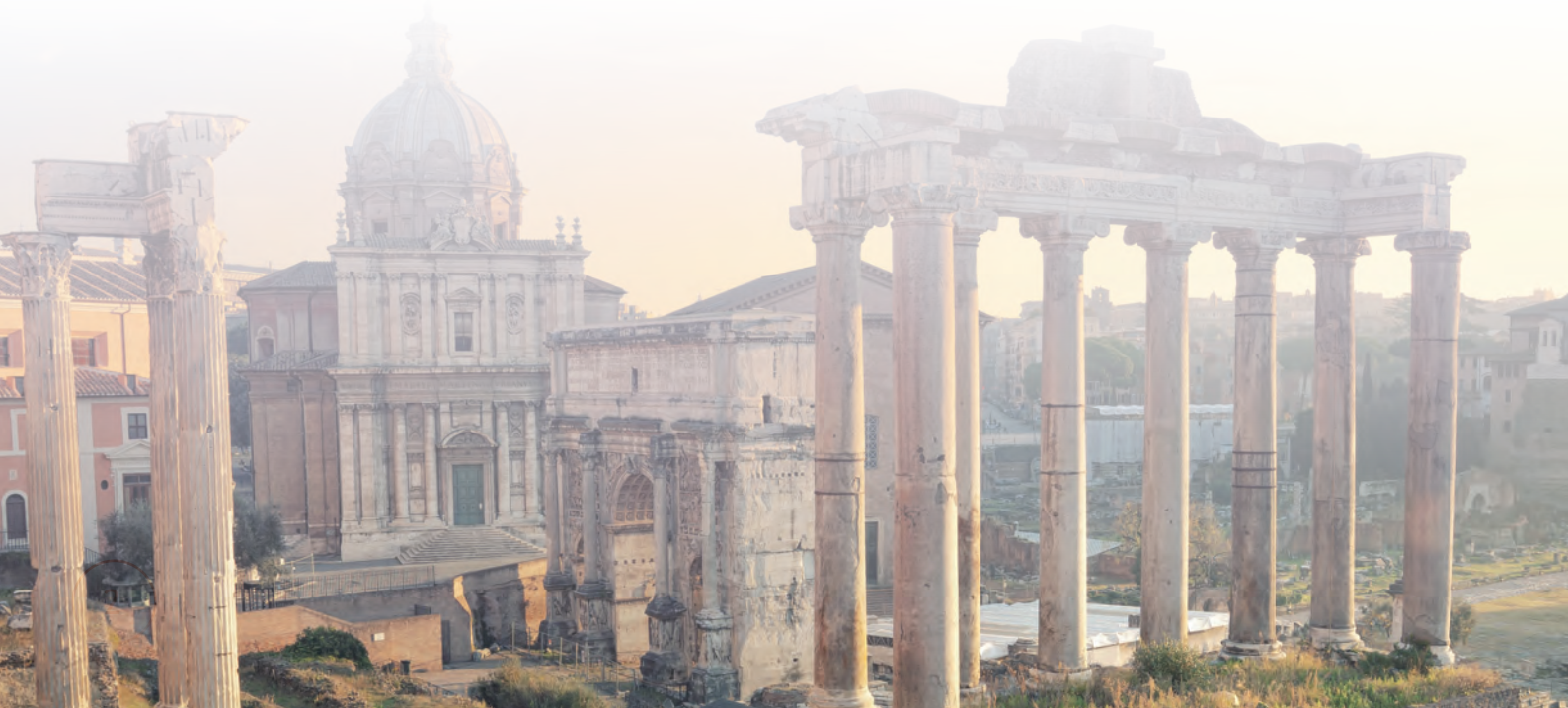
POSTER SESSION 1

WEDNESDAY, SEPTEMBER 27TH, 2017



Characterization of *Legionella* RCC1 domain effector proteins

Authors Leoni Swart A., Schütz S.,Steiner B., Hannemann M., Itzen A., Panse V. G., Hilbi H.

Legionella pneumophila is a ubiquitous environmental bacterium and the causative agent of a severe pneumonia termed Legionnaires' disease. Upon infection the bacterium employs the Icm/Dot type IV secretion system (T4SS), through which it injects approximately 300 "effector proteins" into the host cell. These proteins manipulate various host processes, like signal transduction and vesicular trafficking, thereby enabling the bacterium to form a distinct "Legionella-containing vacuole" (LCV) and preventing degradation of the pathogen via the bactericidal endolysosomal pathway. Proteomic analysis of purified intact LCVs revealed the presence of hundreds of host proteins, including a number of small GTPases implicated in the secretory and endosomal vesicle trafficking pathway, as well as the small GTPase Ran and its effector Ran binding protein 1 (RanBP1). The L. pneumophila effector protein LegG1 harbors an RCC1 domain and has been found to activate Ran, thereby promoting microtubule polymerization, LCV formation and motility, host cell migration, as well as intracellular bacterial replication. The L. pneumophila Philadelphia genome encodes a second RCC1 domain-containing protein, 1 / 2 inserimenti namely PpgA. This study revealed that like the ?legG1 mutant strain, ?ppgA did not have an intracellular replication phenotype in both Acanthamoeba castellanii and RAW264.7 macrophages, but was efficiently outcompeted by the wild-type strain, when amoebae were infected in a 1:1 ratio. Moreover, the motility of LCVs harbouring ?ppgA or ?legG1 was reduced to a similar extent. The phenotype of the ?ppgA mutant was complemented by LegG1; however, the corresponding ?legG1 phenotype was not reversed by overexpression of PpgA. Also, while GFP-LegG1 ectopically produced in Dictyostelium discoideum localized to the LCV membrane, depending on the presence of its CAAX-motif, GFP-PpgA accumulated on the cell membrane. These results suggest that PpgA, like LegG1, modulates Ran activity, yet in a different manner. Currently, the target(s) and mechanism of L. pneumophila RCC1 domain proteins are analyzed in further detail.

LegK4 from *Legionella pneumophila*, a bacterial protein kinase that targets the host cell nucleus

Authors Blache Q., Baïlo N., Michard C., Doublet P.

Background - Crucial for Legionella pneumophila to evade phagocytic destruction and to efficiently replicate within its environmental and human host-cells is the Type IV Secretion System Dot/Icm. Of the large repertoire of Dot/Icm effectors, L. pneumophila secretes 4 protein kinases, called LegK1-K4, that target host cell protein phosphorylation to hijack host signaling pathways (1). LegK1 and LegK2 have been found to target NF-?B (2) and actin polymerization (3), respectively. Here, we decipher the structure and the function of LegK4.

Results - The structure of LegK4 (1-445) reveals a eukaryotic-like kinase domain flanked by a novel cap domain and a four-helix bundle (4). Confocal microscopy shows that GFP-LegK4 is localized into the nucleus of mammal transfected cells. Moreover, structure-guided mutagenesis experiments identified the domain essential and sufficient for nuclear import of LegK4. Consistently with LegK4 nuclear localization, co-purification and phosphorylation assays demonstrate that LegK4 is able to interact with and phosphorylate one core histone, thus inducing a complex host gene epigenetic control.

Conclusion - Together, data presented here suggest that LegK4 would be the first bacterial protein kinase that localizes into the nucleus of the host cell after secretion, and that could trigger nuclear proteins phosphorylation in order to reprogram host cell gene expression to the bacteria benefit.

Disulfide loop cleavage of *Legionella pneumophila* PlaA boosts lysophospholipase A but diminishes glycerophospholipid cholesterol acyltransferase activity

Authors Hiller M., Lang C., Flieger A.

At least fifteen phospholipases A are encoded in the genome of Legionella pneumophila where three, PlaA, PlaC and PlaD, belong to the GDSL lipase family. GDSL proteins are abundant in 1 / 2 inserimenti many bacteria and higher plants and exhibit lipolytic activities. It is currently not known whether and how L. pneumophila phospholipases contribute to the rearrangement of membrane lipids in the Legionella containing vacuole (LCV). We here investigated the role of the GDSL enzymes PlaA and PlaC in L. pneumophila secreted lipolytic activities and the activation mechanism of PlaA. MATERIALS/METHODS Secretion types were assessed by westernblot. For detection of enzymatic activity the proteins were recombinantly expressed, purified and subjected to lipid hydrolysis assay and thin layer chromatography. Additionally the effect of the zinc metalloproteinase ProA on PlaA activity was determined. RESULTS PlaC shows phospholipase A and acyltransferase activities which, as shown before, are activated via ProA-dependent disulphide loop cleavage. PlaA majorly contributed to lysophospholipase A (LPLA) and acyltransferase activity. Western blotting confirmed that PlaA and PlaC are type II-secreted and revealed that both are processed by ProA in a predicted disulphide loop. Interestingly, ProA steeply increased LPLA but diminished acyltransferase activity of PlaA. Accordingly, deletion of 22 amino acids in the disulfide loop resulted in boosted LPLA and reduced acyltransferase activities. CONCLUSION In summary, PlaA and PlaC are processed by ProA via disulfide loop cleavage. While this activates PLA and GCAT activity of PlaC, only LPLA activity of PlaA is boosted. The results help to decipher enzymatic activity and activation of PlaA, an enzyme acting in the Legionella-containing phagosome. We are currently addressing lipid composition, ultrastructure and biophysical properties of LCV membranes in response to infection with Lpn wildtype versus phospholipase mutants. Powered by TCPDF (www.tcpdf.org)

Evolution of virulence traits during mutation accumulation evolution experiment in *legionella pneuomophila*

Authors Carrillo G., Ginevra C., Jaboulay C., Doublet P., Jarraud J., Kay E.

Background: In natural environment, Legionella replicates within many different eukaryotic hosts and this coevolution would allow this bacterium to acquire the adequate virulence tools to become a broad host-range pathogen. Here, we address the question of the mutational robustness of virulence and host spectrum. Notably, we wondered whether bacteria evolving in the absence of hosts for hundreds of generations lose or retain their ability to infect various hosts.
Materials/Methods: To answer this question, we conducted a mutation accumulation (MA) evolution experiment in which several replicate populations, founded from the common ancestor L. pneumophila Paris, were propagated on standard agar medium for hundreds generations.
Such MA experiment allows the accumulation of non-lethal mutations regardless their impact on fitness and virulence. Then, we compared the evolved clones to the ancestor at the phenotypic, genetic and transcriptomic levels.
Results: After several hundred generations of genetic drift, major phenotypic changes have been detected. Evolved clones showed a strong fitness decrease compared to the ancestor and a reduced ability to infect both amoeba and human macrophages. This growth defect is correlated at least in part to a reduced capacity to establish the replication-permissive vacuole.
The most obvious mutations leading to a virulence decrease could target the Icm/Dot secretion system. However, the translocation of effectors into the host cells was not affected suggesting that mutations affect other virulence genes or regulators. To get insights of the genetic modifications underlying these important phenotypic changes, the genome sequences of appropriately chosen evolved clones as well as global gene expression profiles were determined and will be discussed.
Conclusion: Overall, these approaches led us to make connections between genetic mutations and phenotypic outcomes and to identify target genes of the virulence/host spectrum evolution.

Legionella triggers the AIM2 Inflammasome that engages active but unprocessed Caspase-1 to induce noncanonical activation of the NLRP3 Inflammasome.

Authors Cunha D. L., Alexandre L. N. Silva, Ribeiro J. M., Fonseca Lincoln L., P. A. Mascarenhas D., F.S Quirino G., Santos L. L., S.D. Lima-Junior, Zamboni S. D.

The activation of the inflammasomes is a hallmark of many infectious and inflammatory diseases. Many inflammasomes are activated during infections with physiologically relevant pathogens and the current view is that the inflammasomes independently activate and cleave caspase-1 to induce inflammatory cytokines and inflammation. The activation of the NLRP3 inflammasome occurs in response to cell damage and K+ efflux, whereas the activation of AIM2 inflammasome occurs in response to cytosolic DNA. Here, we used the intracellular gramnegative bacteria Legionella pneumophila to dissect the molecular mechanisms related to activation of these inflammasomes and their functional consequences. We found that Legionella trigger activation of the AIM2 inflammasome, a process that leads to caspase-1 activation, but not caspase-1 cleavage. The AIM2 inflammasome preferentially engage active, but unprocessed form of caspase-1 to induce pore formation, which in turn will be responsible for K+ efflux-mediated activation of the NLRP3 inflammasome. In physiological doses of infection, we found that the AIM2 inflammasome is unable to proteolytically cleave caspase-1 in 1 / 2 inserimenti the absence of NLRP3. These data suggest that during physiological conditions of infection, the AIM2 inflammasome orchestrate the activation of the NLRP3 inflammasome, a process that is important to resistance to infection in macrophages and in vivo. Our data show that in physiological conditions of infection the AIM2 inflammasome operates to sense small amounts of PAMPs in the cytoplasm and induce pore formation-induced K+ efflux to amplify signals of infections allowing the non-canonical activation the NLRP3 inflammasome.

Export of the Phospholipase PlaB of *Legionella pneumophila*

Authors Wiebke M. Auraß P., Wissing J., Jänsch L., Flieger A

The cell-associated, highly active phospholipase A, designated PlaB, is a member of a new family of lipases, first described in Legionella pneumophila. In previous studies, PlaB has shown phospholipase A, lysophospholipase A, and hemolytic activity as well as importance in 1 / 2 inserimenti pathogenicity. Furthermore, PlaB was found to localize to the bacterial outer membrane where it seems to become active. The transport mechanism is still unclear since so far described protein export systems were not essential for PlaB export. Therefore, the transport mechanism will be analyzed in this work. MATERIALS/METHODS To identify the responsible exporter of PlaB, interactome studies based on the BioID system are used. The protein of interest is tagged with the E. coli biotin protein ligase BirAR118G BirA*, which biotinylates proteins in a proximity-dependent manner. The biotinylated proteins were purified by neutravidin affinity matrix and identified by mass spectrometry. To prevent a high number of biotinylated proteins, the outer membrane was fractionated by ultracentrifugation. RESULTS PlaB-BirA*-2HA was transferred to E. coli and L. pneumophila and localized to the outer membrane in both, which points to a general transport way in gram-negative bacteria used for PlaB. The expression levels, activity and localization of PlaB-BirA*-2HA were verified and the BioID assay was established. PlaB-BirA*-2HA seems to biotinylate specific proteins interacting with PlaB, which could be identified by peptide sequencing (LC-MS/MS). Knockout mutants of the identified proteins were generated to check the localization of PlaB. CONCLUSION Our PlaB-BirA*-2HA approach suggests first interaction partners of PlaB during the transport to the outer membrane. Knowledge of the transporter, which targets PlaB to the outer membrane, is important for understanding the pathogenicity of L. pneumophila and may lead to the identification of a novel protein transport mechanism, which may serve as a novel drug target

Legionella pneumophila's growth and infection dynamics in Acanthamoeba polyphaga, Dictyostelium discoideum, and U937 macrophages

Authors Moreno A. B., Guy L.

Legionella pneumophila is a gram-negative intracellular pathogen, ubiquitously found in soil and water reservoirs. L. pneumophila has a broad range of hosts, most commonly protozoa, such as amoebae, but also human alveolar macrophages, where it may cause Legionnaire's disease. Although much is known about how L. pneumophila infects and proliferates inside these different hosts, the effect of prey bacteria on the host population has been less studied. Materials/Methods: All fluorescently tagged Legionella pneumophila strains were generated from the Lp Paris strain. We used three different host cells: Acanthamoeba polyphaga strain Linc Ap-1, Dictyostelium discoideum strain FS-11, and Human macrophages U937 cell line. The host cells were seeded on 24-well plates, and different volumes of L. pneumophila culture were diluted as to achieve different MOIs. The plates were incubated at different temperatures: 24, 32, or 37°C, for 72h. Samples were taken at 0, 24, 48, and 72h to quantify the number of host cells. The number of L. pneumophila inside the host cells was measured as function of florescence, using a fluorescence plate reader. Results: Here we examined the growth dynamics of three different eukaryotic hosts of L. pneumophila. We challenged the hosts with L. pneumophila at different MOIs and temperatures for a period of 72h, and measured the number of infected and uninfected host cells at various time points. We also measured the pathogenicity of L. pneumophila for each host, as well as its titer and the number of secondary infections caused by the extracellular bacteria found in the media. Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; msostyle-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minorlatin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;} Conclusions: Amoebae serve as an important reservoir for L. pneumophila, allowing it to replicate and survive even in in sub-optimal conditions and thus can be a possible source of emerging pathogens. This contribution sheds light on the both the host and the prey community dynamics.

Inducing apoptosis to promote *Legionella* clearance by macrophages.

Authors Thomas Naderer

Legionella prevents the induction of apoptotic cell death to promote bacterial growth in macrophages. How Legionella inhibits the activation of apoptosis remains controversial. We have followed Legionella infected macrophages with live-cell imaging to determine the role of apoptosis. This enabled us to visualize mitochondria health, activation of apoptotic caspases and rupture of plasma membrane during infections. We now show that effector proteins that inhibit host protein translation directly affect apoptosis. This is because Legionella infected macrophages rapidly deplete the short-lived survival protein MCL-1. Consequently, L. pneumophila and L. longbeachae depend on the related survival protein, BCL-XL, to prevent apoptosis and to establish infections, both in vitro and in vivo. BCL-XL keeps the death factors, BAX and BAK, in check to prevent apoptotic cell death. Compounds that inhibit BCL-XL, originally developed to kill cancer cells, induce cell death of infected macrophages and reduce L. pneumophila burden in the lungs and prevent lethal bacterial infection in mice. Inducing apoptosis also dampens inflammation associated with Legionella infected lungs. We will provide genetic evidence that targeting BCL-XL strictly depends on the ability of Legionella to inhibit host protein translation.

Effect of human antimicrobial peptides against *Legionella pneumophila*

Authors Vandewalle M., Guillemot J., Chapalain A., Lina G., Doublet P., Jarraud S., Ginevra C.

Antimicrobial peptides (AMPs) are natural antibiotics widespread throughout the animal kingdom, from bacteria to mammals. They are important components of both innate and adaptive immunity, providing protection against a broad-spectrum of pathogens, such as viruses, bacteria, fungi and parasites. The objective of this study was to investigate the effect of two human AMPs (LL-37 and HBD-3) on Legionella pneumophila. Materials/Methods: In this study, we investigated the action of synthetic LL-37 and HBD-3 on L. pneumophila by cultivability assays, membrane permeabilization assays monitored by flow cytometry and transmission electronic microscopy (TEM). Results: We showed that both peptides exhibit a phase dependent bactericidal effect on extracellular L. pneumophila. Flow cytometry assays have demonstrated that LL-37 and HBD-3 treatments induced a rapid permeabilization of the bacterial membranes. TEM experiments showed that the membrane damages induced by the PAM treatments were different between LL-37 treated and HBD-3 treated bacteria. 1 / 2 inserimenti Conclusion: Human AMPs LL-37 and HBD-3 seems to be involved in innate immunity against L. pneumophila by pore forming activity against extracellular L. pneumophila

Orchestration of Dot/Icm bacterial effector expression and secretion by nucleoid-associated proteins and cyclic-di-GMP metabolizing enzymes during *Legionella* infectious cycle

Authors Vianney A., Allombert J., Jaboulay C., Andréa C., Baïlo N., Buchrieser C., Doublet P., Kay E.

Successful Legionella infection requires a functional Icm/Dot type IV secretion system which translocates a large repertoire of effectors into the host cytosol. The synthesis and the kinetic of translocation of these effectors have to be tightly coordinated to ensure their delivery in correct amount and at the precise timing at each step of the infection process. Our objective aims at deciphering the regulatory networks involved in this orchestration. Methods: The role of nucleoid-associated proteins (NAPs) and c-di-GMP metabolizing enzymes were investigated using mutagenesis and transcriptomic approaches as well as reporter gene fusions, intracellular replication and translocation assays. 1 / 2 inserimenti Results: We showed that NAPs are major regulators of virulence genes expression in Legionella. In particular, we demonstrated that Fis proteins jointly control the expression of genes encoding main virulence factors, including Dot/Icm T4SS components and effectors, as well as numerous GGDEF/EAL proteins, which are responsible for the synthesis and hydrolysis of the c-di-GMP second messenger. Three of these GGDEF/EAL proteins are specifically required for bacteria survival at the early stages of infection and for adequate Icm/Dot effector delivery inside host cytosol. More precisely, we established that these three mutant strains exhibit differentially altered translocation kinetics. Overall, these data demonstrate that Fis proteins regulate numerous Icm/Dot effector genes at the transcriptional level while several c-diGMP metabolizing enzymes finely orchestrate their translocation through the Icm/Dot secretion machinery. Conclusion: Our results shed light on the role of sophisticated and inter-connected regulatory networks in the fine-tune regulation of virulence factors at the early steps of the infectious cycle of Legionella pneumophilaactivity against extracellular L. pneumophil

Bacterial transcript analysis reveals a compensatory role for the *L. pneumophila* type IIdependent LapA aminopeptidase and PlaC acyltransferase during infection of Acanthamoeba.

Authors White R. C., Gunderson F. F., Cianciotto N. P.

We have previously shown Type II Secretion (T2S) promotes L. pneumophila growth in both the lung and in macrophages, and have demonstrated an essential role for T2S during co-culture with various species of protozoa. By testing mutants lacking individual T2S substrates, we also documented the importance of NttA, NttC, PlaC, ProA, and SrnA in Acanthamoeba (Ac), Vermamoeba, Naegleria and/or Willaertia. Results and conclusions: Here, we found T2S is dispensable for entry into amoebae but critical for the replicative phase of intracellular infection. Flow cytometric analysis revealed the number of replicating bacteria 1 / 2 inserimenti per cell was greatly reduced upon loss of T2S. We then determined gene expression profiles of proteins secreted by T2S upon replication in co-culture. We identified three genes (lapA, lapB, plaC) that were most up-regulated during growth in Ac. However, these genes had been shown to be dispensable for growth in Ac, based on the behavior of single effector mutants. To determine if these proteins compensate for one other, we assessed gene expression patterns in a lapA lapB double mutant and in a plaC mutant. Upon loss of lapA/lapB, plaC was further upregulated. Conversely, loss of plaC resulted in further up-regulation of lapA and lapB, suggesting LapA and/or LapB compensate for PlaC and vice versa. Hence we examined growth of various double and triple mutants lacking lapA, lapB, and/or plaC. lapA plaC and lapA lapB plaC mutants displayed a significant growth reduction in co-culture, uncovering a previously unidentified role for LapA and PlaC in intracellular growth within Ac. To examine whether aminopeptidase activity of LapA promotes growth, we supplemented the co-culture with free amino acids and found the growth of the mutants significantly improved, suggesting LapA may cleave host peptides/proteins to generate amino acids for uptake and subsequent growth. PlaC may promote the action of LapA or somehow provide an alternative food source

The GDSL hydrolase PlaD is a Dot/Icm secreted effector of *Legionella pneumophila* and confers high toxicity to eukaryotic cells

Authors Hiller M., Lang C., Flieger A.

The genome of Legionella pneumophila encodes for at least 15 phospholipases A which comprise among others three GDSL hydrolases (PlaA, PlaC and PlaD). It is currently not known 1 / 2 inserimenti whether and how L. pneumophila phospholipases contribute to the rearrangement of membrane lipids in the Legionella containing vacuole (LCV). We here examine the role of PlaD in L. pneumophila infection and investigate its mode of secretion and activity. MATERIALS/METHODS Secretion via the type IVB secretion system Dot/Icm was assessed by beta-lactamase translocation assay. Toxicity of PlaD variants was determined by live/dead staining of infected cells. For detection of enzymatic activity the protein was recombinantly expressed, purified and subjected to lipid hydrolysis assay and thin layer chromatography. Competitive infections were performed in the natural host Acanthamoeba castellanii. RESULTS PlaD was found to be secreted via the type IVB secretion system (Dot/Icm) which is dependent on the C-terminus of PlaD. Interestingly, C-terminal truncation of PlaD resulted in increased toxicity towards eukaryotic cells. PlaD exhibits weak lysophospholipase A and acyltransferase activity in vitro and possibly needs to be activated by a eukaryotic factor. In competitive infections of Acanthamoeba castellanii, a plaACD- triple mutant was outcompeted by wildtype L. pneumophila while a plaD- single mutant showed better replication than the wildtype. CONCLUSION Upon L. pneumophila infection, PlaD is translocated into the host cell cytoplasm and gets in contact with host membrane lipids. Thus PlaD might be involved in LCV lipid modifications. We currently address the intracellular localization of PlaD as well as interactions with eukaryotic proteins.

Cytotoxic Glycosyltransferase Effectors of *Legionella*

Authors Levanova N., Belyi Y., Schroeder G. N., Aktories K., Jank T.

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Intracellular growth defect of the iroT mutant of *Legionella pneumophila* despite its hypertoxicity

Authors Miyake M., Tsushima Y., Marukawa T. Kuniyasu K., Sugiyama A., Yoshida I. Abu Kwaik Y., Imai Y.

We had previously isolated many intracellular growth-deficient mutants of the L. pneumophila (Lp) AA100/130b strain designated pmi (protozoan and macrophage infectivity loci) and mil (macrophage infectivity loci). In these mutants, we found four mutants, which are designatged as Toxh mutants, for their hyper-toxicity to host cells. One belongs to the mil mutants (mil-Toxh mutant) while the others to pmi mutants. These Toxh mutants exhibited a cytotoxic phenotype to macrophages and Acanthamoeba polyphaga at levels similar to or higher than the wild type strain. Despite their extremely higher cytotoxicity, these mutants were defective in intracellular replication. In this study, we have characterized a mil-Toxh mutant, the GS147, which is defective in the iroT gene. The IroT protein is known to be involved in ferrous iron transport and be the orthologous protein of L. pneumophila Philadelphia MavN protein, which is an
effector involved in intra-vacuolar iron acquisition. To assess comprehensive gene expression of the GS147 strain within macrophages, microarray analysis using custom L. pneumophila array wasperformed. The relationship between alteration in gene expression and the phenotype of the GS147 mutant will bedeciphered.
The GS147 strain showed the high degree of toxicity to macrophages despite its defect in intracellular replication.
The GS147 strain induces apoptosis of host macrophages through the activation of caspase-3.
Pulmonary infection experiment of A/J mice with L. pneumophila strains demonstrated that despite the intracellular growth defect, the GS147 strain was virulent and lethal to mice similar to the wild type AA100/130b strain.
The mutation of iroT induces to alter the expression of many genes, including the genes encoding components of type IV secretion system and hypothetical proteins and flagella-associated genes.
One of causes of the hypertoxicity of the GS147 strain may be induced by the stringent response to iron limitation.

Caspase-8 participates in the Naip5/NLRC4/ASC inflammasome that is responsible for recognition and restriction of *Legionella pneumophila* replication in macrophages

Authors Mascarenhas D. P. A., Cerquiera D. M., Pereira M. S. F., Castanheira F. V. S., Fernandes T. D., Manin G. Z., Cunha L. D., Zamboni D. S.

Legionella pneumophila is a Gram-negative, flagellated bacterium that survives in phagocytes and causes Legionnaires' disease. Upon infection of mammalian macrophages, cytosolic flagellin triggers the activation of Naip/NLRC4 inflammasomes, which culminates in pyroptosis and restriction of bacterial replication. Although NLRC4 and caspase-1 participate in the same inflammasome, Nlrc4-/- mice and their macrophages are more permissive to L. pneumophila replication compared with Casp1/11-/-. This feature supports the existence of a pathway that is NLRC4-dependent and caspase-1/11-independent. Here, we identified a platform composed of NLRC4, ASC and caspase-8 that operates in response to flagellated bacteria independently of caspase-1/11. Infection of macrophages transduced with NLRC4-GFP or ASC-GFP with flagellin-positive bacteria triggered puncta composed of NLRC4, ASC and caspase-8. Accordingly, NLRC4 and ASC, but not caspase-1/11, were required for caspase-8 activation in response to flagellated bacteria. Silencing caspase-8 in Casp-1/11-/- cells culminated in macrophages that were as susceptible as Nlrc4-/- for the restriction of L. pneumophila replication. Accordingly, macrophages and mice deficient in Asc-/-/Casp1/11-/- were more susceptible than Casp1/11-/- and as susceptible as Nlrc4-/- for the restriction of infection by several species of Legionella. Collectively, our data reveal an inflammasome involved in flagellin sensing that is dependent on NLRC4, ASC and caspase-8 and independent of AIM2, caspase-1 and caspase-11. This platform is critical for the restriction of bacterial infection in macrophages and in vivo.

Legionella effector AnkX disrupts host cell endocytic recycling in a phosphocholination-dependent manner

Authors Neunuebell M. R., Allgood S. C., Badjo B. P., Noll R. R., Lein S., Pike C.

The facultative intracellular bacterium Legionella pneumophila proliferates within amoebae and human alveolar macrophages, and it is the causative agent of Legionnaires' disease, a life-threatening pneumonia. Within host cells, L. pneumophila establishes a replicative haven by delivering numerous effector proteins into the host cytosol, many of which target membrane trafficking by manipulating the function of Rab GTPases. The Legionella effector AnkX is a phosphocholine transferase that covalently modifies host Rab1 and Rab35. However, a detailed understanding of the biological consequence of Rab GTPase phosphocholination remains elusive. Here, we broaden the understanding of AnkX function by presenting three lines of evidence that it disrupts endocytic recycling. First, using superresolution microscopy and immunogold transmission electron microscopy, we determined that GFP-tagged AnkX ectopically produced in mammalian cells localizes at the plasma membrane and tubular membrane compartments, sites consistent with targeting the endocytic recycling pathway. Furthermore, the C-terminal region of AnkX, was responsible for association with the plasma membrane, and we determined that this region was also able to bind the phosphoinositide lipids PI(3)P and PI(4)P in vitro. Second, we observed that mCherry-AnkX co-localized with Rab35, a regulator of recycling endocytosis and with major histocompatibility class I protein (MHC-I), a key immunoregulatory protein whose recycling from and back to the plasma membrane is Rab35-dependent. Third, we report that during infection of macrophages AnkX is responsible for the disruption of endocytic recycling of transferrin, and AnkX's phosphocholination activity is critical for this function. These results support the hypothesis that AnkX targets endocytic recycling during host cell infection. Finally, we have demonstrated that the phosphocholination activity of AnkX is critical for both disruption of endocytic recycling and for inhibiting fusion of the Legionella-containing vacuole with lysosomes.

Comparative whole genome sequence analysis of a *Legionella pneumophila* sg 6 clinical strain and its spontaneus avirulent mutant

Authors Ricci M. L., Equestre M., Salaris S.,Marcantonio C., Orsini M., Scaturro M.

Legionella pneumophila (Lp) serogroup 6 (sg6) is the second most worldwide common serogroup causing Legionnaires' disease. We have already demonstrated that a spontaneous mutant of Lp sg 6 clinical strain is unable to grow into macrophages and escape the lysosome-phagosome fusion. The lack of flagellum and a mutation in dotA gene, affecting the Type IVB secretion system, were also detected. Aim of this study was a comparative genome analysis between this spontaneous avirulent Lp sg6 mutant, named Vir-, and its parental strain (Vir+), to explore genome diversity.

Methods

Genomes were sequenced by Illumina MiSeq platform. Raw reads were evaluated by FastQC and then trimmed using NGS QC toolkit and FASTX. Genome assembly was obtained by SPAdes and ABACAS for contigs ordering, (NC_014125 as reference genome). Gene prediction and functional annotation were done using Rapid Annotation using Subsystem Technology (RAST).

Results

The assembly process returned an horizontal coverage of 99.9%, for both Vir+ and Vir-. Features, including length, GC content, number of coding sequences, RNAs and Subsystems were obtained. Functional comparison showed genetic differences in 42 annotated genes, involving subsystem categories such as: Cell and Wall Capsule, Membrane Transport, Nucleosides and Nucleotides, Protein Metabolism and Virulence, Disease and Defence.

Conclusions

The correlation between the avirulence of Vir- and the observed genomic variations are currently being evaluated and will be of great value in increasing the knowledge of virulence mechanisms of this important Legionella pneumophila serogroup as well as for the pneumophila specie.

Rapid adaptations to the accidental human host in *Legionella pneumophila*

Authors Leenheer D., Pelaz C., Morin M., Hallin E., Klingenberg D., Jarraud S., Ginevra C., Guy L.

Legionella is an amoeba-resistant bacterium found in aquatic environments. In humans, it can cause Legionnaire's disease and Pontiac fever, using similar mechanisms to infect human macrophages. We hypothesize that, despite similarities, the hosts are different enough so that there exist high-selective value mutations that would dramatically increase the efficiency of macrophage infection. As human-to-human transmission is very rare, fixation of these mutations into the population is unlikely, and that mutations in the same genes would be observed in independent human infections, as an example of convergent evolution. Identifying these adaptive mutations would shed light on the specifics of Legionella infection in its accidental human host. By comparing a large number of independent infections, we expect these highly adaptive mutations to appear several times, despite the short duration of the infection. 2 / 3 inserimenti Methods Clinical isolates and isolates identified as their environmental source were sequenced using the Illumina MiSeq platform. Sequences were de-novo assembled using SPAdes, and variants (INDELs and SNPs) were called according to the best practices of the Genome Analysis Toolkit. Variants between samples are compared to identify genes that are likely candidates for humanspecific adaptations in multiple samples. Isolates from 58 independent infections or outbreaks were obtained and sequenced (161 isolates in total, from Sweden, France and Spain). Results A preliminary analysis of 20 of the 102 total comparisons between a clinical sample and its respective environmental source showed that at least six genes were mutated independently two to four times. These genes included the elongation factor Tu and an extracellular serine protease. Conclusion Sequencing and comparing large numbers of Legionella clinical and environmental isolates enable us to identify adaptations that allow Legionella to infect the human host.

The small regulatory RNA lpr0010 plays a role in *Legionella pneumophila's* survival in water

Authors Saoud J., Massé È., Faucher S.

In recent years, small regulatory RNAs (sRNAs) have been identified as important players in virulence and other cellular functions. While many regulatory RNAs (sRNA) have been identified in Legionella pneumophila (Lp), only a few of them have been studied and characterized. Microarray analysis of the response of Lp to water revealed that one sRNAs, Lpr0010, was highly induced, suggesting a potential role during survival in water. 96 Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; msostyle-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minorlatin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin;} Objective: The objective of this study is to determine the role of Lpr0010 in Lp. 1 / 2 inserimenti Methods: Northern Blot was used to evaluate the differential expression of the sRNA, while 5' and 3' RACE were used to confirm its size. A deletion mutant strain was constructed by allelic exchange. Intracellular growth was tested in THP-1 human macrophage cell line as well as in bone-marrow derived macrophages (BMDM) from A/J and C57BL/6 background. Daily CFU counts were done to assess the survival of the strains over a period of 1 week. Survival in water was tested by resuspending cells from CYE plates to chemically defined water (fraquil) at an O.D600 of 1.0, and CFU counts were done on a weekly basis. Results: The expression of lpr0010 increases during the post exponential phase. Gene expression during the post-exponential phase is considered to partially reflect that of the transmissive phase during infection. Expression profiles of this sRNA were analyzed in mutants of known virulence regulators. Lpr0010 is downregulated in a DcpxR background, but upregulated in a DrelA and DrelA/DspoT background. The mutant was not affected for intracellular growth. The survival in water of the lpr0010 mutant was significantly reduced compared to the wild type (WT) strain. Conclusion: Lpr0010 is regulated by the stringent response and essential for optimal survival in water. Targets of the sRNAs will be identified by analysis of the mutant strain's transcriptome by microarray.

Investigating the Use of Metagenomic Sequencing for *Legionella* detection

Authors Carney S., Cox M. J., Cookson W. O.,Chalker V. J., Moffatt M. F.

Current typing efforts for bacterial pathogens are moving towards analysis of whole genome data generated from isolate material. Legionella, however, is difficult to isolate, typically taking from 3 to 5 days. Culture-independent metagenomic sequencing, or sequencing the total nucleic acid content of a sample, may provide information on Legionella presence when it cannot be isolated. It may also provide more information on the landscape of infection such as mixtures of Lp1 sequence types or Legionella species, which have been reported recently. The present study aims to sequence Legionella genomes directly from samples of clinical and environmental origin without a culture step. Materials/Methods: A sequencing validation step was first carried out by preparing metagenomic mock communities to assess the retrieval of Legionella and other bacteria at known proportions in human DNA. Following this, 6 clinical specimens, including known Legionella 1 / 2 inserimenti pneumophila positives, were sequenced to investigate what proportion of microbial DNA can be sequenced and if Legionella can be detected. Results: For five patient samples, the majority of reads sequenced belonged to host DNA. The bacterial proportion of the sequenced Legionella positive samples represented 0.17%, 0.03% and 0.03% of the total DNA. The bacterial proportion of the sequenced Legionella negative samples represented 55.95%, 2.26% and 0.57% of the total DNA. Conclusion: These results confirm prior observations that for metagenomic sequencing either a depletion of host DNA or a target enrichment approach is required to sufficiently and affordably sequence bacterial genomes. Currently a method for depletion of host DNA is being developed and the design of probes to capture Legionella present within the complex microbial background is being investigated. Further clinical and environmental samples received from Public Health England will be sequenced and the presence of mixed Legionella populations will be investigated.

Assessing Genetic Diversity for *Legionella pneumophila* Sequence Type 1 Isolate Discrimination

Authors Mercante J. W., Caravas J. A., Ishaq M. K., Kozak-Muiznieks N. A., Morrison S. S., Raphael B. H.

Current genetic subtyping methods cannot resolve Legionnaires' disease (LD) outbreaks caused by some common, endemic L. pneumophila (Lp) sequence types (ST). In the United States, ST1 is the most prevalent clinical and environmental Lp ST. Recent reports demonstrate the value of whole-genome sequencing (WGS) for providing greater discrimination of Legionella; therefore, our goal was to describe the genomic diversity of ST1 in order to develop improved laboratory investigation tools. Methods: We sequenced 269 ST1 and ST1-variant Lp strains from 36 US states archived from 1982-2016. The addition of 233 international sequences created a 502-isolate dataset that was analyzed using various bioinformatic methods for genetic and geographic diversity and LD cluster resolution. Results: The ST1 population was highly conserved; 98% of core nucleotide (nt) positions were invariant. However, compared to clinical or outbreak subgroups, environmental isolates 1 / 2 inserimenti contained ~65% more nt diversity and a ~30-60% larger accessory genome enriched for transposition and conjugative transfer elements. We noted potential SNP-based geographical clustering, yet considerable conservation existed among strains from multiple continents and decades. WGS combined with a CDC-developed wgMLST scheme was successful in resolving LD outbreaks in our ST1 dataset, and new genomic loci were identified to increase discrimination of ST1 isolates within an expanded SBT subtyping scheme. Conclusion: ST1 legionellae exhibit striking genetic homogeneity overall, but intrinsic differences exist between subgroups. WGS and bioinformatic analyses can provide necessary resolving power for source attribution during ST1 outbreaks, and addition of new, variable genomic loci may help extend existing subtyping schemes for ST1 strains. The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention

Characterising the Genome of *Legionella* longbeachae Serogroup 1 Clinical Isolates

Authors Slow S., Anderson T., Harte D., Murdoch D. R.,Winter D., Biggs P. J.

Background: New Zealand (NZ) has one of the highest incidences of Legionnaires' disease (LD) in the world with Legionella longbeachae serogroup 1 (sg1) causing around two-thirds of cases. Given its clinical significance, we have obtained the genome sequence of 54 L. longbeachae sg1 isolates derived from LD cases from around the country over a 20 year period in order to assess genomic variability. Materials and Methods: Clinical isolates obtained from the NZ Legionella Reference Laboratory and Canterbury Health Laboratories were cultured on buffered-charcoal-yeast-extract agar (72h, 35°C) and DNA was purified using Genomic-Tip 100/G. Sequencing was conducted using the Illumina MiSeq system and the resulting 250 bp paired-end reads were assembled using SPAdes. To create a complete NZ reference genome and obtain epigenome data, one isolate (F1157CHC) was also sequenced using the PacBio RSII system. RSII reads were assembled using the HGAP2 assembly pipeline and polished using Quiver. The MiSeq reads were mapped onto the final RSII assembly using Pilon to assess accuracy. Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline and Prokka. Sequences from the 53 other isolates were aligned with our reference genome and a SNP phylogeny created using Snippy. Results: The reference genome obtained for isolate F1157CHC consisted of a chromosome of ~4.1Mb (Genbank CP020894.1) and a plasmid of ~108 kb (Genbank CP020895.1). A total of 3,691 coding sequences were predicted for the 1 / 2 inserimenti chromosome and plasmid (3,577 and 114, respectively) with 12 rRNAs, 52 tRNAs and 4 ncRNAs. SNP analysis of the chromosome revealed clusters of highly related isolates, areas of recombination and >2000 non-recombinant SNPs. There were clusters of isolates from the same region that appeared to persist over time (>10 years), while others did not. Conclusion: Whole genome analyses shows large scale rearrangements in the L. longbeachae genome and regions of high variability

Characterization of a novel transcriptional regulator in *Legionella pneumophila*

Authors Graham C., Patel P., Brassinga A. K. C.

In its natural and urban anthropogenic aquatic environments, L. pneumophila is an intracellular parasite of protozoa exhibiting a distinct multi-phasic lifecycle, typically alternating between a vegetative replicative form (RF) and the cyst form (CF). The cellular morphology of the RF is a typical Gram negative, non-motile rod. Conversely, CFs are highly motile, irregular coccoid cells with thickened cell walls and multiple membrane laminations enabling their survival between protozoan hosts, and resistance to biocides and heat aimed to eradicate CFs from freshwater. While significant advances have been made in establishing the framework of the regulatory cascade controlling cyst biogenesis, there remains much to understand in identifying and characterizing the pathways involved. Here we report the discovery of a novel transcriptional regulator that is directly associated with the differentiation process. 1 / 3 inserimenti Material and Methods A gene deletion strain was generated and assessed for growth and cellular morphology phenotypes in vitro, and in vivo in human U937-derived macrophage-like cells and Acanthamoeba castellanii protozoa. For comparison and control, the parental L. pneumophila Lp02 and in trans complemented strains were included. GFP-reporter promoter fusion constructs and DNA/ protein binding assays were employed to localize binding sites. Protein levels were determined by immunoblotting with custom-generated antibodies. Results The novel transcriptional regulator is a strong autorepressor with protein levels tightly controlled, but constitutively expressed through all growth phases in vitro. Absence of the transcriptional regulator slightly altered intracellular growth kinetics; however, overexpression, particularly in the parental strain background arrested the differentiation process. Conclusions The novel transcriptional regulator appears to have an important role in the differentiation of the replicative form to the cyst form in host cells

Role of the trans-encoded sRNAs lpr0014 and lpr0059 in the virulence of *Legionella pneumophila*

Authors Mani T., Faucher S.

Small regulatory RNAs (sRNAs) play key roles in regulation of virulence factors and other processes in many bacterial pathogens. Out of the many sRNAs identified in the genome of Legionella pneumophila (Lp) only a few have been studied and characterized. Previous microarray analyses of known virulence regulator identified candidate sRNAs likely involved in the control of virulence factors. The objective of this study was to determine the role of 2 sRNAs, Lpr0014 and Lpr0059. Methods: Northern blot was used to evaluate differential expression of the sRNAs lpr0014 and lpr0059. 5' and 3' RACE were performed to confirm the actual size of the two sRNAs. Deletion and complemented mutant strains were created for the sRNAs to understand their role in bacterial survival and virulence. Survival was tested by resuspending wild-type and mutants in a artificial freshwater medium (fraquil) at an O.D600 of 0.1, and performing CFU counts weekly. To learn whether the sRNAs are implicated in virulence, we compared the ability of the wildtype L. pneumophila, lpr0014 and lpr0059 mutants to infect and multiply in the human monocytederived cell line THP-1 as well as in bone-marrow derived macrophages (BMDM) from A/J and 1 / 2 inserimenti C57BL/6 mice. Daily CFU counts were done over a period of 1 week. Results: Northern blot were performed using total RNA extracted from the KS79 wild-type strain and various mutants of known virulence regulators to see the expression pattern of each of the sRNAs. Successful creation of deletion and complemented strain mutants were confirmed by Northern blot analysis. Preliminary result indicates that both the sRNAs are essential for intracellular growth since their respective deletion mutants show decreased growth compared to the wild-type control. Conclusion: Lpr0014 and Lpr0059 seem to be involved in the control of virulence factors. To identify targets of the two sRNAs, transcriptome analysis of their respective mutants will be performed by microarray.

Looking into microbial dark matter in sediment and soil samples using metagenomics.

Authors Graells T., Guy L.

Legionella spp. are ubiquitous bacteria in environmental water, sediment and soils. They belong to the order Legionellales and are intracellular pathogens of amoebae and protozoa, reproducing inside the host's vacuoles. They can even be accidental pathogens for humans. As far as we know, all members of Legionellales have the ability to reproduce inside a host, but little is known about their lifestyles in their natural environment and hosts. The aim of this study was to investigate the abundance and diversity of Legionellales in various environments, to shed some light on their relationships with their hosts. Methods: Sediment and soil samples were collected from 35 sediment and soil near ponds and in mines in Sweden. Total DNA was extracted using MoBio Soil DNA extraction kit. A 2-step PCR with universal primers (16S/18S) was performed to build amplicon libraries, which were then sequenced on an Illumina MiSeq. After quality control and trimming, forward reads were sorted in Operational Taxonomic Units (OTU), using the imTornado pipeline. Four samples, that were the most diverse and most abundant OTUs belonging to Legionellales deep-sequenced on four lanes if Illumina HiSeq2500. Results: Out of thousands of OTUs found in our soil and sediment samples, as many as 66 belonged to Legionellales. The mine samples displayed 32 Legionellales OTUs. All the samples but one have one or more OTU belonging to Legionellales, but the highest abundance was only 0.5%. These OTUs have been placed in a Legionellales phylogenomic tree and cover the whole diversity of the order. Conclusion: This survey reveals (i) the low abundance (ii) the ubiquity and (iii) the hidden diversity of Legionellales. If they, as Legionella, are able to rapidly (although transiently) multiply in man-made structures, they might represent a source of the large amount of atypical pneumonia for which no etiological agent can be identified.

ABSTRACT BOOK

The 9th International Conference on

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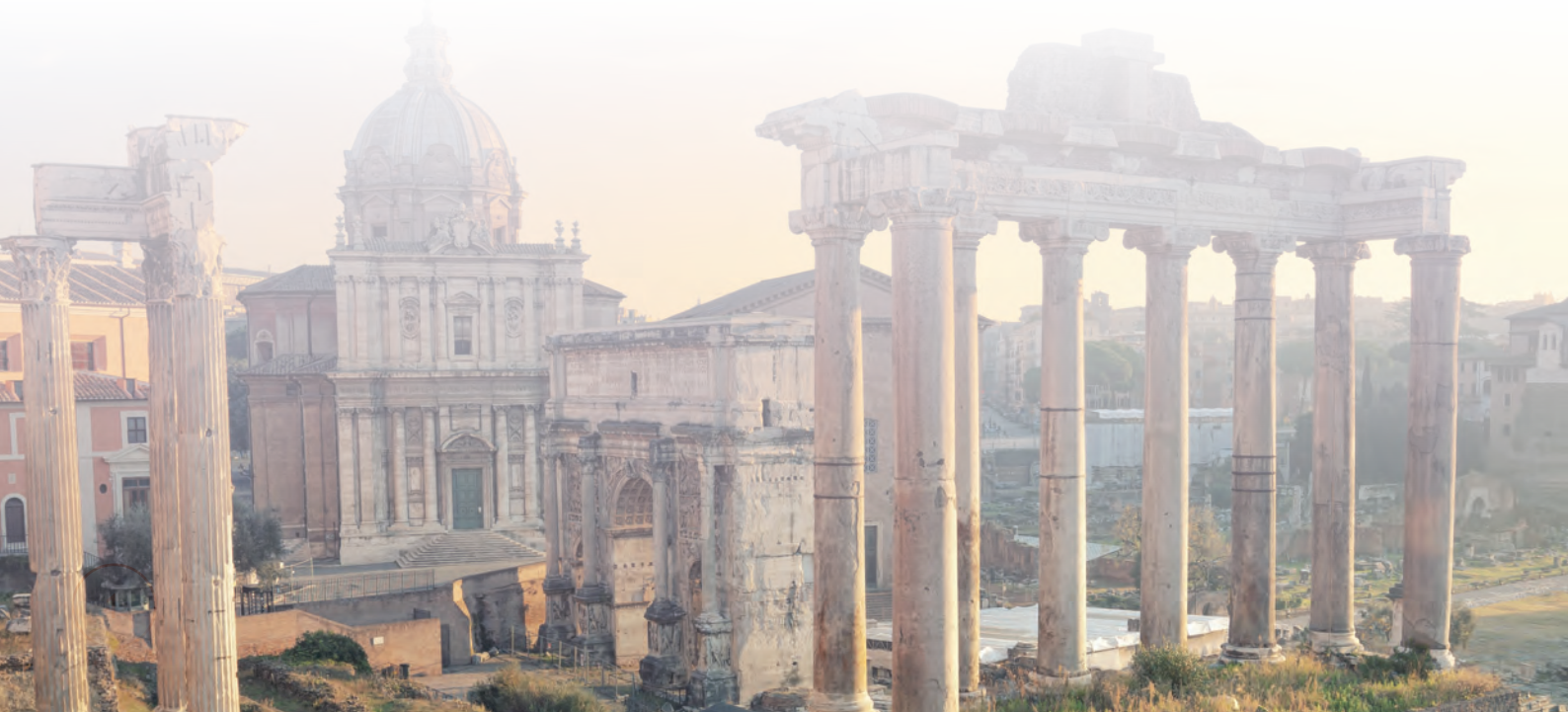
Rome, 26th - 30th September 2017

POSTER SESSION 2

THURSDAY, SEPTEMBER 28TH, 2017



A Legionellosis case linked to Contaminated Hot tub Water: Importance of amoebae to isolate environmental *Legionella pneumophila*

Authors Dey R., Dlusskaya E. A., Tyrrell G. J., Ashbolt N. J.

Amoeba-resisting bacteria (ARB) such as Legionella spp. are potential human pathogens that almost exclusively cause disease via aerosols from water systems. To detect ARB from both human and environmental samples, co-culture with amoebae has been demonstrated as an efficient tool. However, using this procedure, mostly water from cooling towers and hospital water supplies has been investigated as the possible reservoir of ARB. In the present study, we traced the source of a legionellosis case by first isolating the free-living amoebae host Vermamoeba sp. then its ARB from the hot tub of the child patient household. One of the ARB isolates from the amoeba was identified as Legionella
pneumophila by 16S rRNA gene sequencing. The L. pneumophila strain isolated from the patient's sputum shared the same serogroup (6) and sequence type (185) as the one isolated from the hot tub. This appears to be the first reported hot tub-associated infection caused by L. pneumophila serogroup 6, and may have resulted from the patient's immunosuppressed healthstatus.

Molecular and epidemiological analysis of *Legionella pneumophila* strains in an outbreak at bath facilities in Japan

Authors Amemura-Maekawa J., Kuroki T., Ohya H., Furukawa I., Suzuki M., Masaoka T., Aikawa K., Hibi K., Morita M., Lee K. Ohnishi M., Kura F.

In Japan, there are over 1,500 cases of occurrence of Legionnaires' disease per year. Bathing facilities are the most frequent source of infection in outbreaks in Japan. Seven Legionnaires' disease cases occurred among users of hot spring facilities in Odawara, Kanagawa Prefecture between 1 June and 17 June 1015. About 800 people on weekdays and 1,400 on holidays visited the facilities possessing 9 baths for males and females each. All of 7 patients diagnosed by urinary antigen of L. pneumophila SG 1 were male with a mean age of 66.3 years old.

Materials/Methods: Sputum samples from five of seven patients and environmental samples from the hot spring facilities were collected for epidemiological investigations and were cultured for Legionella detection.

Results: L. pneumophila SG 1 (ST1114, the new ST) was detected from the five patients, and SG 13 (ST2113, the new ST) was also detected from one of them. L. pneumophila SG 1 (ST2114) and SG13 (ST2113) were also detected from Baths 1 and 2. Bath 3 contained L. pneumophila SG 1 (ST1447), Baths 4 and 5 contained L. pneumophila SG 10 (ST2115, the new ST), but Legionella was not detected from Baths 6-9. Whole genome sequencing of the ST2113 (one-clinical and two-environmental), ST2114 (five-clinical and six-environmental) and other-STs (four-environmental) strains by MiSeq were performed and SNPs were extracted using L. pneumophila Philadelphia 1 strain as a reference sequence. When the SNPs to the reference sequence were excluded, position of remaining SNPs of strains related to the outbreak had the biased distribution. Approximately 2,100 and 700 SNPs were detected in two genomic regions, respectively. One is the 110 kb region including serogroup-determing gene cluster, suggesting genetic recombination accompanied seroconversion from SG1 to SG13 or vice versa, and the other is the 340 kb region. The isolates were divided into three groups according to the distribution of SNPs: the two SG1 (ST2114) groups with the 700 SNPs each other, and one SG13 (ST2113) group. Intra-group SNP ranged from 1 to 18 in each group.

Conclusion: Molecular and epidemiological investigation of clinical and environmental strains responsible for the outbreak suggested L. pneumophila strains with different serogroups diversified from a common ancestor through mutation, recombination, or gene transfer prior to the outbreak.

Pontiac fever outbreak during all doctors hen party

Authors Scaturro M., Rota M. C., Caporali M. G., Pasticci M. B., Tozzi G., Ciani C., Mencacci A., Ricci M. L.

Pontiac fever (PF) infection, a flu-like disease due to Legionella, is very seldom reported compared to the more severe pneumonia named Legionnaires' disease (LD). It has a shorter incubation period and an extremely high attack rate than LD.

In May 2017 fifteen young female doctors (median age 29.5) were invited to celebrate their colleague hen party in a hotel with a spa pool. All had a bath and after 24-48 hours all reported headache, malaise, fatigue and myalgia symptoms, 13/15 had also fever. A suspect of PF infection immediately arose and before starting specific antimicrobial therapy, urine and sera were collected for testing. Environmental investigation was carried out at the hotel and water samples collected in the spa pool.

PF was confirmed by positive Legionella urinary antigen (Binax Now) test for 12/15 patients after less than 48 hours from the exposure to the spa aerosol. Two urine samples from two patients, collected ≥48 h after levofloxacin was administered resulted negative, while one patient was not tested.

Legionella antibodies detection by indirect immunofluorescence test, in paired acute- and convalescent-phase sera were negative for all patients. Microbiological analyses of water samples found Legionella pneumophila serogroup 1 (106 CFU/L). Monoclonal antibody and SBT typing analyses identified Legionella pneumophila serogroup 1 subgroup Knoxville ST733. Control measures were soon implemented in the spa pool to avoid the occurrence of additional PF or LD cases.

The high concentration of bacteria in the spa pool and a considerable long period of exposure (about 2 hours) caused this outbreak of PF. The young age and the lack of individual risk factors of the patients prevented them to acquire LD. No other PF or LD cases were notified among people who attended the same spa pool before this outbreak. A prompt diagnosis allowed identifying a cluster of PF.

Hotel whirlpool bath as a source of Legionnaires' Disease and Pontiac fever outbreak?

Authors Räsänen P.S., Ruotsalainen E., Broas M., Huumonen K., Metso J., Jääskeläinen A. J., Kakriainen A., Yli-Ilkka T., Jaakola S., Kinnunen M., Mentula S., Kusnetsov J., Lyytikäinen O.

In March 2017, a group of 7 adults with fever, headache and muscular pain, 3 also with pneumonia, sought care 2 days after staying in a hotel in Lapland, Northern Finland. Legionella urinary antigen tests were negative, serology is still pending. The hotel visitors stayed the first night in 3 separate rooms and the following 2 nights they shared a penthouse room with private whirlpool bath, where they bathed many times. After notication of illnesses, local health authorities closed the whirlpool. Hotel water systems and whirlpool bath were sampled, hot water temperature was increased, chlorination (1-2 mg/l) and flushings performed. Whirlpool bath was cleaned with chlorine (12 mg/l). Whirlpool water grew Legionella pneumophila serogroup 6 (1200000 cfu/l) as well as the whirlpool nozzle swabs. Penthouse hot and cold water grew L.p. sg 6 (5-240 cfu/l) and Legionella anisa (up to 6400 cfu/l). Of the 3 separate rooms, 3/3 cold water samples and 2/3 hot water samples contained L. anisa (up to 21000 cfu/l). Hot water temperatures were low in all sampling points (range 42-56 °C). Legionellae were not detected in the incoming water preceding the hotel water distribution system. After the first cleaning, Legionellae were still found in the penthouse hot and cold water systems (2520-13020 cfu/l), but not in the whirlpool. From the separate rooms, Legionellae were isolated from 2/3 cold water sampling sites but no longer in the hot water system. More efficient cleaning is ongoing. Pneumonias and Pontic fever-like symptoms among the hotel visitors were likely caused by L.p. sg 6 of the whirlpool. However, the human laboratory confirmation is still lacking. The abundance of L. anisa findings indicates flavorable conditions for Legionellae. Two thirds of the hotel is only seasonally open, thus stagnation of water is an issue. Rising of hot water temperature decreased Legionella concentration in hot water system and chlorination was successful in the whirlpool bath.

Experience of public health officials in the investigation of a *Legionella* outbreak associated with one Hotel

Authors Serres M., Feliu T., Aguilar C., Minguell S., Coll C., Bieto M.

Legionelosis is an environmental infection produced in most cases by Legionella pneumophila serogrup 1, that can occur as community or nosocomial outbreaks. The inhalation of aerosols or the microaspiration of water containing the bacteria is recognised as the transmission routes. The risk of acquiring the infection depends upon the health status and on the intensity of the exposition. We present here the experience of the public health officials involved on the research of a Legionella outbreak associated to an hotel and that affected 12 persons, of which one died. The establishment provided accommodation for 2000 people distributed in 3 buildings, and have old water installation and have different potential source of infection. During the investigation 4 inspection visits were performed and a total of 23 water samples were taken for the analysis of Legionella, a physiochemical analysis was performed in 10 water samples, and 4 sputum clinical samples. Results evidenced the presence of Legionella pneumophila serogrup 1 in two water samples collected from the hydromassage bathtub (Jacuzzi) and the irrigation hose from well water. The PFGE and the Sequence Base Typing revealed that the isolated strains from one of the sources of infection, hydromassage bathtub, showed the same profiles from 3 of the 4 patients. During all the stages of the outbreak investigation all stakeholders were informed as were also the media. For the public health officials, the importance of this research is related to the difficulty of obtaining a complete epidemiological information, to the complexity of the water installation and the poor collaboration obtained by the owners of the establishment. The experience has enabled us to evaluate the pros and cons of the interventions and has allowed us better define protocols to be implemented in future situations.

Legionella pneumophila on tap: simulating disinfection measures of case study apartment Buildings

Authors Van Kenhove E., Janssens A., Laverge J., Ghent University, Ghent, Belgium

Legionella pneumophila is naturally present in water. The bacteria is almost undetectable in water that enters the building, in Domestic Hot Water (DHW) installations in buildings situations can occur that stimulate the growth of Legionella pneumophila. It is important to determine the points of risk which enhance the growth of Legionella pneumophila. The most important growth factors are temperature and flow rate.

The production of DHW dominates the total energy demand of well insulated and air tight buildings. One of the main reasons for the high energy demand is that hot water is produced, stored and distributed at temperatures above 60°C to mitigate the risk of infecting the DHW system with Legionella pneumophila. At these temperatures, Legionella pneumophila bacteria are effectively killed. For most of the applications of hot water, temperatures of only 30-40°C are required. This disparity doubles the temperature difference between the DHW system and the environment and has a detrimental effect on the efficiency of hot water production units.

A simulation model is developed that allows to investigate the infection risk for Legionella pneumophila in the design phase of a DHW system and to test the effectiveness of disinfection techniques on an infected system. In addition to the simulation model, Legionella pneumophila measurements are performed in buildings.

In this paper the simulation model is used for four case study buildings with 800 apartments to propose best suitable measures to adapt the DHW installation which is infected with Legionella pneumophila. The most effective steps to disinfect such a system and keep it healthy and energy efficient on the long term are investigated by simulation (temperature variations, flow rate, adding of insulation, Ultraviolet Radiation,…).

The overall aim is to update existing design guidelines for DHW installations based on a trade

A 1-year surveillance of Legionnaire's disease including a 2-month outbreak in Parma (Northern Italy)

Authors Calderaro A., Martinelli M., Larini S., Piscopo G., Ruggeri A., Di Maio A., Montecchini S., Dell'Anna M. L., Buttrini M., Arcangeletti M. C., Medici M. C., De Conto F., Chezzi C.

Legionella pneumophila (LP) is recognized as a cause of atypical pneumonia known as Legionnaires' disease (LD). This study reports a 1-year surveillance of LD in patientsattending the University Hospital of Parma, Northern Italy.

Material/methods: During 2016, 1324 urine samples belonging to 1270 patients were submitted to LP urinary antigen testing (UAT) by immunocromatographic and/or immunofluorescence assays. In the same period, 95 respiratory samples (36 bronchoaspirates, BAS, and 59 bronchoalveolar lavages, BAL) for the detection of LP by conventional culture and/or molecular method, and 172 sera (133 patients, 79 of which with UAT performed) for anti-LP antibodies evaluation by indirect immunofluorescence were analyzed. Results: A total of 65 LP positive urine samples was detected. Due to an increased frequency of UAT positive samples, a LD outbreak was disclosed from the end of August to the beginning of November 2016 involving 51 patients, the majority of which (29) lived in the south-east district of the town or attended the same area for working or family reasons. During 2016, among the 95 respiratory samples examined for LP, 1 BAS was positive by culture and 2 BAL (1 included in the outbreak) by molecular method, all positive by UAT. For 133 patients (55 with positive UAT), at least one serum sample was analyzed: for 69 the samples were negative, 20 had a titre ranging from 1:16 to 1:64 and 24 a titre >1/64, and in the remaining 20 cases a seroconversion was revealed.

Conclusion: The annual prevalence of LD in our area is generally low (2% in 2015 by UAT). During 2016, an outbreak was observed involving 54 patients (3 of them revealed only by antibodies detection), most of them were geographically related to a district of Parma. Unfortunately, till now, the source of infection is unknown and this stimulate to the collection of respiratory samples from the patients in order to isolate LP for epidemiological purposes when an outbreak of LD occurs.

Outcome of a 12 month National study of Legionnaires' Disease in New Zealand: TheLegiNZ Study

Authors Chambers ST

Background: Legionnaires' disease (LD) is regarded as a severe form of community acquired pneumonia (CAP), and empiric anti-legionella therapy (ALT) is recommended if the CURB-65 score (CS) on admission is >2 or LD is suspected. Our hypotheses were that mild LD is common, and the outcome is improved by pre hospital ALT, and ALT started 24 hours after admission (early ALT). Materials/Methods: LegiNZ was a prospective cross-sectional study, with active case finding over a 1-year period from 20 hospitals covering 96% of New Zealand's population. Lower respiratory samples from patients hospitalised with CAP were routinely tested for Legionella by PCR. Additional cases of LD were identified through mandatory notification to Public Health Units. CS were calculated at admission. Results: Of the 246 cases (males 154, median age 68 years, range 7-91) Legionella longbeachae was identified in 149 and Legionella pneumophila in 56. On admission 49% had mild (CS 0 and 1), 24% moderate (CS 2) and 22% severe (CS 3-5) CAP (CS missing in 31) with 1 / 2 inserimenti a median length of stay (LOS) of 5, 6 and 8 days respectively. Six cases died in hospital (CS range 1-5) and 39 were admitted to ICU (CS median 3, range 0-5). General practitioners treated 61 before admission. The 11 given ALT (CS median 0) were milder than those given beta-lactams (n=50, CS median 2) and had a shorter LOS (median 4 and 6 days). After admission early ALT was given in 77% of cases including 30 (16%) who were admitted to ICU. Of those given ALT later 9 (16%) were admitted to ICU. For ICU survivors the median LOS was 5.5 days (range 1-30) for early ALT (n=28) and 8 days (range 1-21) for later ALT (n=8) (p=0.2). There were no significant differences in age or severity between these groups.

Conclusions Mild LD was more common than severe, and ALT given prior to admission may reduce severity. Early empiric ALT may improve LOS in ICU but not admissions. Real time results may improve ALT coverage for LD. Larger studies are required.

Improved isolation of *Legionella* longbeachae bacteria from potting mix products

Authors Mohammadi A., Anderson T., Lewis J., Scott-Thomas A., Chambers S. T., Murdoch D. R.

Legionella longbeachae is the main causative agent of Legionnaires' disease (LD) in New Zealand with a peak infection period during the spring and summer months. Recent studies suggest that it is a plant pathogen that can be found throughout the environment and is associated with potting mix and compost. As such L. longbeachae infection and subsequent LD is correlated with gardening, mulching and composting. Acid pre-treatment followed by culture commonly used for the detection of L egionella species in environmental samples but has limited sensitivity because of high contamination loads. Therefore, we investigated a variety of techniques in an attempt to improve upon the current isolation and identification of L. longbeachae from environmental sources. Material/Methods: To develop the techniques, 5g of a commercial potting mix product was autoclaved and then spiked with 106 CFU/ml of L. longbeachae ATCC33462. Subsequent studies were done on non-autoclaved potting mix. Recovery of organisms after acid wash pre- 1 / 2 inserimenti treatment, direct and indirect immunomagnetic separation (IMS), sucrose gradient centrifugation and antibiotic decontamination followed by cultivation on Legionella selective media were compared. MALDI-TOF and quantitative PCR techniques were used to confirm the identity and quantify of the recovered organisms. Results: The results of our comparative study indicated that IMS has greater recovery (105 cfu/ml) compared with the current acid wash-culture standard (103 cfu/ml). Antibiotic decontamination also removed many undesirable organisms within the samples. Conclusion: Our study shows each method has a recovery rate, but all purification techniques reduced contamination with environmental organisms and increased the recovery of Legionella bacteria compared with acid wash. Combining IMS with antibiotic decontamination has further potential to improve the yield of L. longbeachae isolated from potting mix products.

EMA or PMA combined with qPCR cannot be used to detect viable naturally grown *Legionella pneumophila* cells from aquatic environments

Authors Wullings B. A., Van der Wielen P. W. J. J.

DNA based detection methods like qPCR can elucidate the presence of Legionella pneumophila within a few hours. A drawback in using qPCR to quantify L. pneumophila in water, is that qPCR does not distinguish between live and dead cells.. Recently, qPCR preceded by EMA or PMA incubation has been used to quantify viable bacteria. The objective of our study was to determine whether EMA or PMA can be used to reliable quantify viable naturally grown L. pneumophila. Cultures and environmental samples, containing naturally grown L. pneumophila, were non-, heat- or disinfectant-treated. Cultivable and qPCR detectable L. pneumophila were determined in these samples. qPCR was directly done on the DNA or were preceded by an optimized PMA and EMA incubation. L. pneumophila grown under optimal laboratory conditions showed the largest reduction in viable cells after EMA or PMA qPCR. This reduction was 2,5 to 20 times higher than for naturally grown L. pneumophila. No significant differences between EMA and PMA could be observed, indicating that both dyes had the same efficiency. Heattreated samples did not show negative qPCR-results after EMA or PMA incubation, implying that viable L. pneumophila cells were still present. In contrast, cultivable L. pneumophila were no longer detected in these samples, confirming studies showing that heat-treatment is efficient in eradicating L. pneumophila. The tested disinfectants resulted in non-detectable cultivable L. pneumophila, whereas the PMA or EMA qPCR resulted in detectable L. pneumophila and 1 / 2 inserimenti generally less than a 1 log reduction in viable cells was observed. An exception were hypochlorite-treated samples where no signal was detected with EMA or PMA qPCR. However, negative qPCR results for these samples were also observed without EMA or PMA treatment. Overall, we conclude from our study that EMA or PMA combined with L. pneumophila specific qPCR cannot be reliable used to quantify viable L. pneumophila cells in environmental samples.

Validation of a qPCR assay for the simultaneous detection of *Legionella pneumophila* and L. pneumophila SG1 in respiratory specimens and water samples

Authors Bellido B., Pelaz C.

Legionella is a genus of bacteria that can cause outbreaks. The transmission is environmental, so identifying the source of infection is essential. This requires microbiological results that can support epidemiological investigations. Culture is the method of choice, although it is timeconsuming and laborious. Several qPCR assays have been developed for L. pneumophila DNA detection. The main objectives were: 1) Validation of a qPCR assay for the simultaneous detection of L. pneumophila and L. pneumophila SG1; 2) Evaluation of the method with respiratory specimens and water samples. Methods Validation was carried out according to the standard ISO/TS12869: 2012, using L. 1 / 2 inserimenti pneumophila SG1 DNA and a primary DNA standard. Target genes were mip for L. pneumophila detection and wzm for L. pneumophila SG1 as described by ESGLI (Mentsati et al., 2015). The qPCR assay was applied with 125 respiratory samples and with 31 water samples. All samples were analyzed by culture, and Nested-PCR SBT was performed in clinical samples. Results Efficiencies for the target genes mip and wzm were 99.9% and 102.2% respectively. The accuracy of linearity fits with standard's requirements. The limits of quantification and detection were verified. Clinical samples yielded a 56.8% (71/125) of positive results for L. pneumophila, 63 of them were positive for SG1. 48.4% (15/31) of water samples were positive for mip, 11 of them were positive for wzm. Inhibition was observed in 19.4% of water samples. Conclusion The qPCR assay was validated for clinical samples and it is currently pending the evaluation of robustness for water samples. In clinical samples, q-PCR assay increased positive results comparing with culture from 34.4% to 56.8%. The 40% of these samples were positive by Nested-PCR SBT (30.4% achieved a specific ST and 9.6% were positive for 4 - 6 genes). The qPCR assay could be a useful tool for the selection of clinical samples to be analyzed by culture and Nested-PCR SBT.

Performance of the BinaxNOW® *Legionella* Urinary Antigen rapid test in conjunction with the Alere Reader

Authors Beraud L., Montoya A., Ranc A. G., Descours G., Ginevra C., Lina G., Jarraud S. L.

pneumophila serogroup 1 antigen detection in urine samples (US) is widely used for the diagnosis of Legionnaires' disease. Previous studies showed an increase in sensitivity of BinaxNOW Legionella urinary antigen card (UAC) after concentration of US. The Alere Reader used in conjunction with select Alere rapid assays is designed to remove subjectivity from test result interpretation. The objective of this study was to evaluate the performance of the reader when used with the BinaxNOW Legionella UAC. Materials/Methods: Performance was evaluated on concentrated US by ultracentrifugation and non-concentrated US. The card was analysed visually and using the reader. All positive samples were retested after heat treatment. 201 prospective fresh US and 48 positive frozen US (some of them known to be positive only on concentrated US) were analysed. Results: Out of the 200 negative US tested, 3 samples showed false positive results (2 positive results only with the reader and 1 positive by both reading methods). After heat treatment all tests were negative. 3 technical problems were also encountered due to migration defect. Out of 1 / 2 inserimenti the 49 positive US, 41 showed concordant results. 5 UAC were analysed visually as negative on concentrated and non-concentrated US but the reader correctly identified as positive. 3 other UAC were analysed visually as negative on non-concentrated US but positive by the reader. Following concentration both reading methods produced positive result. Conclusion: The use of the reader leads to an increase in false positive results however all US remained negative after heat treatment. The reader correctly identified 8 positive samples in nonconcentrated US which were not identified visually. To conclude, the Alere Reader increases the sensitivity of BinaxNOW Legionella UAC but leads to a decrease in specificity. All positive US should be retested after heat treatment.

Specific real-time PCR for detection and identification of *Legionella pneumophila* serogroup 1 ST1

Authors Ginevra C., Chastang C., David S., Mentasti M., Yakunin E., Chalker V.J., Chalifa-Caspi V., Valinsky L., Jarraud S., Moran-Gilad J.

Legionella pneumophila serogroup1 (Lp1) Sequence Type (ST) 1 is globally widespread in the environment and accounts for a significant portion of Legionella infections, especially hospital-acquired Legionnaires' disease (LD). A rapid and specific detection method for this particular ST is expected to be advantageous and underpin epidemiological investigations and risk assessments. Methods: A collection of 131 Lp genomes, including 49 ST1 genomes was analysed using whole genome sequencing (WGS) and comparative genomics. Of >900 accessory genes interrogated, 12 candidate targets for specific ST1 detection were identified. Further refinement by in silico testing of another 579 international genomes (113 ST1s) resulted in seven unique gene markers for which specific primers and hydrolysis probes were designed. The specificity of the seven primers pairs was evaluated using qPCR on 78 ST1, 19 ST1-related STs and 92 non- 1 / 2 inserimenti related ST. The sensitivity of the assay was evaluated on serial diluted DNA extracted from the reference strain CIP107629. Results: Six PCR assays yielded sensitivities of 2-20 GU/reaction and further evaluated for specificity. The Specificity of the 7 PCR assays was variable and only 2 of them correctly discriminated ST1 and related STs from unrelated ST. Only one PCR target showed sufficient sensitivity and specificity. Conclusion: Based on WGS, we have developed and analytically validated, a sensitive and specific PCR assay that allows the specific detection of isolates belonging to the ST1 clonal complex. Further validation of this assay using environmental and clinical samples is ongoing and will be followed by a multi-site evaluation. ST1-specific qPCR is expected to deliver an added value for Lp control and prevention, in conjunction with other recently developed assays (e.g. ST47 PCR).

Mixed *Legionella* longbeachae infection identified using mip gene sequence analysis

Authors Harte D., Piercy M.

Poster presentation Background: lowing a three-day history of CAP after exposure to compost while gardening. A sputum sample was collected and tested for the presence of the typical respiratory pathogens (Steptococcus pneumonia, Chlamydophila pneumoniae and Mycoplasma pneumonia). These were all negative. Legionella testing on the sputum sample was also included in the testing algorithm using a qPCR method. A positive result was obtained and Legionella longbeachae was the implicit pathogen. Methods: The sputum sample was forwarded to the national Legionella Reference Laboratory where culture isolation of Legionella bacteria was performed. After five days growth on GVPC media Legionellae were isolated from the culture plate. Immunological staining of selected individual colonies suggested a mix of both L. longbeachae serogroups 1 and 2 in the sputum sample. Results: Using mip gene sequence analysis (Ratcliff et.al. 1998), the isolates were shown to match with 100.0 % homology to either L. longbeachae serogroup 1 or L. longbeachae serogroup 2. Conclusions: This is the first reported occurrence of a mixed Legionella longbeachae infection following the use of compost. Unfortunately there was no compost material remaining to test to prove a linkage as it had all been applied to the garden. Culture testing directly from the garden soil failed to culture any Legionellae, although PCR testing of the soil did show the presence of Legionella DNA. Powered by TCPDF (www.tcpdf.org)

Hospital outbreak and post-outbreak investigation of Legionnaires Disease (LD) using whole genome sequencing (WGS)

Authors Decker B. K., Chen L., Kreiswirth B. N., Harris P., Muder R., Merz K. J., Sonel A. F., Clancy C. J.

A 2011-12 LD outbreak at VAPHS resulting in 5 deaths among 22 patients (pts) was caused by L pneumophila (Lp) subsp. pascullei that evolved from a 1982 water system (WS) strain. In response, a WS hyperchlorination and zero-tolerance surveillance policy was instituted. Our objectives were to verify the success of WS remediation and post-outbreak Legionella prevention programs. Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {msostyle-name:”Table Normal”; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-stylenoshow:yes; mso-style-priority:99; mso-style-parent:””; mso-padding-alt:0in 5.4pt 0in 5.4pt; msopara-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:10.0pt; mso-paramargin-left:0in; line-height:115%; mso-pagination:widow-orphan; font-size:11.0pt; fontfamily:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-fontfamily:Calibri; mso-hansi-theme-font:minor-latin;} 1 / 2 inserimenti Methods. We performed Illumina MiSeq WGS on 72 Legionella isolates recovered from pts, pts’ homes, VAPHS WS (3 campuses), and the Pittsburgh community in 1982-2016, and determined phylogenetic relationships with publically-available genomes by comparing core genome single nucleotide polymorphisms. Results. Pt and WS isolates included Lp subsp. pneumophila (5 clades), pascullei (2 clades), and fraseri. WS L. longbeachae (Ll) were also recovered. VAPHS clades were distinct from publically-available genomes. Outbreak and 1982 Lp pascullei strains have not been detected since WS remediation in Nov. 2012. A Dec. 2012 Lp pascullei pt isolate was distinct from outbreak isolates, but identical to an isolate from the pt’s home. Classical and genomic epidemiology verified that no cases of nosocomial LD have occurred since 2012. WGS and epidemiologic data on non-outbreak isolates identified several themes: a) a number of identical Lp isolates were recovered from pts’ samples, homes, WS from different VAPHS campuses, and the greater Pittsburgh community (covering ?50 km); b) some identical Lp isolates persisted in the WS for ?8 years, many of which are indistinguishable from community isolates; c) other Lp isolates evolved by mutations and chromosomal recombination; d) various closely-related isolates of Ll, typically a soil organism, were recovered from WS at different campuses; and e) sequence based typing, in general, was a useful proxy for WGS. Conclusions. A remediation and surveillance program eliminated the outbreak Lp pascullei strain from the VAPHS WS, and prevented new cases of nosocomial LD by the outbreak or other strains. A lack of evolution in some isolates may be consistent with long-term persistence/latency within biofilms or sequestered sites. Legionella clonality in a geographic region may limit WGS as a lone investigative tool. Classical epidemiological approaches are needed to enhance and interpret molecular data.

Simultaneous detection of Legionella spp., *Legionella pneumophila* and Legionella pneumophila Sg-1 using a modified real time PCR recently described assay

Authors Echahidi F., Soetens O., De Mendonça R., Meghraoui A., Piérard D., Roisin S., Wybo I.

Legionellosis is mostly caused by Lpn and particularly by serogroup-1 (Sg-1). Other serogroups as well as non-Lpn species (L. spp.) have also been described as human pathogens. Therefore, correct identification of Lpn. and L. spp. is very important for the correct diagnosis and investigation of Legionellosis. A qPCR for identification of Lpn. and differentiation between sg-1 and non Sg-1 using mip, wzm and gfp targets has been recently described (Mentasti et al., 2015). We describe a modification of this PCR by the addition of the L. spp. 23S-5S target (Cross et al., 2016) to also detect other L. spp. A second modification consists in the substitution of gfp_DNA PCR internal control by the whole Phocine Herpes Virus-1(PhHv-1), with glycoproteine B as target (G. Van Doornum et al., 2003), to have an internal control of the whole process starting from the samples processing step. 1 / 2 inserimenti Material/Methods: DNA extraction from the samples was performed on the EasyMag (BioMérieux). PCR tests of the 2 duplex qPCRs (mip/wzm duplex and 23S-5S/phHv-1 duplex) were performed on the LC480II (Roche). A total of 69 samples from QCMD, ELDSNET and INSTAND EQA distribution panels containing spiked samples with Lpn sg-1, non sg-1 and L. spp. as well as non L. spp. strains were used for the assay validation. A total of 66 reference strains, 6 EQA strains, 42 clinical strains of various L. spp. and a total of 35 Lpn positive respiratory samples as well as 69 negative samples were also tested. Lpn ATCC33152 strain was used for LoD determination. Results: PCR results showed 100% accuracy and analytical specificity for all the targets. PCR efficiency ranged between 95 and 101% and a good reproducibility was obtained. The analytical sensitivity showed a LoD-95% ranging between 122 CFU/ml and 303 CFU/ml for the 3 Legionella targets. Conclusion: This modified qPCR is a useful tool for rapid detection and differentiation between Lpn (Sg-1 and nonSg-1) and L.spp. in respiratory samples.

Antimicrobial Susceptibility Testing Of Clinical And Environmental *Legionella* Spp. Isolates In Greece, By M.i.c. Gradient Strips.

Authors Flountzi A., Velonakis E. N., Koutsiomani T., Vatopoulos A.

Recently, the first ciprofloxacin-resistant L. pneumophila strain, was reported (Bruin JP et al. 2014). Aim of the study was the evaluation of the minimum inhibitory concentrations (MICs) of clinical (ci) and environmental (ei), Legionella spp. isolates against 5 antibiotics used for legionellosis treatment. Materials and Methods: A total of 30 Legionella spp. isolates, 13 (ci) and 17 (ei), previously isolated between 2008 and 2015, were included. The (ei) chosen, came from water samples during TALD's cases investigation, hot springs and potting soil. All isolates were divided into 2 groups: L.pn.sg 1-L.pn.sg. 2-15 (ci) and L.pn.sg 1-Lpn.sg 2-15/Legionella spp. (ei). The in vitro activity of 5 antibiotics, was performed by the MIC Strip Test on BCYE-? agar, for erythromycin (E), azithromycin (AZM), rifampicin RD), levofloxacin (LEV) and ciprofloxacin (CIP). Results were interpreted using the L.pn. AST by EUCAST 09-09-2016. Results: All isolates were susceptible, with low MICs, with two isolates, exhibiting 1.5 mg/L MIC in CIP. In RD, 9 (ci) and 16 (ei) presenting MICs above 0.032 mg/L (EUCAST tent. highest MICs 1 / 2 inserimenti for CIP/RD are 2 mg/L and 0.032 mg/L, respectively). MIC50 and MIC90 values, were calculated for each antibiotic agent, and found both higher for environmental isolates, except for the LEV MIC50, which was equal for all isolates. MIC50 and MIC90 values among different serotypes/serogroups were higher for L.pn. sg 1 isolates, than the non-L.pn.sg 1 isolates for E and AZM. Conclusions: On average, the (ei) exhibited MIC50-MIC90 values, higher than those of the (ci), which seems to differ from other studies until now. Legionella susceptibility seems to be correlated to the serotype/serogroup, for some antibiotics, thus, further studies with more isolates, are required. In our study, (ei) showed lower susceptibility to CIP than the (ci), while, quite all the (ei), revealed lower susceptibility to RD, presenting the tentative highest MIC's values.

C4Diagnostics Lp kit for Rapid Detection of *Legionellosis*

Authors Fugier E., Dumont A., Muller A., Paillusson N., Dukan S.

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The German LeTriWa Project: Microbiological results from community acquired Legionnaires disease (CALD) cases

Authors Gagell C., Lück C., Jahn H. J., Buchholz U., Reber F., Lehfeld A-S., Brodhun B., Haas W., Schaefer B., Stemmler F., OttoC., Bärwolff S., Beyer A., Geuß-Fosu U., Hänel M., Larscheid P.Mähl P., MorawskiK., Peters U., Pitzing R., von Welczeck A.,Widders G., Wischnewski N., EichendorffC., Hinzmann A., Nürnberger E, SchmidtS., Schumacher J., Sissolak D., ZuschneidI. Angermair S., Arastéh K., Behrens S.,Borchardt J., Creutz P., Danckert J., DejaM., Elias J., Gastmeier P., Kahnert H., LaunR., Lehmke J., Leistner R., Naumann M-B.,Pankow W.,
Pross M.Scherübl H.,Stocker H., Sturm A., Wilbrandt B.

Normal 0 21 false false false DE X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:”Normale Tabelle”; mso-tstyle-rowband-size:0; msotstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:””; msopadding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; msopara-margin-bottom:8.0pt; mso-para-margin-left:0cm; line-height:107%; mso-pagination:widoworphan; font-size:11.0pt; font-family:”Calibri”,sans-serif; mso-ascii-font-family:Calibri; mso-asciitheme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso- 1 / 2 inserimenti fareast-language:EN-US;} Background In the German research project “LeTriWa” of the Robert Koch Institute (RKI) in cooperation with the German Environment Agency (UBA) and the reference laboratory for Legionella (RLL) a case-control study in the region Berlin-Brandenburg is carried out to identify risk factors of CALD. The study especially aims to find out if CALD cases can be related to contaminated residential drinking water. Materials/Methods Patient urines were tested by Binax EIA (Alere). Direct subtyping of urines was performed by an in-house EIA with two monoclonal antibodies (mAbs) of the Dresden panel (Helbig et al., 2012). Lower respiratory samples were used for the culture of Legionella sp., L. pneumophila specific PCR and standard sequence-based typing (SBT) method. To identify the putative source of infection isolated strains from water samples were typed by mAbs of the Dresden panel and SBT. Results During the first six month samples from 25 patients were received. In 18 cases the positive test result for Legionella urinary antigen could be confirmed. For nine cases L. pneumophila, mAb subtype Knoxville was identified by direct subtyping of urine samples. Four additional cases were infected by a mAb 3-1 positve strain. Respiratory samples were available from 16 of 25 patients only. Two cases were culture positive and ten were positive in the L. pneumophila specific PCR. Using SBT the sequence type (ST) 182 (mAb subtype Knoxville) was identified in four cases. One case was caused by a ST 213 strain. We received environmental isolates from 16 patients. In just five cases SBT/mAbs typing results from patients matched with environmental strains. In the two culture confirmed cases no match of patient and water isolates was found. Conclusion Direct subtyping by mAbs is a powerful tool to compare patients and environmental samples. However, cultivation of Legionella sp. and /or detection by PCR are still the gold standard for the diagnosis of LD requiring good-quality respiratory samples.

Sequence based typing of *Legionella pneumophila* strains isolated in respiratory clinical specimens between 2008 and 2016 in Emilia Romagna region, Italy.

Authors Fregni Serpini G., Grottola A., Meacci M., Meccugni B., GennariB W., Tagliazucchi S., Forbicini G., Nanni N., Magnani R.,Vecchi E., Simone M.L., Rabacchi C.,Scaturro M., Fabio A., Ricci M.L., Pecorari M.

Molecular typing methods for discriminating Legionella pneumophila (Lp) isolates are essential for prevention/control of infections and outbreaks. Sequence Based Typing (SBT) is considered the gold standard for Lp typing. In this study all Lp clinical strains isolated were retrospectively typed by using SBT and monoclonal antibody (MAb) subgrouping in order to clarify the molecular epidemiology of Lp. Material/methods From 2008 to 2016, 56 Lp clinical strains (53 sg1, 3 non-sg1) were isolated from different 1 / 2 inserimenti respiratory samples in 56 patients and stored at -80°C in Reference laboratory for Emilia Romagna region. All strains were thawed and cultured on BCYE medium and then analyzed by molecular and serological typing assays. Results Sequence type (ST) was assigned to each strain, except for a defective neuA strain belonging to the sg11; overall we identified 23 STs and 3 new ST (ST2402, ST2417 and one to be confirmed). ST 42 was found in 20% of isolates, ST23, ST18 and ST1 in 7.3%, ST20, ST436, ST701 and ST862 in 5.5%. Finally ST37 and ST146 represented 3.6%, while ST94, ST110, ST120, ST233, ST294, ST390, ST593, ST624, ST700, ST901, ST1165, ST1974, ST2212 represented 1,8%. Out of 26 STs, 88.5% were sg1, 6 of which never identified before in Italy (2 Lp non-sg1 identified as ST110, ST390 and 4 Lp sg1 as ST624, ST700, ST701, ST120). MAb assay preliminary data showed 16/56 strains belonging to MAb3/1 positive subgroups. Conclusion The ST42 was the most frequent isolate during 2014-2016, probably for the exposure to a common environmental source or to multiple sources colonized by the same ST, followed by ST18, ST1 and ST23; these last two are the most frequent Lp ST isolated in Italy and worldwide. These data could contribute to clarify the molecular epidemiology of Lp in our region even if only an accurate analysis of the patients files will better clarify the epidemiological investigation of these LD cases.

NF validation of a fast real-time PCR method for the quantification of *legionella* spp. and *Legionella pneumophila* in "clean" water samples

Authors Poty F., Samuels E., Bouton S., Hallier-Soulier S.

Objectives According to standard methods (NFT90-431 ISO 11731), Legionella is typically isolated from water by filtration followed by heat or acid treatment then plating onto BCYE with glycine vancomycin polymyxin cyclohexamide (GVPC) agar. The main drawbacks of this cultural method are linked to its long time to result (up to 10 days) and its lack of sensitivity as only culturable cells are detetected. Moreover, the presence of background organisms may interfere with Legionella growth, leading to an underestimation of the real number of legionellae present in the sample. 1 / 3 inserimenti As an alternative to growth on agar, many alternative qPCR-based methods, certified by AFNOR, have been developed enabling results in
3 to 4 hours. They typically rely on bacterial concentration by filtration, bacterial lysis from the filter membrane and DNA purification on silica column (NF T90-471 and ISO 12869 standards). In order to reduce the hands on time, a new protocol was developed using the GeneDisc® PCR based technology from Pall Corporation for Legionella quantification in "clean" water samples (i.e. tap water, hot tubs, showerheads, whirlpools and spas, and public fountains). Methods Briefly, 100 mL – 1 Liter of clean water samples were filtered through polycarbonate membrane (0.4 µm). The filter membrane is directly inserted in a lysis tube (Pack Environment 3, Pall) and subjected to mechanical lysis by sonication and heating.
Bacterial lysate is either directly analysed by real-time PCR using the GeneDisc Legionella DUO (Pall) or DNA is concentrated concentrated with the Nanosep® centrifugal device 30K before PCR analysis. Results The specificity of each PCR assay was evaluated with 53 and 41 strains for Legionella spp. and L. pneumophila, respectively. Linearity of both PCR assays was demonstrated between 25 and 250 000 Genomic Units (GU)/PCR well with a limit of detection at 5 GU/PCR well. Recovery of the global method was evaluated from two water samples (mineral water and hot sanitary water), at two contamination levels (103 and 105 Genomic Units / L) with 10 independent replicates per contamination levels, i.e. 40 water samples. Results obtained were shown conform to the ISO 12869 standard (> 25 % recovery).
2 / 3 inserimenti Conclusions This new protocol for Legionella quantification in "clean" water samples has been approved and certified NF VALIDATION as it showed results compliant to the standard method requirements. It is an efficient tool offering fast and reliable results for the routine onsite control of water samples.

High resolution identification of *Legionella pneumophila* genotypes in respiratory tract secretions

Authors Jaber L., Amro M., Abu Tair H., Bahader S., R. Zayed A., Al-Alam H., Butmeh S., Abu- Hilal D., Brettar I., G. Höfle M., M. Bitar D.

Molecular diagnosis of Legionella pneumophila is well established and increasingly adopted worldwide, genotyping of L. pneumophila, is important for control of nosocomial outbreaks of legionellosis. Objectives: The overall goal of this study is to monitor, for the first time, the prevalence of legionellosis in pneumonia patients in the West Bank and to identify the Sequence Types (ST's) from respiratory samples and compare them to the ST's of the environmental samples from the same hospital. Methods: 121 sputum and 74 bronchoalveolar lavage specimens (n=195) from suspected pneumonia patients in the West Bank, from September 2014 till June 2016, were cultured for L. pneumophila, genomic DNA was extracted and tested by PCR amplification for L. pneumophila 16S rRNA gene. Nested PCR SequenceBased Typing (NPSBT) according to the European Working Group for Legionella Infections standard scheme, was implemented in situ on genomic DNA of the respiratory PCR positive samples and DNA of environmental samples positive for L. pneumophila. Results: One out of 1 / 2 inserimenti 195 cultured respiratory specimens was positive for L. pneumophila. By PCR, 44/195 (23%) of the respiratory samples were positive for L. pneumophila. Bronchial lavage presented a higher percentage 35%, (26/74) than sputum, which revealed 15% (18/121) positive. Twenty-four PCR positive respiratory samples were analyzed by NPSBT to reveal the following STs; 7/24 (29%) were ST1, 6/24 (25%) were ST 461, 1/24 (4%) was ST 1037, and 10/14 (41.9%) gave an incomplete profile. On the other hand, out of 14 environmental samples analyzed by NPSBT 4/14 (28.6%) were ST 1, 3/14 (21.4 %) were ST 187, 1/14 (7.1%) of each ST 2070, ST 461, and ST 187, while 4/14 (28.5 %) were unspecified Sequence Types. Conclusion: PCR amplification and NPSBT for detection and typing of L. pneumophila in respiratory tract secretions is the best method in developing countries due to heavy use of antibiotics prior to hospital admission.

Comparison of the analytical sensitivity of two rapid point-of-care (POC) urinary antigen diagnostic tests for *Legionella* Serogroups 3, 4, and 6

Authors Lollar R., Grippa L., Baldrica J., Tamerius J.

Diagnosis of Legionnaire's disease (LD) relies predominately on detection of antigen in a urine specimen during the acute phase of the illness. Many of the urinary antigen tests are intended to identify only the dominant Legionella pneumophila serogroup: serogroup 1 (Lp1). However, additional serogroups have been reported to cause LD in certain patient groups (e.g. serogroup 6 in hospitalized immunocompromised patients). A number of the commercially available antigen tests do exhibit limited cross-reactivity for various non-Lp1 serogroups. The reactivity is highly variable, but the analytical sensitivity of the tests for non-Lp1 L. pneumophila is generally much lower. The lower analytical sensitivity made the use of urinary antigen testing unreliable for use in non-Lp1 LD diagnosis. The purpose of this study was to compare the Sofia Legionella FIA with the Alere BinaxNOW for analytical detection of Serogroups 3, 4 and 6. Material/methods: A total of four 10-fold dilutions were made of Serogroups 3, 4, and 6. Testing with the Sofia Legionella FIA and Alere BinaxNOW began from the first 10-fold dilution 1 / 3 inserimenti and continued in duplicate until a negative result (0/2) was determined with each serogroup for each device. The Alere BinaxNOW was read visually and with the Alere Reader. Two-fold dilutions were then prepared from the last positive dilution and tested in duplicate until a negative result (0/2) was determined. Once the dilution resulting in a negative measurement was found, testing continued at the last positive 2-fold dilution. Results: Table 1: Testing Summary – L. pneumophila SG-3, ATCC 30657

Conclusions: Results from this study indicated that the Sofia Legionella FIA is able to detect Serogroups 3 and Serogroup 6 at levels substantially equivalent to Legionella Serogroup 1 (8.43 x 104 CFU/mL). Detection of Serogroup 4 was substantially higher than the Legionella Serogroup 1. The Alere BinaxNOW (visually or with the Reader) failed to detect any of the serogroups used in this study. The results indicate that testing with Sofia Legionella assay could aid in the diagnosis of Serogroups 3 and 6 in addition to Serogroup 1.

Molecular Epidemiology of *Legionella phneumophila* in South Africa, 2015 - 2016

Authors Carrim M., Wolter N., du Plessis M., Stewart R., de Gouveia L., Von Gottberg A.

Sequenced-based typing (SBT) is considered the gold standard molecular typing method for characterization of Legionella pneumophila. Due to limited data, we aimed to describe the molecular epidemiology of L. pneumophila in South Africa. Methods: From March 2015 through April 2016, 44 Legionella isolates were gathered from routine water sampling and case investigations, comprising 4 clinical isolates from 4 patients and 40 environmental isolates from water sampling conducted at 5 hospitals (n=28), 1 child care centre (n=5), 1 hotel (n=4) and 1 household (n=3). Sequences of the 7 SBT genes were extracted from whole genome sequence (WGS) data, and allele numbers and sequence types (STs) were assigned using the online L. pneumophila SBT database. Results: Of the 44 isolates, 25 (57%) were L. pneumophila serogroup (SG) 1, 15 (34%) were L. pneumophila SG2-14 and 4 (9%) were Legionella spp. Isolates were collected from 3 of the 9 provinces (Eastern Cape, Gauteng and Western Cape) where there is an increased awareness for testing and notification of Legionnaires' disease. Among the 40 L. pneumophila isolates we 1 / 2 inserimenti identified 12 STs, with the majority being SG1 ST1 (19/40, 48%). This was followed by SG2-14 ST421 (7/40, 18%), SG2-14 ST1317 (4/40, 10%), SG2-14 ST87 (1/40, 3%), and 8 novel STs (9/40, 23%). Of the 4 L. pneumophila clinical isolates, 2 were SG1 ST1 and 2 were SG1 novel STs (ST2403 and ST2404, 4/7 shared alleles). In one patient with epidemiological links to a hospital and household, for which SG1 ST1 was isolated from both sources, the clinical isolate was more closely related to the hospital isolate based on WGS data. Conclusions: Similar to findings of other countries, we observed a predominance of L. pneumophila SG1 ST1. However, we identified 9 isolates with novel, unrelated STs, of which 2 were clinical isolates.

Molecular typing of *Legionella pneumophila* isolates in Belgium from 2011 to 2016

Authors Belgium from 2011 to 2016 Meghraoui A., Echahidi F., Argudín MA., Deplano A., Soetens O., De Mendonça R., Nonhoff C., Piérard D., Wybo I., Roisin S.

Lpn is the etiological agent of legionnaires' disease. This microorganism can be found in natural aquatic environment and in artificial water systems. Infection occurs mainly through inhalation of contaminated aerosols. To discriminate between Lpn strains, Sequence Based Typing (SBT) has been widely used as typing method. In this study, we have investigated by SBT clinical and environmental isolates of Lpn collected from 2011 to 2016 in the Belgian National Reference Centre. Materials/ methods: Lpn isolates of respiratory samples (n=115) and related environmental samples (n=10) were genotyped using the SBT protocol of the European Working Group for Legionella Infections (EWGLI). The eBURST algorithm v3 was applied to assign the same group to STs that share at least five of seven SBT loci. 1 / 2 inserimenti Results: Clinical isolates of Lpn serogroup 1 (Sg1) (n=109, 95.5%) were classified into 39 STs (Simpson's index of diversity: 0.9). The most frequent STs were ST1 (24.8%) and ST47 (19.3%). The other serogroups (n=6, 4.5%) were represented by 5 distinct STs. Among all serogroups, six STs were newly characterised. The eBURST analysis showed that Lpn Sg1 isolates were distributed into six clonal complexes (CCs) and five singletons. The main lineages were CC1 (n=46, 42.2 %) and CC932 (n=26, 23.9 %). For 10 patients, environmental isolates were available for comparison. In 5 cases, environmental source could be confirmed based on identical ST results. In one case, no similarity was found. In the remaining cases, a matching with the very frequent ST1 should be further analysed with other techniques. Conclusion: This study shows that ST1 and ST47, belonging respectively to CC1 and CC932, are the most frequent STs in Belgium, confirming previous epidemiological observations (Vekens et al., 2012) and in agreement with the epidemiology in northwest Europe. The SBT of Lpn isolates has updated the Belgian database and could confirm the link between clinical and environmental samples.

Evaluation of an Immunoview *Legionella* longbeachae Antigen Test

Authors Podmore R., Schousboe M., Murdoch D.

Evaluation of an Immunoview Legionella longbeachae Urinary Antigen Test Over the last decade the majority of Legionella pneumonia's seen in New Zealand (NZ) have been caused by Legionella longbeachae, predominantly during summer and autumn. Use of the immunochromatographic membrane assay for detecting Legionella urinary antigens is well developed for the diagnosis of pneumonia caused by Legionella pneumophila sg.1. Because of the different epidemiology of Legionella disease in NZ a similar Urinary Antigen Test (UAT) to detect L. longbeachae was sort. The L. longbeachae antigen was characterized by the SSI Diagnostica, Denmark and the anti-L. longbeachae antibody was absorbed onto nitrocellulose membrane and conjugated to visualizing particles. From 2015 to 2017 urines were stored @ - 80°C from patients diagnosed with Legionella pneumonia by PCR and/or culture. Prototype LLVal2 was tested in 2017. The results for concentrated (C) and unconcentrated (UC) specimens were recorded @15 minutes. A small cohort was boiled for 10 minutes and retested. 1 / 2 inserimenti Samples tested, included 19 from patients with pneumonia that were negative for Legionella PCR and/or L. pneumophila sg1 UAT, 39 from patients with L. longbeachae pneumonia by PCR and/or culture and 6 from patients with non-L.longbeachae pneumonia by PCR. Using L. longbeachae PCR result as the gold standard the sensitivities and specificities for prototype LLVal2 on the 61 samples tested, before and after concentration, were 56% and 91% and 67% and 91% respectively. Of the 27 samples that were heated 33% UC and 70% C remained positive. The specificity was increased by heating. This test, using concentrated urine samples, in conjunction with other tests, such as PCR on respiratory specimens, could be used as a rapid diagnosis for pneumonia caused by L. longbeachae in a simple to use format with L.pneumophila sg 1 and/or Streptococcus pneumoniae.

Fast and reliable quantification of *Legionella* spp. and *L. pneumophila* with simultaneous detection of L. pneumophila serogroup 1 in water by real-time PCR including live/dead discrimination

Authors Priller F., Helbig S., Donath M., Ziehbarth H., Junge B., Grönewald C.,Berghof-Jäger K.

14.00 Normal 0 21 false false false DE JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Normale Tabelle"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; msostyle-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; mso-para-margin-bottom:10.0pt; mso-para-margin-left:0cm; line-height:115%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri","sans-serif"; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin;} Background: Case numbers of legionellosis are increasing in Europe as well as the rest of the world also with larger outbreaks occurring. Surveillance so far has been hampered by the reliance on cultural 1 / 2 inserimenti methods such as ISO 11731, which require up to 14 days to confirm the presence of Legionella spp. in a sample. To react more timely to potential threats, rapid molecular methods to monitor drinking water systems, cooling towers, air conditioning, fountains, or spas are in demand. Usability of such methods has however been limited by their inherit property to also detect dead cells, generating false positive results. Materials/Methods: Primers and probes were designed to exclusively target Legionella genus (HEX), L. pneumophila (FAM) and L. pneumophila serogroup 1 (ROX). These were combined with an internal amplification control (Cy5) for prevention of false negative results. A freeze dried master mix was optimized to provide sensitive quantitative results. A newly developed protocol permits recovery, live/dead discrimination and efficient DNA extraction of Legionella from standard laboratory filters. Results: The presented multiplex real-time PCR system is 100% inclusive for Legionella spp. and targeted subtypes and 100% exclusive for other bacteria. The limit of detection is 3-5 GU and the limit of quantification is 10-25 GU/reaction. Taking sample preparation into account, quantification of Legionella only. Conclusion: The developed microproof® Legionella Quantification LyoKit reliably quantifies Legionella spp. and L. pneumophila and, in the same reaction, also measures the abundance of L. pneumophila serogroup 1, which is responsible for > 75% of legionellosis cases. Since only viable cells are detected no false or insignificant results are produced. In addition, PCR results are highly comparable to cfu counts obtained by plating methods.

Method comparison of the Quidel Sofia *Legionella* fluorescent immunoassay (FIA) with the Alere BinaxNow (Binax) assay in urine samples from patients.

Authors Badoux P., Euser S.M., Kosten L., P.F. Ijzerman E.d.

The objective of this study was to compare the clinical performance of the Sofia FIA assay (Sofia FIA, Quidel Inc., San Diego, CA) to the Alere BinaxNOW Legionella Urinary Antigen Card (Binax, Alere Inc., Waltham, MA) using urine specimens from patients suspected of having pneumonia. Method: A total of 97 frozen non-concentrated urine samples were tested. These samples were derived from 47 Legionnaires' disease (LD) cases, and from 50 clinical patients with a suspected lower respiratory tract infection who had tested positive in urine antigen tests, or blood or sputum culture for Streptococcus pneumoniae (18/50), Haemophilus influenzae (14/50), Staphylococcus aureus (6/50), Escherichia coli (2/50), Pseudomonas aeruginosa (3/50), Klebsiella pneumoniae (3/50), or another pathogen (4/50). The laboratory results that were performed for the 47 LD-cases were: urine antigen only 17/47 (36.2%), urine antigen and culture 3/47 (6.4%), urine antigen and PCR 8/47 (17.2%), urine antigen, culture and PCR 18/47 (38.3%), culture and PCR 1/47 (2.1%). After defrosting (into room temperature, followed by vortexing), all urine samples were simultaneously tested with both the Sofia FIA assay, and the BinaxNOW, and read at 10 minutes (Sofia FIA) or 15 minutes (BinaxNOW), according to the manufacturers specification. For the samples that showed a discrepancy between the tests, the urine was concentrated using a static ultrafiltration concentrator with a nominal molecular weight limit of 15KDa (Minicon B15, Merck Millipore Ltd, Billerica, 1 / 2 inserimenti Massachusetts, USA). The discrepant non-concentrated urine samples were also heated at 95°C for 5 min and centrifuged for 15 min at 1000 rpm. Results: All 47 urine samples from LD cases were found positive with both Sofia FIA assay and BinaxNOW, and all 50 urine samples from non-LD cases were found negative with both tests. This resulted in a sensitivity and specificity of both the Sofia FIA and the BinaxNOW of 100.0% (47/47) and 100% (50/50). Conclusion: Both Sofia FIA assay and BinaxNOW showed a high sensitivity and specificity for the detection of Legionella antigen in urine samples from clinical patients with a suspected lower respiratory tract infection. This work was initiated and funded by Quidel Inc., San Diego, CA.

Legionnaires' disease in immunocompromised patients: beware of toilets!

Authors Ginevra C., Nesa N., Descours G., Campèse C., Tankovic J., Beraud L., Ranc AG., Jarraud S., Barbut F.

Background: Two cases of nosocomial Legionnaires' disease (LD) were diagnosed by urinary testing in the same room (room no.1) of Haematology Unit 5 months apart. Broncho alveolar lavages from the two patients grew L. pneumophila serogroup 1 Paris strain, ST1, Philadelphia subgroup. As water from shower & bathroom sink was filtered and as L. pneumophila was isolated from the toilets (1100 CFU/l), a contamination by airborne aerosols from toilets flushing was suspected. Materials/Methods: The 2 clinical strains and 9 environmental strains isolated from cold water samples collected during hospitalization of patient no.2 (4 from the water tank of toilets in room no.1, 2 from the water tank of toilets in the room next door (room no.2), 3 from sampling point located elsewhere in the water network of the building) were explored by whole genome sequencing using Nextera XT Illumina technology. Phylogenenetic analyses based on mapping and SNP calling against the Paris reference genome were performed on 46 ST1 genomes including the 2 clinical and 9 environmental related isolates and 35 unrelated isolates. 1 / 2 inserimenti Results: Phylogenetic analyses demonstrated that the 2 clinical and the 9 environmental isolates clustered together and shared the same most recent common ancestor. In particular, the genome from the patient 2 isolate had no SNP difference when compared to the 4 strains isolated from the water tank of the toilets of his own room (room no.1). Legionella genome of patient 1 differed by 19 and 10 SNPs from patient 2 and from the closest environmental genome, respectively, suggesting a microevolution of the environmental L. pneumophila population during the 5 months between the 2 cases. Conclusion: These data provide strong evidence that colonized hospital toilets could represent risks of exposure to Legionella aerosol inhalation, especially in immunocompromised patients

Contribution of molecular biology to surveillance of Legionellosis risk in Hotels and Industry in Abidjan

Authors Coulibaly-Kalpy J., Monemo P., Koffi K.S., Sylla A., Ehuié P., Kissiédou E., Kacou-N'gazoa S., Koffi-Akoua C., Dosso M.

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Comparative performance Legiolert™ vs. standard methods for the quantification of *Legionella pneumophila* in potable and nonpotable water samples

Authors Broder D., Knight T., Pednault A., Newport V., Swalla B.

Legionella pneumophila is an opportunistic pathogen found in both potable and nonpotable water systems, and is the primary cause of a pneumonia-type illness termed Legionnaires' Disease. A key step in mitigating disease risk is to routinely monitor premise water for the presence of L. pneumophila, for which there are several versions of culture methods in routine use internationally. The objective of this study was to illustrate the benefits in sensitivity and specificity of the MPN-based culture method, Legiolert, by comparing it to multiple standard culture methods. Methods: Independent, international field trials were conducted to evaluate methods for quantification of L. pneumophila in environmental potable and nonpotable samples collected during routine lab operations. Samples were analyzed using both Legiolert and a colony counting method based on either ISO-11731, SM9260J, the CDC method, or custom laboratory methods. Statistics relevant to the test countries and local regulatory bodies were applied. Results: For potable water samples in either Germany or the U.S., Legiolert showed greater sensitivity than ISO-11731-2 or SM9260J, respectively, for quantifying L. pneumophila. For German potable water (100mL) the mean relative difference was +89.3% (n=445) and for N. American potable water, a Wilcoxon Signed Rank test (n=74) showed statistically higher sensitivity for Legiolert. German and N. American trial sites reported 97.9% and 100% specificity, respectively. For N. American nonpotable water, a Wilcoxon 1 / 2 inserimenti Signed Rank test showed Legiolert statistically equivalent at 3 trial sites and less sensitive at 1 site (N=291). Specificity for all nonpotable samples was 96.5%. Conclusion: The simplified usability, increased counting range, confirmed result, and reduced subjectivity of Legiolert make it an excellent method for routine testing laboratories to increase their throughput and consistency without compromising sensitivity and accuracy.

Weather factors affect *Legionella* positivity differently across two hospital water systems (WS)

Authors Decker B.K., Kelly M.B., Walker J.D., Sonel A.F., Clancy C.J.

Background: Waterborne Legionella cause Legionnaire's disease (LD), a potentially severe pneumonia that is more common in summer and fall. Prior studies have associated LD cases with humidity and precipitation. After a 2011-12 LD outbreak, VA Pittsburgh Healthcare System (VAPHS) initiated a comprehensive program of water surveillance and remediation. Our objective in this study is to develop a prediction model for days of Legionella WS positivity based on weather or water chemistry factors. Methods: Hot and cold WS samples were tested for Legionella and water chemistry (pH, chlorine, temperature) at 2 VAPHS campuses (A, B) between Jan 2014-Dec 2015. Water at both campuses comes from the same source, but is processed at different plants. Past 7-day average weather data were obtained from NOAA (temperature, relative humidity, precipitation) and date-matched to sample collection. Logistic regression was conducted separately for each water system. Results: 3,316 samples were collected over 216 unique days. On 185 days, there was no 1 / 3 inserimenti positive test at either campus (86%, 185/216). Both campuses were sampled on 37 days, with no positive results. Days of Legionella positivity in hot and cold water, by campus are shown in the Table. Logistic regression modeling indicated that effects of weather factors and water chemistry on Legionella positivity for a given day varied by water temperature and campus. Conclusions: Humidity and precipitation were inversely related to WS positivity, a finding at variance with prior reports for LD cases. Previously reported weather effects on the prevalence of LD may not be actuated through potable water sources of Legionella. Weather and WS factors may affect positivity rates differently in hot and cold WS, even within the same building. Decision tree analyses are in progress to further explore factor relationships, which should allow us to create predictive algorithms for Legionella positivity. Such algorithms might facilitate preemptive, rather than reactive, WS remediation protocols. Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; msostyle-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:10.0pt; mso-paramargin-left:0in; line-height:115%; mso-pagination:widow-orphan; font-size:11.0pt; fontfamily:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-fontfamily:Calibri; mso-hansi-theme-font:minor-latin;} table.MsoTableGrid {mso-style-name:"Table Grid"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-priority:59; mso-styleunhide:no; border:solid windowtext 1.0pt; mso-border-alt:solid windowtext .5pt; mso-paddingalt:0in 5.4pt 0in 5.4pt; mso-border-insideh:.5pt solid windowtext; mso-border-insidev:.5pt solid windowtext; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widoworphan; font-size:11.0pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-themefont:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin;} C a m p u s Da ys tes ted N (%) pos itiv e Risk factor for positivity H ot A 11 3 8 (7.1 %) Decreased 7 day average relative humidity (p=0.02) H ot B 10 5 12 (11 .4 %) None identified C ol d A 12 0 19 (15 .8 None identified 2 / 3 inserimenti %) C ol d B 10 5 7 (6.6 %) Decreased 7 day average pr ecipitation (p=0.04), and increased pH (p=0.02)

Adaptation of Legiolert™ for amoebal co-culture of VBNC cells

Authors Dey R. and Ashbolt N.J.

Legionella pneumophila is a water-based pathogen responsible for Legionnaires' disease, which has evolved to utilize free-living protozoa as its main site for environmental growth and in engineered water systems. After disinfection, L. pneumophila has been detected in a "viable but non culturable" (VBNC) state, cells that can be resuscitated by intracellular amoeba culture. Recently Legiolert™ from IDEXX Laboratories was released for simple culture and confirmed detection of Legionella pneumophila from drinking water. We hypothesized that by combining a freshwater amoeba, Acanthamoeba polyphaga with Legiolert™ that VBNC L. pneumophila could be resuscitation for increased enumeration of viable pathogens. We used co-culture approaches to investigate possible "resuscitation" of VBNC L. pneumophila in LegiolertTM medium incubated at 30 o and 39 oC. In the absence of A. polyphaga, L. pneumophila became non-culturable after chlorination treatment, but the bacteria were 1 / 2 inserimenti resuscitated and proliferated robustly within A. polyphaga in LegiolertTM compared to no growth in LegiolertTM alone. Further evaluation of this approach is necessary to clarify what other amoeba-resisting bacteria may also grow/interfere in this novel co-culture system.

Prevalence and Diversity of *Legionella pneumophila* in the Defense Setting

Authors Yakunin E., Ohayon S., Schnaidman B., Marva E., Agmon V., Eizenkraft A.,Wagnert L., Grotto I., Valinsky L., Moran-Gilad J.

Legionella pneumophila (Lp) causes human infection, especially among susceptible individuals, following exposure to contaminated water aerosols generated by manmade water systems. Prevention of legionellosis builds on routine water inspection and corrective actions per national regulations. There are no data concerning the prevalence of Legionella spp. in water systems in the defense setting. This study thus aimed at estimating the prevalence of Legionella in samples obtained from a wide range of water systems in Israeli defense settings and explore the molecular epidemiology of these strains. Methods: During 2015-2016, multiple samples were collected from water systems in 31 sites across Israel. Legionella was detected and enumerated per the ISO11731 method. Sequencebased typing (SBT) was performed at the National Reference Laboratory for Legionella on a 1 / 2 inserimenti subset of Lp isolates and compared to the national database. Results: Of 191 water samples tested, 92 (46%) were positive for Legionella spp. and 71% of positive sampling points harboured Lp serogroup 1. In 45% of sampled sites, Legionella spp. counts exceeded the regulatory threshold of 103 CFU/L. SBT was performed on 88 strains and revealed 16 different sequence types (STs) including novel STs, which were subsequently deposited in the international database. The most abundant ST was ST1 (49%), which is also the most common ST found in clinical and environmental samples throughout other sectors in Israel. Conclusions: This is the first study looking at the prevalence and diversity of Legionella in the defense setting in Israel. While the military population is generally considered healthy, the findings of this study suggest that the risk for acquiring legionellosis in defense settings should be carefully assessed, especially with respect to use of installations in emergency situations.

Detection and identification of *Legionella* species in aerosols from the area nearby asphalt roads and bath water in public bath facilities in Toyama Prefecture, Japan

Authors Kanatani J., Isobe J., Norimoto S., Kimata K., Uchida U., Kura F.,Amemura-Maekawa J., Watahiki M.

Legionellosis may be caused by inhalation of aerosolized water contaminated with Legionella species, especially L. pneumophila. To date, the presence of Legionella species in aerosolized water has rarely been investigated. In this study, we investigated the presence of Legionella species in aerosols from the area nearby asphalt roads and nearby bath water in public bath facilities. Materials/Methods: A total of 115 air samples (99 and 16 from the area nearby asphalt roads and bath water in public bath facilities, respectively) were collected in Toyama Prefecture, Japan in 2016. We attempted to detect Legionella by means of cultures, cocultures with amoeba, and quantitative PCR. In addition, next generation sequencing (Illumina MiSeq) was carried out using DNA extracted directly from air samples to perform16S rRNAbased metagenomic analyses. Results: Legionella species were isolated neither with culture nor co-culture methods from any of the 115 air samples. However, Legionella DNA was detected in 69 of 99 samples (69.7%, roads) and in 12 of 16 samples (75.0%, bath facilities). The mean copy numbers of 16S rRNA genes per m3 were 72.9 (roads) and 79.0 (bath facilities). In one sample from a bath facility, the amount of Legionella DNA was increased 1.1 × 105 fold after coculture with amoeba. Metagenomic analyses revealed that reads of Legionella species accounted for 0?1.76% (roads) and 0.001?0.25% (bath facilities) of the selected 23 and 9 1 / 2 inserimenti samples, respectively. Among the total reads of Legionella species, those of L. pneumophila accounted for 20.5% (roads) and 37.0% (bath facilities) of the samples. Conclusion: Our findings suggest that there may be a risk of exposure to Legionella species not only in bath facilities but also in the surrounding area nearby asphalt roads. Considering the release of free DNA by membrane-impaired cells, further studies are necessary to evaluate the risk of legionellosis and to design prevention strategies.

Genomic relatedness of virulence *Legionella* strains from different water supply sources

Authors Kazanova T., Kalediene L.

Legionella pneumophila, the causative microorganism of Legionnaire‘s disease (LD), is an aquatic bacterium that can be found in numerous water. Legionnaires’ disease remains an uncommon, mainly sporadic respiratory infection with low notification rates in EU countries . In Lithuania dispite the desinfection strategies the incidence of LD has steadily increased. The first case of LD was recorded in 1985. In 2012, 4 cases were reported, in 2013 – 1 case, in 2014 – 8 cases, in 2015 – 7 cases. In 2016, 10 case numbers of LD in Lithuania were the highest ever observed. Legionellosis outbreaks occurred in January 2017, when 3 from 5 people died, there were contaminated with legionella apartment building water system. Identifying the genetic factors that influence the pathogenicity of bacteria is the greatest importance in trying to gain better control of infectious deseases. The genetic basis for virulence differences in the serogroup strains have been shown to be related to the presence or absence of certain virulence genes among the strains. The distribution of 7 major virulence genes (lvh, mip, pilB, pilD, rtxA, dotA, hsp60) were surveyed in 22 strains belonged to serogroups 1, 6, and 2-14, were selected randomly from 239 Legionella isolates. The results showed unexpected similarity of PGR and PFGE patterns of unrelated isolates of Legionella from different water supply. The results showed that environmental Legionella isolates have a very high virulence. The virulence genes lvh and rtxA have a strong association with legionellosis.The strains from water had a significantly high frequency of lvh and rtxA.Virulence lvh gene expression in all water samples 1 / 2 inserimenti were higher than 72 %; rtxA - higher than 86 %; dotA –higher than 82 %; pilB and pilD -higher than 90 %; mip, hsp60 genes were found in all environmental isolates. The results of our study provide data of the possible associations between virulent clinical isolates and strains detected in various water sources.

Challenges and Opportunities for *Legionellosis* Surveillance Using Information Technology

Authors Kilgore P. E., Zervos M. J., Alaga K. C., Alsaghayer A., Salim A. M., McElmurry S. P.

Background: In the U.S., Legionellosis surveillance has become increasingly sophisticated through the use of information technology. In addition, web-based surveillance systems enhance case data collection and transmission from lower to higher levels in public health systems. We investigated the status Legionellosis surveillance to anticipate new opportunities for enhancement. Methods: We reviewed electronic state-level Legionellosis surveillance systems to identify characteristics, methods for displaying data and accessibility of Legionellosis data. We also conducted a systematic review of peer-reviewed publications to identify health technology best practices now in use or in development by local, state and national health agencies. Results: To access state-level Legionellosis databases, end-users may be required to create a user account and/or agree to usage terms that require adherence to data privacy rules. Health department sites frequently post reports in PDF format that illustrate county- and city-level case data. In some cases, incidence rates are reported. In addition, health departments are increasingly posting additional guidance such as frequently asked questions (FAQ), water 1 / 2 inserimenti system maintenance recommendations, and reference to laboratories that perform Legionella testing. Conclusion: Variations in methods for making Legionellosis data available and reporting of Legionellosis cases suggest that there are significant opportunities for application of existing technologies (e.g., web-based systems) and further integration of newer technologies (e.g., wireless/mobile applications) that enhance Legionellosis surveillance quality. As surveillance systems develop further, we foresee deployment of health information technologies that facilitate data transfer and integration between medical records, clinical and environmental laboratories as well as public health surveillance systems.

Climatic conditions as risk factors for the colonization of hotel water systems by *legionella* species: preliminary results of an 12-year study in Crete (Greece)

Authors Papadakis A., Chochlakis D., Yachnakis E., Keramarou M, Sandalakis V., Tselentis Y., Gikas P., Psaroulaki A.

Background Recent studies have raised queries on the role of whether environmental conditions on the dispersal of Legionella. The aim of the current study was to evaluate the potential role of climatic conditions on the colonization of hotel water systems (WS) by Legionella species. Materials/Methods From 2006 to 2017, 945 environmental samples from 43 hotels (Heraklion, Crete, Greece) were tested (ISO 11731) for Legionella. Identification was achieved by MALDI Biotyper (Bruker Daltonics, Leipzig, Germany) and serogrouping by agglutination. 1 / 2 inserimenti Data on air temperature, mean wind direction (MWD), mean relative humidity (MRH) and total rainfall (TR) were collected from the Hellenic National Meteorological Service and the National Observatory of Athens. The statistical analysis was performed using Epi-Info and SPSS (p .05). Descriptive analysis was used to describe the basic features of the data. Multiple linear regression analysis was performed to investigate if and how the number of positive results depends on the quantitative variables by means of a liner regression model. The variables were analyzed by means of the Fourier transform for accessing their important frequencies. For understanding the deterministic or non-deterministic nature of the variables, the correlation dimension (D2) was used. Results Legionella colonization was statistically significantly influenced by mean maximum temperature (MMAXT), mean minimum temperature (MMINT), mean temperature (MT), TR and mean MRH. Legionella pneumophila sg 1 was statistically significantly influenced by MWD and MRH, whereas Legionella species (except from L. pneumophila) were statistically significantly influenced by MMAXT, MMINT, MT and MRH. Conclusion Climatic conditions seem to play a role in the colonization of WS. Their potential role should be investigated in concordance with other risk factors. The current study is part of a multi-level approach on Legionella colonization. A linear model is expected to be produced.

Population structure and minimum core genome typing of *Legionella pneumophila*

Authors Qin T., Zhang W., Zhou H., Ren H., Xu J.

Legionella pneumophila is an environmental organism and an important human pathogen causing nosocomial and community-acquired pneumonia. A large number of subtyping techniques have been used for epidemiological typing purposes, including sequencebased typing (SBT) and pulsed field gel electrophoresis (PFGE), which typically take several days to obtain results. However, the discriminatory power of SBT could not meet the need for distinguishing outbreak isolates or non-outbreak isolates. In this study, whole genome sequencing (WGS) was used to study the characteristics and population structure of L. pneumophila strains. Materials/Methods?We sequenced and compared 53 isolates of L. pneumophila covering different serogroups and sequence-based typing (SBT) types (STs). Results? We found that 1,896 single-copy orthologous genes were shared by all isolates and were defined as the minimum core genome (MCG) of L. pneumophila. A total of 323,224 singlenucleotide polymorphisms (SNPs) were identified among the 53 strains. After excluding 314,059 SNPs which were likely to be results of recombination, the remaining 9,165 SNPs were referred to as MCG SNPs. Population Structure analysis based on MCG divided the 53 L. pneumophila into nine MCG groups. The within-group distances were much smaller than the between-group distances, indicating considerable divergence between MCG groups. MCG groups were also supplied by phylogenetic analysis and may be considered as robust taxonomic units within L. 1 / 2 inserimenti pneumophila. Among the nine MCG groups, eight showed high intracellular growth ability while one showed low intracellular growth ability. Furthermore, MCG typing also showed high resolution in subtyping ST1 strains. Conclusion?The results obtained in this study provided significant insights into the evolution, population structure and pathogenicity of L. pneumophila.

Research of *Legionella* spp in Bosnia and Herzegovina

Authors Obradovic Z., Besic A., Obradovic A.

Legionellae are ubiquitos aquatic organism. There are different serogroups and some of them are patogenous for people and cause the diseases: Legionnaires' Diseases and Pontiac fever. This diseases are transmitted by inhalation of aerosolized water and /or soil contaminated with the legionellae. It is not transmitted from person to person. This diseases can occur in general population and in hospital enviroment where can cause nosocomial infections. Until now in Bosnia and Herzegovina there were no registered cases of domestic illness, but some cases were registered by tourists who visited our country. Aim:To investigate the link between the presence of Legionella with measured parameters of temperature and residual chlorine in the sampled water, as well as to assess sanitary conditions of the facilities and to evaluate the risk of legionella in the given facilities 1 / 2 inserimenti Material and methods: Samples of water in tourists facilities in Bosnia and Herzegovina and questionnaire for assessing sanitary conditions. In the field the values of water temperature and residual chlorine were measured, and water was tested in microbiological lab. Statistical analysis was based on statistically significant tests. Results The largest number of samples positive for the presence of Legionella spp. is in the group which ranges in temperature from 20 °C to 50 °C. In the group of samples with a residual chlorine concentration of less than 0.2 mg/l significantly higher number of samples which tested positive for the presence of Legionella spp. was detected. According to sanitary conditions in the group of samples which have been categorized as unfavourable, there was a significantly larger number of samples positive for the presence of Legionella spp. with respect to results of the group of samples which have been ranked as hygienically satisfactory. Conclusion Legionella spp was detected in some tourist s facilities in Bosnia and Herzegovina and this is an objective threat for occurrence of diseases which are caused by this bacteria Key words: legionella, tourist facilities, Bosnia and Herzegovina

First results of the case-control “LeTriWa” study on community-acquired Legionnaires’ Disease in Berlin: No indication of infection among household members of Legionnaires’ Disease cases

Authors Jahn H.J., Buchholz U., Reber F., Lehfeld A.S., Brodhun B., Haas W., Lück C., Gagell C., Schaefer B., Stemmler F., Otto C.,Bärwolff S., Beyer A., Geuß-Fosu U.,Hänel M., Larscheid P., Mähl P., MorawskiK., Peters U., Pitzing R., von Welczeck A.,Widders G., Wischnewski N., EichendorffC., Hinzmann A., Klosinski M., NürnbergerE., Schilling B., Schmidt S., SchumacherJ.Sissolak D., Zuschneid I., AngermairS., Arasteh K., Behrens S., Borchardt J.,Creutz P., Danckert J., Deja M., Elias J.,Gastmeier P., Kahnert H., Laun R., LehmkeJ., Leistner R., Naumann M-B., Pankow W.,Pross M., Scherübl H., Stocker H.,Sturm A., Wilbrandt B.

Normal 0 21 false false false DE X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:”Normale Tabelle”; mso-tstyle-rowband-size:0; msotstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:””; msopadding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; msopara-margin-bottom:10.0pt; mso-para-margin-left:0cm; line-height:115%; mso-pagination:widoworphan; font-size:11.0pt; font-family:”Calibri”,”sans-serif”; mso-ascii-font-family:Calibri; mso-asciitheme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso- 1 / 2 inserimenti fareast-language:EN-US;} Background Residential drinking water (RDW) is suspected of causing a substantial proportion of communityacquired cases of Legionnaires’ disease (CALD). Interestingly, household clusters of LD are reported rarely. It is unclear if cases among household members (HHM) are simply underdiagnosed or really occur extremely rarely. As part of the pilot phase of a larger study in Berlin (the “LeTriWa study”) we investigated possible hints for recent Legionella infections among HHM of laboratory confirmed cases of RDW-associated cases of CALD (RDW-CALD). Methods We collected data from December 2016 until May 11, 2017. We interviewed cases and took water samples from patients’ homes as well as potential non-RDW sources. Cases who could not be confirmed microbiologically as being infected through a non-RDW source were assumed to be infected through RDW. We interviewed HHM for the occurrence of acute respiratory infections in the previous six months. Because a positive urinary antigen test (UAT) can be positive for up to 8 weeks after a Legionella infection, we took urine samples from consenting HHM and tested with UAT (BinaxNOW™, Alere, Waltham, USA). Results Until May 11, 2017, 35 CALD cases were reported. Twenty of them were included in the study, 15 were RDW-CALD. These 15 cases had 12 HHM of whom 11 agreed to be tested. Two of those 11 HHM reported that they suffered from an acute respiratory infection in the past six months. None (0%; upper 95% confidence interval 24%) of the 11 UATs were positive. Conclusion Preliminary results do not suggest that Legionella infections have recently occurred among HHM in addition to the already known cases of CALD. However, we cannot rule out earlier infections. Because source attribution is difficult it is possible that in a portion of cases the true source has not been identified. One hypothesis for the lack of clusters is that the immunological status or genetic parameter of a person plays a decisive role for the acquisition of a Legionella infection.

Healthcare-associated Legionnaires' disease: surveillance data from 20 states and one large metropolitan area — United States, 2015

Authors Smith J.C., Barskey A., Soda E., Shah P., Cooley L.

Legionnaires' disease (LD) is a severe pneumonia typically acquired through inhalation of aerosols containing Legionella bacteria. Vulnerable individuals in a healthcare setting may be at increased risk of LD, as healthcare facilities often have large, complex water systems where Legionella can amplify, particularly if the water system is not well maintained. We describe healthcare-associated LD in the United States to reinforce the importance of LD prevention and response in healthcare facilities. Materials/Methods: Our analysis included national surveillance data from 20 states and one large metropolitan area that voluntarily reported exposure information for ?90% of confirmed Legionella infections in 2015. We assessed healthcare exposure status, defining hospitalization or long-term care facility residence for the entire 10 days prior to onset as "definite" and exposure to a healthcare facility for any part of 10 days as "possible." We excluded all other cases as non-healthcareassociated. Results: Of 2,809 total LD cases, 85 met definite and 468 met possible healthcareassociated case definitions. Sixteen (76%) of 21 jurisdictions reported at least one definite healthcare-associated case. Among definite cases, exposures occurred at 72 different facilities (15 hospitals and 57 long-term care facilities), with a 25% case fatality rate. Inpatients and outpatients accounted for 351 possible healthcare-associated cases (75%), followed by 77 visitors/volunteers (16%) and 36 staff (8%); 4 were missing exposure type (1%). Conclusions: 1 / 2 inserimenti Exposure to Legionella in healthcare settings can result in LD, and cases are not limited to patients alone. The engagement of healthcare providers, healthcare facility leaders, and public health staff is critical for the success of LD prevention and response efforts. Implementation of effective water management programs and rapid case identification and investigation could reduce the number of healthcare-associated LD cases.

Monitoring and control of *Legionella* in a private hospital group in South Africa, 2015 -2016

Authors Stewart R., Cleghorn J., Thomas T.,Wolter N., Carrim M., Duse A., Von Gottberg A.

1 / 4 inserimenti A private hospital group with 53 hospitals throughout South Africa encourages group hospitals to routinely test for Legionella annually. If detected, treatments are implemented including flushing taps, heat treatment, and chlorination. If positive, repeat samples are submitted after treatment. Methods We reviewed samples collected from group hospitals from 2015 through 2016. Samples were sent to NHLS Infection Control Laboratory, Johannesburg and were cultured according to ISO 11731. Results Table 1: Number of hospitals & water samples tested & positive by year Year Hosp itals teste d Hosp itals posit ive n (%) Sam ples teste d Posit ive s ampl es n (%) 2015 13 8 (61. 5) 111 30 (2 7.2) 2016 26 14 (5 3.8) 265 74 (2 7.9) 2 / 4 inserimenti Table 2: Legionella serogroups identified by year Year Isola tes SG 1 n (%) SG 2-14 n (%) Spec ies n (%) 2015 30 8 (26. 6) 10 (3 3.3) 12 (4 0.0) 2016 74 36 (4 8.6) 26 (3 5.1) 12 (1 6.2) Note: Results from sequence-based typing will be available for the presentation Summary for 2015 – 2016 The percentage of positive hospitals remained high (>50%) and percentage of positive samples remained consistent although the number of hospitals submitting samples and the number of samples tested increased. 15 hospitals (38.5%) supplied sample temperatures. The breakdown of samples (n=376) taken from recommended high-risk areas versus low-risk 3 / 4 inserimenti areas was 295 (78.5%) versus 81 (21.5%). Repeat samples (n = 35) from positive sites were sent by 9 (40.9%) of 22 hospitals of which 5 (55.5%) were negative. 22 repeat samples were negative (62.8%) and 17 positive (48.5%). Total samples plus repeats was 411. Conclusions Over 50% of hospitals tested had Legionella detected in their water systems. Only 40.9% submitted repeat samples and half of these were negative. This indicates a lack of effectiveness or compliance with treatment protocols. One fifth of samples submitted (21.5%) were from lowrisk areas indicating a need for training.

A Snapshot of the Prevalence and Molecular Diversity of Legionella pneumophila Strains in Water Systems of Israeli Hotels

Authors Yakunin E., Kostyal E., Yavlovich A., Agmon V., Grotto I., Valinsky L., Moran-Gilad J.

Exposure to Legionella spp contaminated aerosols in hotels and resorts confers a risk factor for travel associated Legionnaire's Disease (TALD). In this study we investigated the prevalence of Legionella contamination and its molecular diversity in hotels across Israel. Methods: The study comprised of a convenience sample of water systems in 169 hotels and resorts countrywide, routinely inspected between March 2015 and February 2017. Detection and quantitation of Legionella were performed in a single laboratory per the ISO11731 method. The distribution of Legionella isolates was analysed according to geography and source. The genetic diversity of a subset of isolates was analysed by Sequence-Based Typing (SBT) at the National Reference Laboratory for Legionella and compared to the national database. 1 / 2 inserimenti Results: Out of 2853 samples tested, 483 (16.9%) obtained from 102 different premises (61.8% of hotels) were positive for Legionella spp. In 234 samples (48.6% of all positive, 8.2% of total samples), accounting for 37.3% of hotels, Legionella spp. counts exceeded the regulatory threshold of 103 CFU/L. The leading contaminated water sources were cooling towers (37.5%) followed by faucets, whirlpools, water lines and containers (14-17% each) and 30.6% and 16.1% of samples obtained from cooling towers and whirlpools, respectively, exceeded regulatory threshold. SBT was performed on 83 strains and revealed 28 different sequence types (STs) including two novel STs. The most prevalent STs found were ST1 (25%), ST87 (9%) and ST93, ST461 and ST1516 (6% each). Several L.pneumophila strains have been found to be limited to certain geographical regions. Conclusions: This is the first study looking at the prevalence and diversity of Legionella in the hotel setting in Israel. Interestingly, the prevalence of ST1 was lower than reported in surveys of other settings in Israel. These findings will inform risk assessment, surveillance and control measures of TALD.

How is travel-associated legionnaires' disease reporting rate associated with travel volume?

Authors Robesyn E., de Jong B., Beauté J., Payne L., Stålsby Lundborg C., Faes C.

Background Legionnaires' disease is a severe form of pneumonia for which travel is expected to be a risk factor through a variety of pathways. The European Legionnaires' disease Surveillance Network (ELDSNet), coordinated by ECDC, runs a specific surveillance system for travelassociated Legionnaires' disease (TALD). Given its relation to travel, we expect the reporting rate of TALD to fluctuate with travel volume. However, other factors also influence the reporting rate, e.g. diagnosis and notification, travel destinations, and demographic, weather, and behavioural changes. Consequently, assessing the reason for increases in reporting rate is 1 / 2 inserimenti challenging. To be able to account for varying travel volume in surveillance reports, we assess whether there is a short term association between monthly travel volume and TALD reporting rate, and which strength and form this association has. Materials/Methods We used data on tourism from 1990 to 2016 (EUROSTAT) and data on TALD cases from The European Surveillance System (TESSy). We fitted a Poisson generalized linear model, controlling for long term and seasonal effects. We analyse model assumptions, apply several model variations, both marginal and country specific, and compare them using Akaike Information Criterion (AIC). Results Over 50 billion nights were spent by travellers in tourist accommodations in the EU in the 26 years long time series. In the same period 15 327 TALD cases were reported by public health authorities. An overall increasing trend and marked seasonality are observed in the time series. We found an association (p

Travel-Associated Legionnaires' Disease United States, 2015

Authors Edens C., Barskey A., Cooley L.

Legionnaires' disease (LD) is a severe pneumonia predominantly affecting persons with underlying illnesses or older age. It is caused by aerosolized exposure to Legionella bacteria, a waterborne organism that can grow in building water systems that are not well maintained. Since the first recognized outbreak at a Philadelphia hotel in 1976, the association of LD with overnight stay away from home has been well established. We describe travel-associated LD in the United States (U.S.) in 2015, with a focus on accommodation types during the days when exposure likely occurred. Materials/Methods: The Supplemental Legionnaires' Disease Surveillance System captures exposure information on laboratoryconfirmed cases of LD in the U.S. that are reported to CDC. Travel association was defined as an overnight stay in any location other than the patient's primary residence in the 10 days before symptom onset. Complete (?90% of cases) reporting of exposure data was available for 20 states and one large metropolitan area. Results: In 2015, 2,809 confirmed LD cases, including 369 (15%) with a reported travel association, were reported by 21 complete reporting jurisdictions. Patients with travel-associated LD were mostly male (63%) and white (63%) with a median age of 59 years; 54% stayed at a public accommodation (hotel, etc.) only, 30% stayed at a private accommodation only, (5%) stayed at both, and 2% had cruise-related travel. The median length of stay (in days) was longer for cases associated with private (5, 1–220) vs. public (2, 1–191) locations. Travel was reported in 42 states and 2 jurisdictions. Conclusions: 1 / 2 inserimenti Public accommodations with inadequate water management programs can put guests at risk of exposure to Legionella bacteria. We report that most cases with a reported travel history had exposure to public accommodations such as hotels. Owners and building managers should consider implementing effective water management programs to protect guests from LD.

Colonization and persistence of *Legionella pneumophila* ST328 in a hospital.

Authors Graells T., Guy L., Padilla E.

Background Legionella spp. can colonize man-made water systems. Immunosuppressed patients are susceptible to develop Legionnaire's Disease (LD) when aerosols produced by showers, faucets or cooling towers containing these bacteria reach the lung alveoli and infect macrophages. Monitoring the concentration is critical and regulated in healthcare facilities in many countries. The aim of this study was to characterize different Legionella spp. found in a hospital in Spain between February 2015 and January 2017. Methods 1 / 2 inserimenti Water samples were screened for Legionella spp. according to ISO 11731. Upon positive result, Latex kit (OXOID) was used to discriminate between L. pneumophila serogroup 1, serogroups 2-14 and other Legionella spp. Pulse Field Electrophoresis Gel (PFGE) was performed for all isolates and for one clinical strain. Sequence Type (ST) was determined by Sequence-Based Typing (SBT) of flaA, pilE, mip, mompS, asd, proA and neuA genes. Results All environmental isolates are L. pneumophila serogroups 2-14, whereas the clinical isolate belongs to serogroup 1. The PFGE shows clones very closely related (slightly different patterns) among water samples, but a different one for the clinical strain. However, all water samples share the same allelic profile and ST- ST328. The clinical isolate belongs to ST266. Conclusion Legionella pneumophila ST328 could be recurrently isolated although the hospital water was sanitized. As far as we can trace, this colonization lasted for almost two years indicating that these measures are not appropriate. During this time, two cases of hospital-acquired LD were diagnosed using the urinary antigen test (UAT) which only recognizes L. pneumophila serogroup 1. One of these strains was recovered. Surprisingly, not even one L. pneumophila serogroup 1 was isolated from water samples. Even though L. pneumophila serogroup 1 is not predominant, screening using UAT could lead to underdiagnosing LD caused by other L. pneumophila serogroups.

Distribution of *Legionella* species in windshield washer fluid of motor vehicles in Toyama, Japan

Authors Isobe J., Jun-ichi K., Keiko K., Amemura-Maekawa J., Fumiaki K., Masanori W.

Introduction: Legionella sp. is a bacterial pathogen that lives in various environments and causes legionellosis. However, the sources of Legionella transmission are not clear. Recently, it was reported that Legionella spp. were isolated from the windshield washer fluid of motor vehicles. In this study, we investigated the distribution of Legionella spp. isolated from windshield washer fluid. In addition, we investigated the ability of L. pneumophila serogroup (SG) 1 and 5 to survive in washer fluid. Methods: A total of 193 samples (100 mL) of windshield washer fluid were collected in Toyama Prefecture in Japan. We attempted to detect Legionella by means of culture (193 samples) and PCR for amplifying Legionella genus-specific 16S rRNA genes (142 samples). The strains of L. pneumophila were typed by Sequence-Based typing (SBT). In addition, we investigated the survivability of Legionella in the commercially available washer fluid that contains biocidal surfactants. Results: Five different species of Legionella (L. moravica, L. pneumophila SG5, L. quateirensis, L. dumoffii and L. ruburilucens) were detected in 18 out of 193 samples (9.3%). Among them, the maximum concentration of Legionella spp. in a sample was ?10,000 CFU/100 mL. 16S rRNA gene was detected in 88 out of 142 samples (62.0%). Two sequence types of L. pneumophila SG 5 were detected as new types on SBT database (ST1532 and ST1620). L. pneumophila was not able to survive in the commercial washer fluid diluted by 40-fold and spiked with the bacteria, under the condition that 60% of the 1 / 2 inserimenti bacterial CFU remained in the spiked PBS solution as a control. Conclusion: Our findings suggest that windshield washer fluid of motor vehicles may be a possible source of legionellosis when surfactant in washer fluid are diluted enough to permit survival to microbes.

Legionella detection in wastewater using culture and real-time quantitative PCR methods

Authors Zamfir M., Bartha B., Walser S.M., Brenner B.,Huber S., Höller C., Seidel M., Herr CEW.

Legionella detection in wastewater using culture and real-time quantitative PCR methods 1 / 4 inserimenti Zamfir M1 , Bartha B1 , Walser SM1 , Brenner B1 , Huber S2 , Höller C2 , Seidel M3 , Herr CEW1 1Occupational and Environmental Health, Epidemiology, Bavarian Health and Food Safety Authority, Munich, Germany 2Hygiene, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany 3Chair of Analytical Chemistry and Water Chemistry, Institute of Hydrochemistry, Technical University of Munich, Munich, Germany Background The detection of Legionella with the standard culture based method from complex environmental matrices like wastewater is difficult and influenced by many uncertainties. In many cases the background bacterial flora overgrows Legionella and the agar plates are not evaluable. In this case, culture independent methods, like real-time quantitative PCR (qPCR), give a better estimation of exposure levels and provide results within a few hours. Our aim was to compare the standard culture based method with the culture independent qPCR method. Methods Water samples were collected from municipal and industrial (brewery and dairy) wastewater treatment plants (WWTPs), altogether from 17 activated sludge tanks and secondary settling basins. 2 / 4 inserimenti A detection method including homogenization by ultra turrax, dilution and heat treatment of the samples followed by incubation on GVPC agar plates was established. Additionally, Legionella spp. and Legionella pneumophila were detected by qPCR using commercial kits. Results Using the qPCR method, Legionella spp. was detected in all examined WWTPs, while L. pneumophila was found in three municipal WWTPs. From them, the cultural detection was successful in two cases. Additionally, L. pneumophila from one activated sludge tank was only detected by the cultural method. Conclusion The standard culture based method is not suitable to detect Legionella in wastewater samples because of the strong accompanying bacterial overgrowth. Using the culture method, only L. pneumophila was detected, but not other Legionella species. The qPCR method was more efficient to detect both Legionella spp. and L. pneumophila, providing better results faster than the cultural detection method. Nevertheless, colony isolation is still important for further subtyping methods, like sequencebased typing (SBT) or monoclonal antibody typing. Therefore, there is an urgent need for optimization of the culture detection method.

Surveillance of *Legionella* Speciation and Serotypings from Environmental Water Samples in Taiwan - A 74 institution Survey

Authors Jui-Chen H., Wan-Rong Y., Yusen E. L.

Legionnaires' disease is an important cause of hospital and community acquired pneumonia. Hospital-acquired Legionnaires' disease is directly linked to the presence of Legionella in the man-made water systems. The objective of this study is to investigate the presence of Legionella and its colonization rate in hospitals (water systems + cooling towers) and industrial cooling towers in Taiwan. Methods: Thirty-seven hospital water systems, 10 hospital cooling towers and 26 industrial cooling towers were routinely monitored from 2013 ~ 2016 for Legionella. We followed the standardized protocol to perform environmental cultures using (1) water samples; (2) BCYE and DGVP culture media; (3) latex agglutination test (LAX) and direct fluorescent antibody (DFA) technique for L. pneumophila speciation and serotyping. Results: Total of 4,295 water samples were collected and processed during 2013 ~ 2016 period. L. pneumophila comprised 95.5% (470/492)of all positive water samples. Lp1 remained the most recurrent serotype which was 44.9% (218/492). Lp6 comprised 27.4% followed by Lp3 (4.9%, 24/492), Lp5 (4.5%, 22/492) and Lp2 (0.6%, 3/492). Legionella species comprised 12.6% (62/492). This study allow healthcare professionals to use a variety of laboratory methods and not just rely on urinary antigen tests for diagnosis of Legionnaires' diseases to provide better 1 / 2 inserimenti patient care quality.

Use of rep-PCR for Molecular Genotyping and Inventory among Isolates from Nosocomial Legionnaires' Disease Infections

Authors Jui-Chen H., Wan-Rong Y., Yusen E.L.

Nosocomial Legionnaires' disease infection has emerged as a challenge for healthcare professionals. Identifying the source of infection is an important task for hospitals to develop a cost-effective strategy for prevention. Molecular subtyping is a powerful tool for determining the source of infection by their genetic relatedness among isolates. However, the conventional method (ie. pulsed-field gel electrophoresis), is time consuming and skill intensive for interpretable results. Currently repetitive-sequence-based PCR (rep-PCR) technique has been developed as an automated procedure that may provide a rapid method for molecular subtyping of bacteria. Such rapid method provides same-day genotyping results and enables infection control practitioners to eliminate further Legionella transmission. The reproducibility and digital output of rep-PCR also provide the opportunity for hospitals to inventory isolate patterns. Objective: To evaluate the feasibility of using rep-PCR for nosocomial infection associated isolate typing. Methods: Twelve Legionella pneumophila isolates (2 from patients, 10 from the environment) were used in this study. A rep-PCR assay and a capillary electrophoresis system (Bioanalyzer 2100, Agilent) was used for molecular analysis. We followed the standardized procedures for analysis including: (1) extract DNA from the isolated cultures; (2) 1 / 2 inserimenti amplify samples using rep-PCR and the specie-specific, in-house typing kits; (3) separate fragments via electrophoresis performed in a microfluidics DNA LabChip; and (4) analyze data using dendrogram, similarity matrix, and overlay analysis. Results: A patient isolate of L. pneumophila matches the isolates cultured from the patient room faucet. During an outbreak investigation, our Legionella subtyping results suggested a >99.3% match between patients and the isolates from water taps in patients' rooms. Healthcare facility can build an isolate database based on rep-PCR and capillary electrophoresis results which can be used to determine the possible source of infection in future outbreak investigation. In-house rep-PCR protocol/reagents/condition and generic labchip may be a cost-effective alternative compatible to the brand name kits and chips, which can reduce the cost per isolate significantly. Conclusions: The signal-base electrophoresis by rep-PCR provide more reproducible results than the analog image-base results by PFGE. Times to results were 6 to 8 hr for rep-PCR compared to 3 to 5 days for PFGE. Rapid, standardized results and high reproducibility make the rep-PCR a valuable tool for use in nosocomial transmission investigations.

Legionella longbeachae in England and Wales - results of enhanced surveillance, October 2013 - December 2016

Authors Collins S., Afshar B., Mentasti M.,Naik F., Smith R., Kirrage D., David S., Ready D., Chalker V.

L. longbeachae (LL) infection is rare in Europe. In England, five cases were detected from 1997-2013. This is likely an underestimate as LL infection will not be detected by urinary antigen tests (UAT). Cases in England and Wales are detected using culture of respiratory samples or Legionella spp. PCR - both are not in widespread use in diagnostic labs. Infection has been linked to horticultural growth media (HGM) and gardening. In 2013, there was a significant increase in LL cases in Scotland. In response to this, and to better understand the burden of LL in England and Wales, enhanced surveillance was initiated LL infection. Materials/Methods Surveillance criteria: Any hospitalised patient 1 / 2 inserimenti in ICU with community acquired pneumonia (CAP); and negative for Legionella UAT and other common causes of CAP; and known contact with HGM in the 14 days prior to onset or a keen gardener Isolates and lower respiratory tract specimens (sputa, BAL, etc.) were referred to the National Legionella Reference Lab. Patient specimens and HGM used by two of the identified cases were cultured for Legionella. Isolates were identified by partial mip gene sequencing. Representative patient and environmental isolates were analysed by whole genome sequencing (WGS). Results Three confirmed cases were identified, representing 0.3% of all LD cases in the same period. Two cases ere identified due to enhanced surveillance while a third was identified from a referred isolate. All cases were occasional or keen gardeners. LL was isolated from HGM in use by two cases, however WGS showed isolates were distinct to those from patients and suggested the phylogenetic diversity was large. Conclusion The prevalence of LB infection in England and Wales is unknown and under-ascertainment is still likely. Cases do occur and testing of patients meeting the enhanced surveillance criteria should be considered. Sampling HGM is complex and given the apparent large phylogenetic diversity, is not routinely recommended in sporadic LL case investigations.

Risk factors of legionella growth in water system: 29 years epidemiological study in Crete (Greece)

Authors Papadakis A., Chochlakis D., Keramarou M., Sandalakis V., Tselenis Y., Gikas P., Psaroulaki A.

Background The aims of the study were: a) to research the frequency and severity of contamination of water distribution systems by Legionella spp, b) to identify the Legionella spp. in environmental samples, c) to identify the risk factors associated with Legionella colonization, d) to evaluate the implementation of Water Safety Plans (W.S.P.) against Legionella colonization. Materials/Methods From July 1998 to May 2017, 1161 environmental samples from 43 hotels at the prefecture of Heraklion (Crete, Greece) were laboratory tested. Data on water temperature, pH, chlorine concentration, disinfection methodology, hotel star rating, number of rooms/beds, presence of WSP, etc, were recorded. Laboratory analysis was performed according to ISO 11731 and ISO 11731-2. Isolated colonies were identified and serogrouped by agglutination tests while from 2010 onwards, a MALDI Biotyper (Bruker Daltonics, Leipzig, Germany) was used for the identification of all Legionella colonies. The statistical analysis was performed using Epi-Info and SPSS. Results 251 (21,6%) out of the 1161 samples were positive (>50 cfu/L). The risk of having a human infection was higher in small municipalities and in the absence of a fully developed water supply network, equipped with a regular chlorination system. A statisticaly significant result was calculated when an innovative disinfection method (stabilized chlorine dioxide; copper ions) was not used at a hotel 1 / 2 inserimenti in case of concomitant use of a solar water heater, at temperatures out of range, in the absence of an automated chlorination system, at a chlorine concentration less than 0.2 ppm, in hotels of

ABSTRACT BOOK

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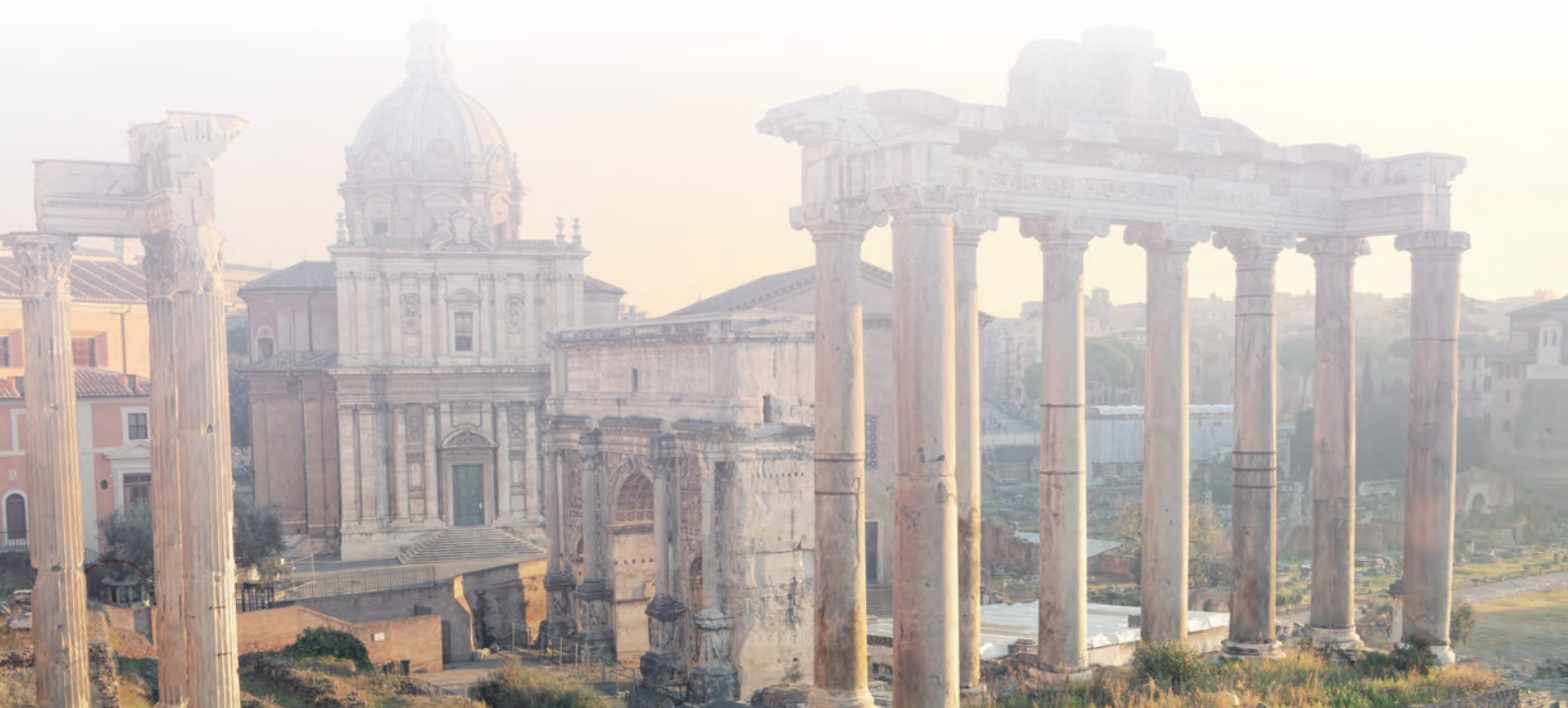

Effectiveness of monochloramine at different dosage in reducing *Legionella* water contamination avoiding formation of potentially toxic by-products

Authors Paduano S., Marchesi I., Vecchi E., Saini N., Bolognesi A., Borella P., Sircana L., Bargellini A.

Background. Several technologies are utilized to disinfect hospital water contaminated by Legionella spp. This study evaluates the efficacy of monochloramine in reducing Legionella pneumophila contamination without formation of toxic by-products.

Methods. Three devices applied in the same hospital building are investigated: device A is working since 2009, B and C were installed in 2015 in water networks previously treated with chlorine dioxide. Monochloramine is generated in situ and injected or in hot water (A and B) or in the cold water (C) in order to reach 2-4 mg/l at distal points. Municipal cold water and hot water from distal points and return-loops of treated and untreated networks were collected. N-nitrosamines, nitrite, nitrate, free-ammonia, trihalomethanes (THMs), chlorite, chlorate and TOC were measured by standard methods. Legionella spp was quantified by culture.

Results. During 18 months of study, Legionella was found only in 2/148 samples treated with monochloramine. All samples were negative for the presence of N-nitrosamines, except two samples below WHO limit (0.10 µg/l), one in the network A and the other in the untreated network. THMs at very low concentrations, nitrite, nitrate, and TOC at levels below the limits were detected in all samples. The chlorite was observed only when chlorine dioxide was used as disinfectant, not after introduction of monochloramine. Chlorate >700 µg/l and free ammonia >0.50 mg/l were occasionally found due to hypochlorite reagent degradation; after corrective actions (draining and cleaning of storage tanks, use fresh and more stable hypochlorite) these parameters decreased below the limits.

Conclusion. Our results confirm the efficacy of monochloramine in controlling Legionella contamination in hospital buildings, without production of undesirable toxic substances. The basic conditions to obtain satisfactory results and guarantee the safe of patients and personnel comprehend a regular maintenance and the right proportions of reagents dosage.

Influence of climate on *Legionella* contamination in automobile windshield washer fluid

Authors Schwake D. O., Brown C., Marr L. C.

Background: While epidemiological evidence has shown professional drivers to be at increased risk for legionellosis, there has been limited research on transmission related to vehicles. Our previous work revealed Legionella contamination in washer fluid reservoirs of buses in the southwestern U.S., suggesting that spraying of windshield washer fluid could be a transmission route for legionellosis. The goal of this study is to expand on these previous findings by testing the hypothesis that warmer climates and their associated lower levels of methanol in washer fluid would favor Legionella occurrence in windshield washer fluid. Materials/Methods: Washer fluid was collected from vehicles at seven sites across the U.S. with differing climates. Samples were collected in the summer and winter, and cultivation was performed to determine concentrations of Legionella. In a lab experiment, L. pneumophila was inoculated into washer fluid from Arizona (containing 1% methanol by volume) and Michigan (containing 35%) and ncubated at different temperatures. Cultivation and microscopy were performed to evaluate changes in culturability and viability. Results: A positive association was observed between mean monthly air temperature and 1 / 2 inserimenti Legionella occurrence. During wintertime, Legionella was detected only in washer fluid from the warmest site. In the lab experiment, loss of culturable Legionella was inhibited at 4 °C and in washer fluid containing a high level of methanol. Microscopy revealed large portions of viable but non-culturable (VBNC) cells for nearly all conditions, often contrasting with cultivation results. Conclusions: We have provided evidence of widespread occurrence of Legionella in windshield washer fluid. It is more common at higher ambient temperatures, but other factors likely play a role. These findings highlight the need for more detailed research on this novel Legionella transmission route, as well as VBNC physiology.

Comparison of FISH and Culture Methods in the Examine of Heterotrophic Bacteria, *Legionella* Bacteria and Free - Living Amoeba in Cooling Tower Waters and Biofilm Samples

Authors Zeybek Z., Doğruöz güngör N., Türetgen I.

Background: The various microorganisms that live in the water of the cooling towers can affect both human health through inhalation of aerosolized water as well as effects on industrial processes. In order to check such man-made water systems, some microbiological analyses are needed that can give results in a short time. In this study, the presence of heterotrophic bacteria, Legionella bacteria and free - living amoeba, FLA, including Acanthamoeba in cooling-tower water and biofilm samples were investigated. Materials/Methods: 40 water and biofilm samples were taken from 16 different cooling towers in Istanbul. All samples were examined by using two different methods, one being fluorescent in situ hybridization (FISH), the second culture. CY3-labelled EUB 338 probe, CY3-labelled LEGPNE1 probe, FAM-labelled ACANTHA probe were used for the examination of heterotrophic bacteria, L. pneumophila and Acanthamoeba respectively in the FISH method. Hybridization R2A agar medium, BCYE agar supplemented with GVPN and NNA were used for cultivation of the same microorganisms.. Results: With these two methods, the numbers of heterotrophic bacteria within both the water and the biofilm samples were detected above the treshold values (>105 cell. mL-1). Despite Acanthamoeba detection in all cooling tower specimens, Legionella pneumophila serogroup 1 were detected in the biofilm of only one cooling tower. Conclusion: According to the results of this study, both methods are recommended to be used in conjunction. As FLAs show great biodiversity, new studies are being planned to use FISH method, which provides short-term sensitive and reliable results for the investigation of other FLAs such as Hartmanella sp. and Naeglaria sp.

Isolation of amoeba associated *Legionella pneumophila* in water systems of three South African public hospitals

Authors Muchesa P., Leifels M., Jurzik L., George Barnard T., Bartie C.

The prevalence of bacteria, viruses, fungi and protozoa in hospital water systems may pose a potential risk of nosocomial infections to immuno-compromised patients and health care workers. Among protozoa, free-living amoebae (FLA) are known to occur in high numbers in the natural aquatic environment as well as in man-made water systems. FLA can interact with and allow the survival, growth and transmission of clinically important bacteria such as such as methicillin-resistant Staphylococcus aureus, Vibrio cholerae, Legionellaspecies including Legionella pneumophila and environmental Mycobacterium species. The purpose of this study was to investigate the occurrence of Legionella pneumophila associated with amoebae in hospital water networks of Johannesburg, South Africa. A total of 98 water and biofilm samples were collected from thesterilization unit, theatres, neonatal ward and intensive care units. All the samples were filtered and analyzed using an amoebal enrichment technique. Observed autochthonous amoebae were then purified and lysed to release potential intracellular bacteria, which were then inoculated on buffered charcoal yeast extract and selective Glycine-Vancomycin-Polymyxin-Cycloheximide Legionella agar. L. pneumophila DNA was extracted from cultures and detected using conventional and real time PCR. Amoebae were isolated from 71 (72.4%) samples. Isolated amoebae were analyzed using qPCR and culture methods to test for the presence of Legionella. L. pneumophila did not grow on selective media in any of the samples. A total of 7 out of the 71 (9.9%) amoeba positive samples showed a positive reaction for L. pneumophila using qPCR. Although relatively few samples were positive for Legionella in this preliminary study, the association with amoeba still present a potential public health risk to immuno-compromised patients when exposed to contaminated water

Activity of *Legionella* bacteria in the cooling water of metal industry

Authors Räsänen P.S., Pitkänen T., Kusnetsov J.

Applications of modern molecular detection methods with quantitative polymerase chain reaction (qPCR)offer possibility to estimate activity of Legionellae. Legionella bacteria can lose their cultivability but remain viable e.g. after a biocide treatment and still cause risk of infection. According to ESGLI Technical Guidelines, cooling systems should be treated efficiently with biocides if Legionellae are present in numbers exceeding 1000 cfu/l. The aim of the study was to estimate the activity of Legionella bacteria in cooling water of metal industry. Legionella counts were analyzed using the standardized culture method (ISO 11731), and a combination of propidium monoazide (PMA) treatment with qPCR and the ratio of ribosomal RNA (rRNA) to rRNA genes (rDNA) was used for bacterial activity evaluation (n=17). Legionellae were isolated from the 82 % samples (85–100000 cfu/l) by culture. Depending a sampling site both viable and damaged Legionellae were observed using the PMA-qPCR method (ΔCT -2.1–5.5). The copy number of Legionella spp. rRNA transcripts was higher than the copy number of rDNA, potentially indicating activity of Legionellae in the investigated water system. 82-88 % of samples were Legionella spp. positive by qPCR. L. p. was cultured from 77 % and detected by qPCR from 65 to 71 % of samples, depending on the nucleic acid extraction method. Molecular techniques tested herein, especially L. spp. PMA-qPCR and rRNA-based approach could estimate activity of the targets. Further, analyzing rRNA offered better sensitivity for Legionella spp. detection. Culture also gave good results, showing remarkable tolerance against to inhibitors. In the future, molecular methods need further emphasis especially as regards of the quality assurance procedures and the result interpretation. Other microbes, iron compounds, organic material and inhibitors are challenging both culture and qPCR based methods for detection of Legionellae from industrial water samples.

Diversity of *Legionella* in lab-scale activated sludge systems

Authors Nogueira R., Pal R., Rosenwinkel K. H., Purohit H.

Background Legionella can multiply in biological treatment plants receiving municipal as well as industrial wastewaters. This is not surprising considering that amoebae and ciliates, the natural hosts for Legionella, are common inhabitants of the complex biocenose - the activated sludge - contained in these systems. However, the diversity of Legionella in the activated sludge has not yet been described. This particular knowledge is of relevance for estimating the health risks coming from the exposure to aerosols contaminated with pathogenic species of Legionella produced in several steps in the treatment plants.
The proposed study intends to analyze the diversity of Legionella species in lab-scale activated sludge reactors operating at 15 °C and 35 °C.

Materials/Methods
Two sequencing batch reactors were inoculated with activated sludge containing L. pneumophila and operated for 4 weeks at 15 °C and 35 °C. To cover the diversity of Legionella species to a deeper level, a 16S rDNA primer was designed and optimized for 16S rDNA clone library preparation. DNA was extracted from activated sludge samples, amplified with the new primer pair and sequenced.

Results
This study reveals a higher diversity of Legionella at 35 °C compared to 15 °C. L. pneumophila was the dominant species in activated sludge at 35 °C, while L. longbeachae was retrieved at a considerably lower density.
At 15 °C only a small percentage of the sequences was affiliated with described Legionella species being L. drancourtii one of them. These results suggest that yet-uncultured Legionellae are common members of the microbial communities in activated sludge at low temperature.

Conclusion
The diversity of Legionella in activated sludge is influenced by the temperature, being L. pneumophila the dominant species at 35 °C. L. longbeachae that has been mainly reported in association with composts and potting soils was retrieved in the present study from activated sludge at 35 °C and L. drancourtii, a Legionella-like amoebal pathogen, at 15 °C.

New concentration method for drinking water samples improving *Legionella* detection developed in Aquavalens project.

Authors Saucedo G., Puigdomènech C., Arnedo M. J., Juárez R., Galofré B., González S.

Background
The Aquavalens project (http://aquavalens.org/) is a European Commission financed project within the 7thFramework Programme with the purpose of protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation.
The main objective of the large drinking water system field demonstrations is testing and demonstrating the suitability of the developed and validated techniques of the project as a tool to improve the management and safety of water quality. Even though Legionella is not a gastrointestinal pathogen that it is not included in drinking water regulation, it has been analysed due to its interest as a causing agent of Legionnaires' disease.

Materials/Methods
Field studies have been carried out with the aim to test these new techniques. A novel concentration protocol, developed in the project is tested opening up the possibility of sampling viruses, bacteria and protozoa using a unique filter able to concentrate high water volumes. Sample analysis are done by using PCR kits developed in the project compared to verification analysis with validated methods.

Results
Preliminary results of Legionella are presented for sampling campaigns at different treatment steps of the Drinking Water Treatment Plant (DWTP) and Distribution Network (DN) of Barcelona. Roughly 60 samples were analysed for Legionella spp and L.pneumophila by PCR. The initial results shows positive values in the first steps of DWTP and detection of Legionella spp in few cases in DN.

Conclusion
This study proves the capacity of the newly developed techniques to concentrate large volumes of water improving the detection of Legionella species. The results show a great potential to improve the microbiological sampling and detection in terms of time, money and human resources needs.

Acknowledgments

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Prevalence of *Legionella pneumophila* at thermal spas in Algeria

Authors Boilattabi N., Bouanane-Darenfed A.

Legionella bacteria are omnipresent in both natural and anthropogenic aquatic environments. Natural environments do not support extensive Legionella growth but anthropogenic systems can promote its proliferation to high concentrations. In case of their proliferation which plays an essential role in assessing the risk of disease transmission; environmental laboratories would detect them.

Abatement of Legionella bacteria appears to be difficult and environmental eradication is not possible. Legionella can transform itself into viable but non culturable and persistent forms, as well as grow on necrotrophic substrate and survive in protozoa and biofilm, compromising the efficiency of control strategies based on chemical, mechanical and physical disinfection systems. Official methods for Legionella detection are based on the growth of the microorganism in selective media (ISO 11731). Long assay time, low sensitivity, loss of viability after collection or sample treatment, presence of interfering microbiota. Quantitative polymerase chain reaction (qPCR) has been proposed as method for monitoring Legionella in environmental systems , but the interpretation of qPCR results from environmental samples remains difficult. The main problem of qPCR is that it enumerates DNA of both live and dead cells leading to an overestimation of the actual health risk .

In this study we compared the cultural and molecular method and the results were significant. Water samples were collected from therapeutic thermal spas in Algeria: 75% of the samples contained Legionella Pneumophila ; the predominant isolates (60%) belonged to Legionella pneumophila serogroup 1.A total of 50 water samples were assayed by the two techniques (culture and qPCR method). Of these, 38 were recorded as positive by at least one test. Legionella spp was detected by culture in 15 of these 38 samples. Eighteen (80%) of the 40 samples were positive by were PCR positive. Each sample was mixed well by shaking by hand then filtered through a 2.7 µm glass fiber pre-filter (Filterlab) and a 0.4 µm nylon filter (Millipore) overlapped. Pre-filtration allowed separation of bacteria from bigger particles and this was discarded after filtration. The filter was then removed from the filter holder and placed with 20 ml of the diluent L0 (Biótica) in a 100 ml sterile plastic container, vigorously vortexed for 2 minutes.

Each 20 ml concentrated sample was thoroughly mixed and then divided into two portions. One 10 ml portion was assayed by qPCR (Applied Biosystems) for Legionella spp. with internal process controls in order to assess inhibition or suboptimal reaction conditions. T

he second 1 ml portion was assayed by culture for Legionella species following ISO 11731. Culture procedure followed the ISO standard 11731-1:2004. 0.1-0.5 ml portions of concentrated sample were cultured onto the selective medium GVPC without pretreatments, at 36°C for 10 days. Presumptive colonies were cultivated on buffered charcoal yeast extract media, BCYE and BCYE-Cys, at 36°C during at least 2 days. Colonies grown on BCYE but not on BCYE-Cys were confirmed as Legionella. Moreover, agglutination latex test was also applied for suspicious colonies .

There is a significant discrepancy between qPCR results and culture results for Legionella in water samples, because positivity rates for qPCR are usually greater than those obtained by culture. The methods tested in the present study might be used in laboratories for routine water analysis because the large number of positive Legionella pneumophila samples indicates a potential risk of infection to patients, especially those undergoing inhalation treatment with thermal water, or those using a whirlpool or taking a shower.

Co-occurrence of *Legionella* spp. and free living protozoa in drinking water supply systems in Latvia

Authors Valcina O., Pule D., Malisevs A., Trofimova J., Makarova S., Grantina-Ievina L., Berzin A., Krumina A.

Background: L. pneumophila is known as the causative agent of Legionnaires' disease, while free-living protozoa (FLP) are considered as a vector and reservoir for bacterial population and serves as additional protection for pathogenic bacteria against high temperatures and disinfectants.
The aim of this study was to investigate the co-occurrence of Legionella spp. and FLP in drinking water supply systems.
Materials/Methods: In total 184 water samples from taps and shower heads were tested for presence of free-living protozoa and detection of Legionella spp. in accordance with standard ISO method and MALDI-TOF. Isolation and cultivation of FLP was performed using previously described protocols (Schuster, 2002; Thomas, 2006). Identification of FLP was performed by microscopy and 18S Ribosomal DNA PCR and sequencingprotocol (Schroeder, 2011) for Acanthamoeba.
Results: Overall, Legionella spp. was detected in 70 samples (38%). L. pneumophila was found in 63 (34%) samples, L. rumbrilucens in 6 samples while L.anisa was observed in one sample. The most common L.pneumophila serogroup was SG 3, which was isolated from 37 samples (59% of L.pneumophila positive samples). SG 1, 2, 6 and 9 ranged between 8% and 13%.
FLP were detected in 129 samples (70%). Most frequently observed FLP genus was Acanthamoeba, which was found in 100 samples (54%). Vermaoeba and Valkampfia were found in 17 (9%) and 12 (7%) samples. In 36 samples at least two genera of FLP were observed. Identification of Acanthamoeba revealed diversity of acanthamoebas in drinking water, including T4 genotype, A.quina, A.triangularis, A. castellanii, A.polyphaga and A. lugdunensis.
Occurrence of FLP was statistically higher ($p<0.0001$) in Legionella positive samples with FLP present in allLegionella positive samples.
Conclusions: Implementing water treatment and disinfection strategies for inactivating free living protozoa should also improve control opportunities for pathogenic microorganisms including Legionella spp.

Analysis Of Enviromental Drivers And Geographical Distribution Of Relevant *L. Pneumophila* Mlva Genotypes From The West Bank

Authors Zayed A.R., Marina P., Butmeh S., Salah A., Al-Allam H., Abu Tair L., Bahader S.A., Brettar I., Höfle MG., Bitar D. M.

Legionella pneumophila is responsible for a severe community-acquired and hospital-acquired pneumonia. The study of the genetic diversity and the geographical distribution of L. pneumophila strains are essential for the development of public health control strategies. In this study, high resolution molecular typing and environmental drivers were used for a better understanding of the geographical distribution of this pathogen. Methods: Two year proactive environmental surveillance of L. pneumophila in the water distribution systems of eight hospitals was carried out. A subset of 180 isolates were analyzed using Multiple-Locus Variablenumber tandem repeats Analysis (MLVA). Also, twenty five physiochemical parameters were measured to correlate with L. pneumophila prevalence. Results: L. pneumophila was detected in all hospitals' water systems. Low Legionella counts correlated with the high impact of magnesium (rs= -0.511) in the West Bank ground water. Most of the isolates were identified as serogroup 1 (62.2%). The 180 isolates were typed in 26 MLVA-8(12) isolates (ID=0.790, 95% CI 0.739-0.841). The most frequently isolated genotype was Gt4 (17) (n=71, 41.1%). All MLVA genotypes were clustered into four MLVA clonal complexes (VACCs). Conclusion This study showed the biogeography of L. pneumophila strains in the West Bank, remarked the differences with world strains

The potential adversary effect of Bacillus species on *Legionella pneumophila* colonization of cooling towers

Authors Paranjape K., Faucher S. P.

The bacterium Legionella pneumophila (Lp) is the causative agent of Legionnaires' Disease. Cooling towers are a major source of outbreaks. Interestingly, cooling towers are colonized at different levels. Lp continuously contaminates some towers despite treatment, whereas others are free of Lp for extended periods of time. The reason for the latter is not understood. One possibility could be the presence of microorganisms that inhibit the growth of Lp. In this study, two isolates were identified and characterized for their inhibitory effect on Lp. Materials/Methods: Fifty millilitres of water was sampled from the basin of a cooling tower in Montreal, Canada. The sample was serially diluted and plated on R2A and NA. The plates were incubated at 30°C overnight and different colonies were isolated using the streaking method. Next, the different isolates were spotted on a mat of Lp JR32 on CYE at 37°C for 3 days. Genomic DNA was extracted from both isolates and 16S rRNA was amplified by PCR, using the 1 / 2 inserimenti 27F and 1492R primers. The PCR product was sequenced and compared to the NCBI gene database using BLAST, in order to identify the two strains. Phylogenetic trees were created using MEGA. Results: Two isolates, KMP29 and KMP30, inhibited the growth of Lp on CYE. The inhibition zones were respectively 18mm and 45mm in diameter. These two isolates were rod shaped and gram positive. BLAST and phylogenetic analysis revealed that these two isolates were Bacillus species. More specifically, KMP29 was closely related to B. amyloliquefaciens and KMP30 to B. licheniformis. 0 0 1 282 1556 Universite de Montreal 12 3 1835 14.0 Normal 0 21 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Tableau Normal"; msotstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-paramargin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin; mso-ansi-language:EN-US;} Conclusion: In conclusion, two bacterial isolates identified as B. amyloliquefaciens and B. licheniformis are reported to inhibit the growth of Lp. The presence of such species could help explain why certain towers have low numbers of Lp.

Distribution and molecular characteristics of *Legionella spp*. strains isolated from cooling tower and hot spring in Kobe City, Japan

Authors Nakanishi N., Tanaka S., Arikawa K., Iwamoto T.

Water systems contaminated Legionella are the implicated source of Legionnaires' disease. Prospective surveillance of the extent of Legionella pollution was conducted at various freshwater environments in Kobe city, Japan. It is important from a public health perspective to understand molecular characteristics of environmental Legionella spp. In this study, we aimed to clarify the genetic characteristics and virulence traits of L. pneumophila and the distribution of Legionella spp. isolated from cooling tower (CT) and hot spring (HS) in Kobe City. ?Materials and Methods?A total of 396 environmental strains of L. penumophilla including CT (n = 161) and HS (n = 235) were analyzed by using sequence-based typing (SBT) and multiple-locus variable number tandem repeat analysis (MLVA). We examined the presence of the two virulence genes, lvh and rtxA by PCR. A total of 190 Legionella spp. including CT (n = 64) and HS (n = 126) were characterized using mip gene and 16S rRNA gene sequencing for species. ?Results?The 161 L. pneumophila isolates from CT, which were classified into 30 sequence types (STs) including 15 new STs, were divided into three clonal complex by minimum spanning tree (MST) based on SBT and MLVA. Legionella spp. Strains other than L. pneumophila isolated from CT were identified as follows: L. erythra (20/64, 31.3%), L. anisa (12/64, 18.8%), L. rubrilucens (9/64, 14.1%) and L. quinlvanii (8/64, 12.5%). The 235 isolates from HS were divided into 101 STs including 62 new STs. 8.5% (20/235) of tested isolates 1 / 2 inserimenti carried both of lvh and rtxA. We found that L. londiniensis (54/126, 42.9%) and L. israelensis (40/126, 31.7%) predominated in HS. ?Conclusion?Our analysis revealed the genetic distinction and distribution of Legionella isolated from CT and HS. This study highlights the importance of the epidemiology and ecology of Legionella from the standpoint of public health.

Occurrence of *Legionella* in UK household showers

Authors Stevenson D., Collins S., Walker J., Bennett A.

Household water systems have been proposed as a source of sporadic Legionnaires' disease. Showers represent a frequently used aerosol generating device in the domestic setting yet little is known about the occurrence of Legionella spp. in these systems. This study investigated the prevalence of L. spp. by culture and qPCR in UK household showers. Materials/Methods Sample packs were provided to allow collection of 1L water samples and if possible, swab 1 / 2 inserimenti samples from the shower hose. These were then tested, according to traditional culture methods and qPCR, for presence of Legionella species. Ninety nine showers from 82 separate properties in the South of England were sampled. Two L. spp. positive showers were further investigated with cyclone and Andersen air samplers to detect any Legionella present in the aerosols generated. Results Clinically relevant L. spp. were isolated by culture in 8% of shower water samples representing 6% of households. L. pn. sg1 ST59 was isolated from two showers in one property and air sampling demonstrated its presence in the aerosol state. A further 31% of showers were positive by L.spp. qPCR. By multi-variable binomial regression modelling L. spp. qPCR positivity was associated with the age of the property ($p = 0.02$), the age of the shower ($p = 0.01$) and the frequency of use ($p = 0.09$). The concentration of L. spp. detected by qPCR was shown to decrease with increased frequency of use ($p = 0.04$) and more frequent showerhead cleaning ($p = 0.05$). There was no association between L spp. qPCR positivity and the cold water supply or the showerhead material ($p = 0.65$ and $p = 0.71$, respectively). Conclusion Household showers may be important reservoirs of clinically significant Legionella and should be considered in source investigations. Simple public health advice may help to mitigate the risk of Legionella exposure in the domestic shower environment. Further refinement of aerobiology techniques to improve sensitivity with regards to Legionella detection is required.

Detection of *Legionella spp*. by a New Colorimetric Probe Alternation Link Self-Assembly Reaction (PALSAR) Method

Authors Morinaka R., Amemura-Maekawa J., Kanatani J., Sasaki M., Isobe J., Haraguchi H., Futo S., Kura F.

A rapid and readily available colorimetric PALSAR method was developed to detect genus-specific 16S rRNA of Legionella spp.. Using this new method, Legionella spp. can be detected within 4.5 hours without cultivation, DNA amplification and any measuring equipments. In this study, we tried to assess the sensitivity and specificity of the colorimetric PALSAR method for the detection of Legionella spp. using pure culture samples and bath water samples. Materials/Methods: A total of 79 strains of 53 Legionella spp. and 18 strains of 15 nonLegionella spp. were used. All of Legionella strains were cultured on a BCYE agar from stock culture, then inoculated the fresh colonies to AYE broth for overnight cultivation. According to the McFarland values, 2-fold dilution series from 7.3x 104 to 7.1 CFU/mL were prepared and colorimetric PALSAR assay was performed. In the meantime, the bacterial counting was determined by plating the serial dilutions on BCYE Agar. For non-Legionella strains, 1x106 CFU/mL samples were prepared and detected by the colorimetric PALSAR assay. Bath water samples from 79 public baths were also tested both by the conventional culture method and the colorimetric PALSAR method. Results: All of the 79 Legionella strains could be detected by the colorimetric PALSAR method effectively. The range of LOD was 1 to 104 CFU per well. For the 18 non-Legionella strains, all samples were tested as negative where no false positive were 1 / 2 inserimenti found. Furthermore, Legionella spp. was detected in 23 out of 79 bath water samples by conventional culture method. On the other hand, 34 out of 79 bath water samples were positive when detected using colorimetric PALSAR method. Hence, the relative sensitivity and relative specificity were calculated as 87% and 75%, respectively. Conclusion: The new colorimetric PALSAR method proposed here appears to be an efficient and innovative means for rapid detection of Legionella spp. in bath waters from public baths.

High prevalence of *Legionella* in non-passenger merchant vessels - is new guidance required?

Authors Collins S. L., Stevenson D., Mentasti M., Shaw A., Johnson A., Crossley L., Willis C.

The provision of safe, potable water on board ships is critical for the health of passengers and crew alike. The WHO provides guidance on managing the risks associated with Legionella on board merchant vessels. Despite this, and other country-specific guidance, studies have shown a high prevalence of Legionella contamination in passenger vessels. However, there is a paucity of information on the risk from potable water in non-passenger merchant vessels (NPMVs) particularly with regards to Legionella and other bacteria. Materials/Methods During Port Health Authority inspections water was sampled from 550 NPMVs docked in eight UK ports. A total of 1027 samples from 412 NPMVs were examined for total aerobic colony counts (ACC), coliforms, E. coli and enterococci. Eight hundred and three samples from 360 NPMVs were cultured specifically for Legionella. 1 / 2 inserimenti Results Forty one percent of samples yielded ACC above the action level (>1x103 CFU/ml) and 4.5% contained actionable levels (>1CFU/100ml) of faecal indicator bacteria. More than 50% of vessels were positive for Legionella and 27% of samples showed levels greater than the UK upper action limit of 1x103 CFU/L. Cabin showers (49%) and hospital shower (45%) were frequently positive. A subset of 106 samples was analysed by qPCR for Legionella and identified a further 11 Legionella positive NPMVs, returning a negative predictive value of 100%. There was no correlation between NPMV age or size and any microbial parameters (P = >0.05). Legionella pneumophila sg1 was isolated from 46% of NPMVs and sequence based typing of 17 isolates revealed four sequence types previously associated with human disease. Conclusion These data raise significant concerns regarding the management of Legionella risks on board NPMVs and suggest that better guidance and compliance are required to improve control. Public Health England are revising current ship guidelines to include specific advice on controlling Legionella risks in NPMVs.

A new Legionella Monitoring Plan in an old hospital water network in continuous hyperchlorination: first results.

Authors Marinelli L., Del Cimmuto A., Cottarelli A., Di Bella O., Barbato D., La Torre G., Renzini V., and De Giusti M.

Background: To control the presence of Legionella spp, in the old Teaching Hospital Policlinico Umberto I in Rome (TH-PUI), under continuous hyperchlorination (0.5-1.0 mg/L at distant points), a new Legionella Monitoring Plan named Acquapol was implemented according to the criteria of the new National Guidelines. Methods: The new plan involved a systematic monitoring repeated every six month of the water system in 183 sampling points from 23 buildings based on a specific risk assessment of the water network including: a) distance from the chlorine pump; b) thermal power plant; c) water temperature. New sampling points were introduced within the wards and dressing room (shower and washbasin). Extra sampling points in other 3 buildings were analysed after maintenance work on the water network and after suspected cases of nosocomial legionellosis. Results: 757 samples of cold water supply (326/757; 43.06%) and hot water supply (431/757; 56.94%) were collected from October 2014 to May 2017, determining residual free chlorine (0.81 ± 0.036 µg/L), pH (7.28 ± 0.02) and temperature (32.18 ± 1.06 °C). Legionella was isolated in 24 out of 757 samples (3.17%) in 13/26 (50,0%) buildings (L.spp 50%; L.pneumophila sg1 16.7%; L.pneumophila sg 2-15 29.2%; L. anisa 4.2%). In 16/24 (66.7%) samples the level exceeded 102 cfu/L. There is no significant difference in detection of L.spp according to different concentration of free chlorine (2.8% for >0.5 vs 4.3% for <0.5; p=0.341). However, an inverse correlation with residual free chlorine was found only for L. pneumophila sg1 (Spearman's Rho = -0.073; p<0.05). Independent risk factors for L.spp isolation were: temperature between 20°C and 59°C (OR 3.95; 95% CI 1.08-14.38) and pH (OR 8.10; 95% CI 1.24-52.78) when residual free chlorine was > 0.5 µg/L. Conclusions: According to our data the chlorination does not have an impact of the detection of L.spp. It is perhaps time to think about a new disinfection strategy.

Investigation the effects of various stress factors on *Legionella pneumophila* in biofilm layer

Authors Vatansever C., Turetgen I.

Background: Microorganisms found in a biofilm structure are quite resistant against adverse environmental conditions. Legionella pneumophila is one of the most common pathogenic bacteria in the biofilm. L. pneumophila found in biofilm structure can cause health and economic problems. The aim of this study was identify the most effective elimination strategy for combating biofilm and planktonic L. pneumophila and prevent biofilm-related problems which are important in terms of human health.

Materials/Methods: In this study, L. pneumophila ATCC 33152 was inoculated in the cooling tower model system with potable water and formed biofilm on surfaces of glass coupons for 14 weeks. 14 weeks biofilm and planktonic phase bacteria were treated with various stress factors including different temperature, pH solutions, salt solutions, different doses and types disinfectants, desiccation, starvation and UV radiation. After exposing to stress factors, viability of both the biofilm and planktonic phase L. pneumophila were determined using conventional culture and EMA (ethidium monoazide bromide)-qPCR methods.

Results: Results indicated that L. pneumophila in biofilm structure were more resistant than planktonic counterparts against stress conditions. It was identified that 65°C heat treatment, acidic pH, 10 ppm chlorine, hypertonic salt, dessication and UV treatments are the most effective techniques for elimination of biofilm and planktonic phase L. pneumophila. Live bacteria were detected by EMA-qPCR in after treatments where L. pneumophila were not cultured conventionally.

Conclusion: Temperature of 65°C, acidic pH, hypertonic salt and UV treatments were effective applications that can be implemented easily and inexpensively in combating with L. pneumophila. It has been determined that EMA-qPCR technique may become advantageous in PCR techniques in order to enable the detection of the presence viable microorganisms in the sample following the appropriate optimization steps

Use of terminal filters to prevent Legionnaires' disease: measure filter efficacy

Authors Antonioli P., Perrone P., De Lorenzi S., Salvatorelli G.

Water in the mains plays an important role in the onset of hospital-acquired infections, 1 / 3 inserimenti particularly given the rise in pulmonary disease due to Legionella pneumophila. The advantage of using antibacterial terminal filters is that applicat ion can be limited to the wards with high risk patients plus the fact that installation and maintenance are simple and inex pensive. Our study assessed the efficacy of SBS water main terminal filters (Nu ova S.B. System S.r.l., Cinisello Balsamo, MI-Italy), model WF1 for wash basins and bidets and model WF2 for show er s an d water birth tubs, composed of a polypropylene pre-filter (1 ?m) and a double layer polyethersulphone filtration membrane (0.2+0.1 ?m). Materials/Methods Ferrara University Hospital , opened in 2012, has 710 beds. It is served by 6 different water subplants, each with different features and contamination history and problems. Hospital areas are classified in 3 risk levels (High, Medium, Low). Since 2012, a Water Safety Plan was designed, with 115 sampling points , identification of specific risk factors and preventivecorrective interventions for environmental and plant factors, care activieties to manage the risk f or patients and operators. The methods adopted are: - maintenance temperature in safety range -weekly flushing and limestone disintegration 2 / 3 inserimenti -disinfection with chlorine dioxide -ultrafiltration at the input point -water terminal filters. In High Risk wards are permanently installed 138 terminal filters and filter efficacy is performed, in each ward, during 2 years. Results In High risk wards there was no cases of Legionnairs' disease , despit e, over time, the water of the subplant was contaminated with Legionella beyond the limits of acceptability (eg. Onco-Hematology hot water subplant: 24.000 CFU/l). Conclusion Water terminal filters guarantee microbiologically safe water dispens ing. So, filters are also used temporarily to safely place an area with con taminated water to allow the analysis and the consequent technical eng ineering interventions.

Legionella prevention in water systems in hospitals: Stakeholders and the process seen from Facility Management

Authors Leiblein T. W., Tucker M., Ashall M., Al Khaddar R., Lee S., Gollnisch C., Hofer S.

An ongoing research project systematically uncovers the situation of Legionella prevention in water systems in healthcare organisations. Healthcare facilities (i.e. hospitals) can be part of a Facility Management (FM) portfolio. The aim of the overall research project is to work out a framework for FM. The discipline FM is present in healthcare and operates within a range of regulations specific to a country. According to the International Facility Management Association an organisation has to take precautions to manage risks properly. Data on six case studies of three countries were collected in a two-stage sequential exploratory research study design. Expert's interviews were followed by a questionnaire study including further groups of stakeholders. The study focuses on the organisational structure with respect to the process of Legionella prevention, seen from a FM's perspective. Findings demonstrate fundamental differences in the organisational structure in hospitals and thus, the different process owners working for Legionella prevention in water systems. The UK follows recommendations of the WHOs water safety plan by maintaining a defined group of specialists (water safety team). In Germany and Switzerland the 'hygiene commission' implements decision-making and takes responsibility for infection prevention measures. 1 / 2 inserimenti Potentially the organisational structure has an impact on the awareness of topics related infection prevention of hazards related to water hygiene. Usually a technical operations unit (or similar) is responsible for maintaining water systems. Present-day economically-driven budget cuts, delayed reconstruction works or incomplete risk assessments may be recognised and counteracted by those in scope of liability. For that reason a process-scheme for Legionella prevention in water systems may be helpful to identify every stakeholder working for the joint process. This study may be a headstone, considering FM's needs and duties within the context of healthcare.

Rapid detection of *Legionella pneumophila* by IMS and flow cytometry

Authors Aguilar C., Rachmühl C., Stöckli M., Ehlert A. K., Julian A., Morger D., Keserue H. A.

Legionella is an opportunistic pathogen of high public health concern. It is the causative agent of atypical pneumonia known as Legionnaires' disease as well as the acute febrile illness known as Pontiac fever. Legionella is responsible for both nosocomial and community-acquired infections. Potable water systems are the primary source of Legionella infections, causing over 50% of all drinking water-related outbreaks, increasingly adding to annual health costs. Currently, the ISO 11731 culture-dependent method is the standard for the isolation and enumeration of Legionella from water samples. This approach is inaccurate, timeconsuming and cannot detect viable but non-culturable (VBNC) bacteria, thus largely underestimating the population numbers of Legionella present in water. Materials/Methods: We developed an automated, culture-independent immunomagnetic separation (IMS) method in a standalone device. Magnetic nanoparticles were conjugated to highly specific monoclonal antibodies against L. pneumophila SG1. A second set of antibodies was used for fluorescent detection in a flow cytometer. We analyzed different matrices for the 1 / 2 inserimenti presence of L. pneumophila SG1. Results: Combining the automated IMS with flow cytometry, we accurately enumerated total or viable Legionella cells present in water samples, including VBNC. The sensitivity increased from five to ten times over the traditional, culture-dependent method. We successfully detected Legionella in different matrices like tap, bottled water or more complex or highly contaminated matrices like cooling tower water. We showed that in addition to quantification by flow cytometry, the samples prepared with our IMS method could be used for quantification of Legionella by plating on selective media. Conclusion: We present here an automated IMS method for the detection of L. pneumophila SG1, shortening the time from sample-to-result for detection from several days to 1-2 hours.Max length 2,000 characters

Usefulness of flow cytometry to detect, quantify and evaluate cytotoxicity of *Legionella* Viable But Not Cultivable

Authors Allegra S., Girardot F., Riffard S.

Our team has been working since 2006 on the improvement of Legionella detection and quantification in their environmental reservoirs. Several flow cytometric assay (FCA) have been set up to:

(i) – detect and quantify Legionella in monomicrobial (1) or polymicrobial samples ((2), works in progress).

(ii) – characterize the physiological state of Legionella and their percentages under stress conditions: thermal, chlorine stresses and after nebulization experiments (3–5).

(iii) – evaluate the pathogenicity of Legionella VBNC forms against amoebae and human mucosa respiratory cells (6,7).

FCA is now an appropriate tool to rapidly detect and quantify Legionella cells. As this technique can also discriminate all physiological states, it brings new insights to prevent Legionella risks through the anticipation of their virulence and their reservoirs colonization capabilities.

Sampling of *Legionella* In Bioaerosol: An Inail Patent

Authors Gioffrè A., Samele P., Iavicoli S.

The detection of microbial contamination in the air is particularly important for protection of population health. There are numerous techniques for the detection of pathogenic microorganisms, such as Legionella, in environmental matrices. A selective and rapid sampling minimizes analytical disadvantages for identification and quantization. The objective of this study is to use a new system for capture of L. pneumophila in bioaerosol: is a bubbling device for sampling of environmental matrices such as air and aerosol, patented from INAIL. Materials/Methods Dilutions of L. pneumophila ATCC 33152 were nebulized by an aerosol system in a test chamber. The dilutions is sampled by the bubbling device contains 1 ml of sterile water with 100?l of sample buffer and 50?l of Dynabeads® anti-Legionella (IMB). Then, the suspension was placed in 1.5 ml tube. The IMB were recovered by a magnetic plate and washed for three times. The samples were analysed with Gold Standard method, PCR, innunomagnetic ELISA, microscopy fluorescence with spectroradiometer. Results Cultural method: we noted a loss of the sample after the heat treatment, while the recovery with the IMB is almost 100%. PCR: The sample was captured by IMB, where there is the amplification band, while the supernatant is negative. Immuno-magnetic ELISA: this system has allowed us to quantify Legionella bound to IMB from a concentration of 107 CFU/ml, up to 103 CFU/ml. Fluorescence Analysis: we measured the photons emitted by fluorescence, trying to quantize the cells present on the glass slide. The calculation was made by measuring the integral 1 / 2 inserimenti between 510-560nm. Conclusion The development of this device will allow to reduce the time of post sampling analysis and support the use of numerous analytical protocols. In addition, the extreme versatility in its use allows you to change the target organism by simply replacing the antibody bound to IMB.

Comprehensive hospital water system (WS) surveillance identifies factors associated with recovery of *Legionella*

Authors Brooke K., Decker M. D., Harris P. L., Toy L. D., M. Cloud-Woods S., E. Baumgart L., Muder R., J. Clancy C., F. Sonel A.

VA Pittsburgh Infection Prevention (VAPHS IP) has performed Legionella cultures (cx) on almost 10,000 WS samples since a 2011-12 nosocomial outbreak of Legionnaire's disease. Methods. WS samples collected during VAPHS IP surveillance were analyzed. Chi-squared and logistic regression analyses were used to determine correlations between WS factors and Legionella cx positivity (+). Results. Type of sample. 2147 matched pairs of 1-liter (1L) water and swab cx from the same fixtures in 2012-13 were compared. Cx were Legionella + by ? 1 method in 4.1% of pairs. Water and swab + was 3.7% and 1.3%, respectively (p=0.001); sensitivity was 89.9% and 30.3%, respectively. Water samples were superior for detecting L. pneumophila (Lp) serogroups 1 and 2-14 (p=0.001 and 0.0002, respectively); there was no difference in detecting 1 / 2 inserimenti non-Lp spp (p=0.42). WS temperature. Legionella + rates from hot and cold water loops in 2013-14 were 2.5% and 3.8%, respectively (p=0.03). Hot and cold water were more likely to yield Lp

Prevention of legionellosis in oil industry

Authors Carducci A., Janis B.

The risk of illnesses caused by Legionella is widely analysed in hospital and community settings like hotels, spas, apartment buildings for which extended guidelines have been produced. The prevention and control of legionellosis at workplace requires the application of specific procedures according to the different jobs and processes besides the general rules applied for communities. It is then necessary that, following the institutional guidelines, companies elaborate internal procedures and protocols to be followed in different sites and for different plants and tasks. Matherials/Methods: An important Energy Company (ENI) with more than 36.000 employers all over the world, has produced Internal Operative Guidelines (IOG) for the implementation of the risk assessment and control of Legionellosis at work. To this aim a wide literature review has been carried out producing a report specifically focused on oil industry contexts as refineries and offices. After an internal process of revision and synthesis, the final document has been released for application. 1 / 2 inserimenti Results: the IOG take in consideration: industrial cooling towers and evaporative condensers, hot and cold water plants in office buildings and air treatment systems. The management of plants follows the approach of Water Safety Plans recommended by international and national guidelines, focusing on: design and test, functioning, maintenance, cleaning and prevention treatments, control and monitoring (inspections, microbiological and chemical analyses), emergencies

Conclusions: the application of ENI IOG is aimed to unify the prevention and control of legionellosis in all the contexts of the Company. This model could be useful for similar settings.

Evaluation of the efficacy of 3 and 4 months of use microfilters on *Legionella pneumophila* in real life conditions in a healthcare facility

Authors Cassier P., Coudrais S., Gardes S.,Gerbier-Colomban S.,Vanhems P., Raymond M.

Point-of- use (POU) filters are now validated in laboratory by manufacturers for 3 and even 4 months, using bacterial challenges according to the American Standard Test Method (ASTM) F838. However, evaluation of POU filters in real life conditions in healthcare facilities is rarely conducted. The aim of this study is to assess our supplier's 3 or 4 months of use (MOU) new POU filters (tubular membrane microfilters with a 0.1mm pore size) placed on showers known to be contaminated by L. pneumophila in our hospital. We studied 6 showers equipped with 3-MOU filters, 6 showers equipped with 4-MOU filters, and a control shower equipped with 1-MOU filters. All MOU filters were placed in the same clinical unit in one of the hospital building, on the same day and in the same conditions of use. The microbiological culture and quantification of L. pneumophila was conducted regarding the French standard AFNOR NF T90-431 by an external laborataory. For test showers, a water sample (1st flush) of 500 mL was collected without filter (Day (D) 0) and with filters (D0, Month (M) 1, M2, M3, and M4). Control shower water was also collected monthly before and after changing filter. Water temperature was measured for 1st flush, and 2 minutes after opening. 1 / 2 inserimenti Without filters, all the 12 showers samples grew positive for L. pneumophila (range: 630 CFU/L-750,000 CFU/L) as well as control shower (max: 25,000 CFU/L). With filters, 6/6 (100%) of 3-MOU filters and 6/6 (100%) of 4-MOU water samples were negative for L. pneumophila in the conditions of the study. Despite the limited number of POU filters tested in this investigation, we showed in real life conditions (i.e. mild to heavy contamination of the water network), L. pneumophila retention by 3-MOU and 4-MOU filters. Their use should be considered instead of the actual 1or 2-MOU filters to remove more durably L. pneumophila from the water.However, it would be also interesting to evaluate these new filters for other waterborne pathogens.

Evaluation of *Legionella* contamination in water distribution systems of prisons in Sicily (Italy).

Authors Coniglio M. A, Laganà P., Giammanco A., Calà C., Fasciana T., Distefano S., Mascarella C., Piricò V., Pulvirenti D., Mortellaro S., Lavima G., Melfi M., Ingallinella V., Buonora C., Quartarone G., Calì A., La Valle S., Delia S., Palermo M.

Cases of Legionnaires' disease at prisons have been reported in the last years worldwide. In the majority of the penitentiaries involved, the hot water distribution systems (WDS) were the ultimate sources of the infection. This study evaluates Legionella contamination in WDS of the 22 prisons located in Sicily (Italy). Materials/Methods: The project was proposed and funded by the Regional Health Department. Samples were collected by the three Sicilian Legionella Reference Laboratories in collaboration with the local Public Health Departments. Samples were from central lines (water storage tanks and boilers) and from distal sites (faucets and showers selected on the basis of distance from boilers), comprising cold and hot water. For each sample, the temperature was documented. Isolation and enumeration of Legionella was performed by cultural method (ISO 11731). The isolates were identified on the basis of cultural and serological features. Results: 149 samples were collected. Legionella was isolated from central lines in 6 prisons and from distal sites in 13 prisons: 52.63% of all central and 41.53% of all distal sites were contaminated. At distal sites, cold water (temperature range: 18-25°C) was more frequently contaminated with Legionella compared to hot water (temperature range: 1 / 2 inserimenti 35-55°C) (60.00% vs 33.33%; Fisher's test, two-tailed P=0.0900). Legionella SG 2-14 was frequently isolated but comparison with the other serogroups was not statistically significant (Fisher's test, two-tailed P=0.1762). Conclusion: Data show a higher contamination of cold than of hot WDS, suggesting that not only hot water supply may be a source of infection. Anyway, Legionella monitoring of cold and hot WDS should be recommended in prisons because penitentiary populations contain vulnerable people. Prison health services should work closely with national and local health services, so that the prisons can provide the same standard of care as local hospitals and communi

Legionella and Amoeba in hospital cooling tower: monitoring and control

Authors Demarie V., Avanzini C., Giorgione N., Carcieri A., Franzin L.

Cooling tower (CT) water is favourable environment for microbial growth. CT is the most important source of infection associated to legionellosis outbreak. Aim of the study is Legionella monitoring of a hospital CT water in order to prevent aerosol contamination. Materials/Methods: 50 water samples from 4 CTs were periodically collected during a 6-year period of regular use. Hospital CT water is disinfected by sodium hypochlorite and cleaned each month; basin water is untreated. After 1-liter water filtration, Legionella was quantitative detected by culture on BCYE, BMPA, MWY, directly and after acid and heat treatment. 1 / 2 inserimenti Suspected colonies were typed and identified by mip sequencing. Quantitative detection of L. pneumophila and Legionella spp. was performed by real-time PCR. Amoeba was detected by MPN at 25°C and 37°C. Aerobic bacteria count (TVC) was performed by serial dilutions on PCA plates at 30°C. Temperature, free chlorine and pH were also determined. Aerosol (1 m 3 air) was collected by Coriolis ? for Legionella detection in 12 samples. Results: Legionella was 100 fu/L except in 8 (16%) samples (2 for CT in 2 instances; 103 cfu/L). The strains isolated were L. pneumophila 1 Bellingham ST59, L. rubrilucens, L. anisa, Legionella spp. Legionella was positive by qPCR in 100% of samples up to 107 GU/L, L. pneumophila in 56%. TVC was ?104 cfu/mL in 34%, 104 in 24%, 105 in 38% and 106 in 4%. Temperature was 9°C-25°C, free chlorine was ?0.1 ppm and pH was 7.7-9.6. Amoeba was isolated from 82% of samples. Legionella was not detected from bioaerosol. Conclusion: Legionella detection by culture is underestimated in these CTs as shown by PCR and predicted by Amoeba high contamination. When biocides are not used in such complex environment, a greater attention should be given to the water system proper maintenance and a strict program including monthly disinfection must be employed to minimize scale, corrosion, solids deposition and to control microbial growth.

Monochloramine-based “SANIKILL®” patented technology for shock and long-term disinfection of housing water distribution systems

Authors Di Marino O., Doniselli N., Comazzi A., Viganò S.

The contamination of plumbing systems by Legionella spp. represents a severe problem not only in the healthcare and recreational facilities and industrial sites, but also in housing building. Legionella can easily colonize pipes reaching sinks, showers and all the other domestic hot water distribution points. If micrometer drops containing Legionella are inhaled by susceptible individuals (e.g. immunocompromised, smokers, elderly, etc.), reaching the lower respiratory tract, the bacteria can cause Legionnaires’ disease, a severe and often lethal form of pneumonia. Once Legionella colonizes the plumbing systems, forming biofilm with other bacterial species and taking advantage of its ability to reside within some protozoa, it is not easy to remove it completely with the generally adopted traditional physical or chemical methods (e.g. increasing temperature up to 70 - 75 °C or filling up the pipes using a chlorine dioxide solution 1 / 2 inserimenti at high concentration for a limited period of time). Methods In autumn 2014, about 350 flats in Milan were found to be extremely contaminated by Legionella, posing a severe risk to the health of their inhabitants. Considering the high contamination rate and the extension of the contaminated plumbing system, it was decided to apply an innovative remediation strategy divided in two steps: a 10 hours shock treatment with monochloramine at 10 mg/l, produced using the SANIKILL® patented technology (Sanipur Srl, Italy), followed by the reduction of dosage to 2 - 3 mg/l, to maintain the long term system disinfection. Results and Conclusions Four days after the high dosage treatment, none of the twenty-three samples analyzed were found to be positive (100 CFU/liter), compared to the 17/23 positive samples found before monochloramine application. The monitoringof Legionella presence after the reduction of the biocide to the maintenance concentration showed that this disinfectant is extremely effective in the long-term treatment of the Domestic Hot Water distribution systems against this harmful pathogen.

Preliminary data on detection and quantification of viable and VBNC *Legionella spp*. by culture end PMA-qPCR in water distribution system treated with monochloramine.

Authors: Ditommaso S., Giacomuzzia M., Ricciardia E., Gremob F., Griffab D., Spinac E., Procaccic V., Barezzanid L., Mezzogorid A., Vetroned A., Zottia C. M.

Legionella spp. a facultative intracellular human pathogen, can persist for long periods in natural and artificial aquatic environments. Eradication of this bacterium from plumbing systems is often difficult. Monitoring Legionella spp.in water networks is generally done by enumeration on standard solid medium. This method does not take into account VBNC bacteria. These cells constitute potential sources of contamination and should be taken into account in monitoring water networks. Methods: A prospective environmental study is conduct in order to investigate the impact of monochloramine disinfection on the viable and VBNC Legionella spp. in hospital water system. In October 2016 monochloramine solutions were applied on biofilms at concentrations ranging from 2 to 3 mg/L as Cl2 mg/L, 18 samples have been collected from hospital water system 1 / 2 inserimenti exposed to monochloramine for after six and even month after monochloramine introduction. Legionella spp. survival has been tested after monochloramine treatment using culture method and qPCR based on propidium monoazide (PMA) treatment to selectively remove DNA from membrane-compromised cells. Results: After six month after monochloramine introduction, Legionella colonization ranged from 5% (1/18) and 88% (16/18) when investigated by culture when tested by PMA-qPCR. Monochloramine level in hospital water system were increased to 3 mg/L as Cl2 and after 1 month, colonization fell significantly to 16% (3/18) of samples analyzed by PMA- qPCR but there was no change in colonization rate calculated by culture (5%). Conclusion: Addition of monochloramine, at concentrations of 3 mg/L as Cl2 into a hospital potable water system reduced legionella colonization and ultimately resulted in undetectable levels of legionella in 84% of samples collected. Further study should be conducted to assess the long-term efficacy of monochloramine treatment.

Inspection Body Accreditation according to DIN EN ISO/IEC 17020 – A Quality Assurance Measure for Cause Identification of Legionella contamination in Water Installations

Authors Gollnisch C.

Inspection Body Accreditation according to DIN EN ISO/IEC 17020 – A Quality Assurance Measure for Cause Identification of Legionella contamination in Water Installations What does accreditation mean? 1 / 5 inserimenti Accreditation is a third-party evaluation and demonstration of competence. It is the assessment of independence, objectivity and competence of an entity for the performance of defined activities. Accreditation is a public authority activity and is awarded by national accreditation bodies such as UKAS (United Kingdom), COFRAC (France), ACCREDIA (Italy), DAkkS (Germany)1,2 . Based on European Parliament and Council regulation, EC Regulation 765/20083 , Germany have adapted an accreditation authority law. It regulates the accreditation as a responsibility of the national authorities Accreditation Body DAkkS. DAkkS state that the value of certificates, inspections, tests and calibrations is reliant on the competence of the person performing the assessment. Many organisations therefore undertake quality assessment programs to assure their work4 . Independent accreditation organisations confirm the professional competence of their activities under consideration of legal and normative standards with an accreditation which is reviewed regularly by independent DAkkS professionals. How does an Inspection Body work and what types of Bodies exist? According to the international standard DIN EN ISO/IEC 170205 , an Inspection Body examines and inspects an in-premise water system and provides expert judgement and risk assessment based on appropriate requirements. The Inspection Body is graded type A, B or C, where Type A has the highest level of “Independence” and Type C the lowest. For example, a DIN EN ISO/IEC 17020 Type A Inspection Body will ensure and assure that they are not engaged in the operation, maintenance, sampling, testing or control of the in-premise water system or any activities that may conflict with their independence of judgment and integrity in relation to their inspection activities. What is the procedure of the Inspection Body to determine Legionella spp. contamination in in-premise water systems? A DIN EN ISO/IEC 17020 Type A Inspection Body for in-premise water systems will focus on identifying the cause and source of Legionella contamination with target-performance comparisons with numerous national and European standards and guidance, including 2 / 5 inserimenti supporting documents from the World Health Organisation6, 7 . What conclusions are there following 10 years since Accreditation of Inspection Bodies for in-premise water systems? 1. Compliance with key points within the Code of Practice is the basis for minimising microbial contamination 2. Microbial, chemical and physical/hydraulical balances in water
systems can affect each other and should be inter-coordinated 3. Installation and operation of disinfection systems without further control measures rarely leads to long-term sustainable results (see photograph) 4. 100 % Legionella elimination from a water system is not possible
5. An engineering intervention in a water system without consideration of the complete system or impact on user behaviour can lead to increased hygienic problems 6. Solving hygienic problems in water systems, especially Legionella contaminations, should always be the work of a Water Safety Team, not a single individual 3

A new fast method to control the legionellosis risk : the Legio EZ-TestTM.

Authors Dumont A., Fugier E., Muller A., Paillusson N., Dukan S.

Background: Legionella pneumophila is the main cause for legionellosis, a common form of severe pneumonia. Over the last few years, numerous outbreaks of this infection have been documented. These outbreaks have occured in the home, offices, hotels and hospitals where warm waters facilitate the growth and dispersion of L.pneumophila.
The regulation imposes to monitor these bacteria in hot water systems by conventional cultural method but it takes more than 10 days to get results which increase the risk of L.pneumophila dispersion.

Materials and methods :To better control this microbiologic risk, we have developed a new method enabling to detect in just 2 days and on site the presence of this alive pathogen.
This method is based on a disruptive technology protected by 3 patents and consisting in specifically functionalize the membrane of L.pneumophila via the natural assimilation and then the integration of a « hook » within the membrane. Afterwards, this anchorpoint allows, via a « click » reaction, to highlight the presence of the bacteria by colorimetry.

Results: We have compared our method on a large pool of hot water system samples with the conventional cultural method. The sensitivity of our method is 85,19% and the specificity is97,62%.

Conclusion : Results obtained in this study strongly suggest that our method offers a fast and sensitive alternative to the cultural method for L. pneumophila detection in hot water systems on site and without any particular competence required.

CYTO-WATER: A new system for rapid detection and quantification of *Legionella* in industrial water samples

Authors Soria E., Catalán V., Yáñez A., Fernández-Fuentes M. A., Mellado V., Parker A., Buxton A., Trouchet D., Pérez J. M., Coll T., Amaya W., Hurth C., Jofre M., Martínez P., Pruneri V., Götzen R., Viader G., González S.

Analytical methods applied in diagnostic microbiology laboratories are usually performed manually and have some drawbacks, the major one being the long time to results. This is noteworthy in cases such as Legionella, where culture isolation can take up to 12 days. Rapid detection methods may overcome the disadvantages of traditional microbiological methods and achieve a fast detection which will help to prevent the spread of waterborne pathogens and outbreaks of waterborne diseases. The objective of the CYTO-WATER project is to deploy a new platform for on-site monitoring of Legionella and E. coli in water samples. 1 / 2 inserimenti Methods The CYTO-WATER system will integrate technologies uniquely available to the consortium partners: an automated water concentration module that includes Celltrap® concentrating filters, a microfluidic system where Legionella cells from the concentrated sample will be labelled to facilitate detection and measured by a newly designed CMOS-based fluorescence image cytometer. The whole integrated CYTO-WATER system will be validated according to ISO 16140-2:2016 in the laboratory comparing with conventional analysis methods (culture isolation and qPCR) in order to determine whether the method is suitable for detecting and quantifying microorganisms in water samples. Results The system will be capable of processing automatically four water samples. The automated water concentration module is completed and a microfluidic cartridge for labelling and measuring microorganisms has been designed and manufactured. The cytometry reader will be able to measure Legionella labelled with a specific fluorescence marker. Conclusion CYTO-WATER platform has been designed to be a portable device suitable for on-site applications. It will be a low cost solution for rapid microbiological analysis. The system will avoid water sampling, transport and manual concentration in the laboratory and will enable onsite detection of Legionella and E. coli in a reduced timeframe for early decision making

Can Total Aerobic Bacteria Predict The Presence Of *Legionella Spp*. In Cooling Towers?

Authors: Figueras M. J., Sanchis M. and Barbany Salas J. First author Figueras M. J.

The genus Legionella is composed by more than 60 species that are ubiquitous in aquatic habitats and water distribution systems. Legionella pneumophila serogroup 1 is the most important pathogen responsible for ca. 90% of Legionellosis cases that occur mainly in industrialized countries as result of the inhalation of contaminated aerosols. Cooling towers are the most important focus of larger outbreaks. The Spanish legislation for cooling towers requires a verification of the established preventive auto-control plan for Legionella through the analysis of total aerobic bacteria (TAB) every month and of Legionella spp. (Lp), every three months. No action is needed if results for Lp are 100CFU/L and those of TAB are 10.000 CFU/mL. However, it isunclear to wich extend the 10.000 CFU/mL established standard for total aerobic bacteria is the appropriate one for predicting the presence of Lp. Evaluate this, is the objective of this study. The results obtained from 1376 water samples after determination of geometric mean (GM) of counts of TAB in Plate Count Agar (PCA) and Lp obtained in GVPC were analyzed using the statistics program SPSS. Only 238 (17.3 %) of samples were positive for Lp (PLp) and showed a GM of 177 CFU/L and of 135 CFU/ml for TAB. The TAB showed a GM of 83 CFU /ml in the 1138 negative samples for Lp (NLp). Significant differences were observed between 1 / 2 inserimenti the GM of the TAB in the PLp and the NLp samples. In fact, it was observed that when the concentration of TAB increased the number of PLp samples also increased almost in a linear mode. The present study shows that, the standard for aerobic bacteria should be reduced considerably because at ?119 CFU/mL we found 53.3 % samples positive for Lp with concentrations > 100 CFU/L, that would require some early interventions.

Effect of nanosecond pulsed electric field on *Legionella pneumophila* in cooling water

Authors Guionet A., Helmi K., Zaepffel C., Packan D., Garnier J. P., Jaffrezic M. P., Ingrand V., Blanckaert V., Teissié J., David F.

Legionella contamination of cooling towers has been identified as the cause of sporadic cases and outbreaks of legionellosis. Efficient water treatment is essential to control Legionella concentration in cooling water in accordance with legislation. One of the different ways to eliminate microorganisms could be the use of pulsed electric fields (PEF) being able to limit the use of chemicals. Material/Methods: The nanosecond pulsed electric fields delivery system (nsPEF) was composed of a high voltage generator and a flow chamber of treatment. Escherichia coli (E. coli) and environmental 1 / 2 inserimenti Legionella pneumophila sg1 (Lps1) suspensions (synthetic cooling water) were exposed to nsPEF according to various conditions (frequency, flowrate, concentration). In order to evaluate treatment efficiency, effect on bacteria was analyzed before and after pulse sequences (Plate count method and a FACSCanto II flow cytometer). Results: The efficacy of nsPEF treatment was initially optimized with E. coli. With specific conditions, using culture-based standard method, 2 Log10 reduction within 5 min was achieved. This treatment was less efficient on Lps1 with 1 Log10 in 2 hours. Lps1 were therefore 50 times more resistant than E. coli. Flow cytometric analyzes suggested that there is no effect of nsPEF on bacteria DNA but an impact on membrane was observed, confirmed with/by a loss of ability to cultivate. Conclusion: The results obtained demonstrated a lower susceptibility of Lps1 to nsPEF with the field values that wee achievable with our generator. Longer treatment time is necessary on Lps1 for the same reduction effect than E. coli. Action of nsPEF on the membrane of E. coli seems more effective than on Lps1.

Comparison of the Anti-*Legionella* Fill Material against Standard Polypropylene Fill Material in Recirculating Model Water System

Authors Türetgen I., Vatansever C., Dobrita D.

Cooling towers are heat rejection systems which were used in some industrial applications and they have the potential to develop infectious concentrations of Legionella pneumophila. Anti-Legionella fill material and standard polypropylene fill material were compared in terms of biofilm formation potential and anti-Legionella activity within 4-month period using laboratory-scale recirculating water system similar to cooling tower water system. Materials/Methods: The experimental study was performed using a lab-scale cooling tower model system. A supply of network water was used to replenish water lost by evaporation and blowdown. Both test materials were fixed with hangers over the bulk water surfaces without any contact to each other. Test materials were not submerged into bulk water; surfaces were only in contact with sprayed water through the nozzles. System was experimentally infected with L. pneumophila standard strain suspension and operated continuously until all experiments had been completed. No chemicals were added to the system, to exclude their negative effects on microorganisms and biofilm formation. Results: Legionella colonization occurred on both test material surfaces beginning at the first month of the experimental period. Legionella bacterial counts on surfaces were increased over time. Statistically, significant difference was found between two test materials in terms of Legionella growth on surfaces. Product with antiLeigonella activity showed significantly lower Legionella colonization in comparison to standard 1 / 2 inserimenti polypropylene fill material after four-month test period (P

Microarray-based rapid verification and risk assessment of *Legionella* by on-chip amplification and live/dead differentiation

Authors Kober C., Niessner R., Seidel M.

Today, cultivation is still the gold standard for the detection of Legionella, though it takes 10 days and only a general detection of Legionella spp. is possible. Often legionellosis is only caused by the species Legionella pneumophila (90%, L.p.). Therefore, and especially for the monitoring of condensation recooling plants (c.r.p.), it is important to quantify Legionella spp. and L.p. culture-independent. A live/dead differentiation is additionally necessary to monitor biocide effects. Materials/Methods: A chemiluminescence DNA microarray on the automated flow-based analysis platform MCR 3 was established for the isothermal on-chip recombinase polymerase amplification (RPA). For quantification and differentiation of Legionella spp. and L.p. the genomic sequences of 16S rRNA and mip gene were used, respectively. To differentiate between living and dead Legionella, the sample is pretreated with the DNAintercalating dye PMA, which intercalates exclusively into the DNA of dead Legionella with permeable cell membranes. By subsequent on-chip RPA only living Legionella are detected. Results: Calibration curves were obtained with specific RPA primers, reaching detection limits of 87 genomic units (GU) per µL for Legionella spp. and 26 GU / µL for L.p. With the viability onchip RPA, predefined proportions of living Legionella could be measured in a range from 10 - 100,000 GU / µL with recovery rates of 75 to 105%. Conclusion: A combination of both methods allows the quantification of the sum parameter of living and dead Legionella and the 1 / 2 inserimenti determination of the proportion of living Legionella in the sample on one microarray with two flow cells in less than one hour. By addition of preconcentration methods like monolithic adsorption filtration, c.r.p. water was measured within a few hours and the effect of biocides was demonstrated

Long term effectiveness of chlorine dioxide disinfection against *Legionella spp:* evidence from a large teaching hospital in Rome

Authors Laurenti P., Raponi M., Boccia S., de Waure C., Sezzatini R., Bruno S., Damiani G., Vincenti S.

The high lethality rate of legionellosis among hospital-acquired cases requires a proper maintenance of the hot water systems by routine using of biocides in the hospital settings. Former studies have reported the effectiveness of different disinfection methods in health facilities, though very few investigated of the over time effectiveness of chlorine dioxide in eradicating Legionella spp. from water distribution system. Materials/Methods: We conducted our study in a health care facility with 1,500 patient beds located in Rome, Italy. Continuous chlorine dioxide treatment is active in the entire water distribution system of hot water since 2011, combined with an environmental and clinical surveillance according to the national guidelines. The study is based on an historical 1 / 2 inserimenti surveillance of legionella from water samples collected from selected bathroom outlets and heaters of the hospital wards, in the period from August 2011 until December, 2016. A life table analysis was used to investigate the over time effectiveness of chlorine dioxide against Legionella spp contamination. Results: Overall, 167 sampling points were included in the analysis, of which 18 (10.8%) were positive for Legionella spp. The cumulative proportion of samples free from Legionella spp was 99% at 30 days, 97% at 90 days, 96% at 180 days, 95% at 270 days, 94% at 360 days, and 93% at 450, 540, 630 days and at 2-years. Serotyping performed on isolates revealed that 5 (27.8%) of 18 positive water samples were L. pneumophila Sg 1, 10 (55.5%) samples were L. pneumophila Sg 2-14, and the remaining samples were other species. Conclusion: Our findings show that the use of chlorine dioxide biocides in hot water system, in combination with an active environmental surveillance system, provides a high proportion of Legionella free outlets and boiler samples at two years of follow-up.

Water safety in the operating rooms-benefits of using filters to prevent microbial contamination

Authors Manoni N., Belgiovine R., Brigida L.

Background: The hospital has 1.368 in-patient beds and 7 large operating room wards, complex systems in which numerous risk factors of infection can be present, including water contamination. The water may constitute a source of infections caused by opportunistic pathogens, including Legionella spp., Pseudomonas aeruginosa, etc..

Materials/Methods: The water is regularly monitored by the research of :Legionella spp., Escherichia Coli, Enterococci, Staphylococci,, Pseudomonas Aeruginosa, total bacterial contamination. Despite chlorination, monitoring the microbiological quality of the water, we found microbes in the water for surgical hand washing. As Legionella and other microorganisms have used the terminal antibacterial filters for several years in preventing contamination of the water, the hospital multidisciplinary team for the prevention of waterborne microorganisms, decided to use permanent terminal filters, to solve the problem. In the operating theatres were installed 103 antibacterial terminal filters on the tab for surgical hand washing.

Results: We assessed the efficacy of the filters with periodical monitoring. The filters guarantee safe water free from microorganisms.
There were also organizational advantages: the reduction of impossible use of taps for hand washing due to maintenance work and disinfection, reduction of access by external operators, that causes discomfort.
The good performance of the filters antibacterial allowed the reduction of microbiological testing with a consequent reduction of the sampling and analyzes costs.

Conclusion The terminal antibacterial filters installed in the operating rooms have proved effective in the prevention and control of contaminations by Legionella pneumophila, Pseudomonas Aeruginosa,
as well as by other microorganisms. The daily costs of the filtration is counterbalanced by the reduction of maintenance work, the reduction of controls, and crowding of operating rooms.

Control Measures for Legionellosis in Italian Hospitals: A National Survey

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Risk assessment, environmental monitoring and disinfection of water system are the key elements to prevent legionellosis (LG) risk. In this context, GISIO-SItI and SIMPIOS working groups carried on a national survey to know LG control and prevention measures adopted in Italian healthcare facilities (HF). METHODS. A sample of 163 HF on a voluntary base completed an online questionnaire using the network of the infection control professionals (ICP) joining GISIO-SItI and SIMPIOS. The questionnaire was built using SurveyMonkey platform and included 71 questions regarding HF general characteristics, and adopted LG control and prevention measures. Data were analysed through STATA12 software. RESULTS. A multidisciplinary team dedicated was present in 52.8% of HF, performing risk assessment in 79.9%. Legionella was routinely tested in water systems of 93.3% HF, 66.4% of them detected 1 / 2 inserimenti al least a positive result. L. pneumophila. sg 2-15 was the most frequent species (58.8%). The investigation for Legionella in air was conducted in 9.8% HF, but no positive data appeared. The common control measures were disinfection of water system (68.7%) mostly by thermic shock (37.4%) and chlorine dioxide (34.4%), substitution (65%) or cleaning (65.6%) of the tap and showerhead. With regard to clinical surveillance, laboratory diagnosis was performed by urinary antigen (80.4%), single antibody titer (39.9%), seroconversion (26.4%), sputum culture (28.8%) and molecular diagnosis (22.1%). Targeted training courses were organized in 35.6% HF, involving nurses (86.2%), physicians (81%) and technicians (74.1%). CONCLUSIONS. This study underlines that control and prevention measures for LG are present in Italian HF, but some critical aspects have to be improved: a well-done risk assessment, use of more sensitive diagnostic tests and more careful environmental sampling. Moreover, training courses should be implemented and ICP should play a leadership role to prevent LG risk.

Aptamers: New frontiers in *Legionella* detection

Authors Saad M., Faucher S. P., Tabrizian M.

Background: Transmission of Legionella to humans is exacerbated by management failure of man-made, engineered water systems. Quick, cost-effective detection strategies are important for effective prevention. Sensor based techniques such as Surface Plasmon Resonance (SPR) are promising detection technology and the use of aptamers should help improve their limits of detection. Aptamers are short, single stranded DNA or RNA oligonucleotides that can bind to whole cells with high affinity and specificity. Therefore, our objective is to create highly specific aptamers for detecting Legionella in complex matrices by using Cell-SELEX, an iterative process which involves incubating Legionella cells with a random pool of oligonucleotides, separating the cell bound and unbound oligonucleotides and amplifying the cell bound sequences via PCR for the next round of selection. Our first task is to optimize the SELEX procedure.

Materials and Methods: A random library of oligonucleotides was exposed to Legionella cells. Bound oligonucleotides were eluted, amplified by PCR and labelled with FITC and biotin. Streptavidin coated magnetic beads were used to make the sequences single-stranded prior to the following SELEX round. Different growth conditions, library amounts and PCR conditions were tested.

Results: We optimized various steps of the cell-SELEX procedures. We found that: 1) using bacteria suspended in water minimized contamination with gDNA, 2) Amplification by PCR was optimal when using 1:1000 dilution of eluted oligonucleotide and 30 cycles, and 3) a minimum of 1 nmol of oligonucleotide is necessary for the first round while 2-100 pmol is sufficient for subsequent rounds. Following 7 rounds of selections, the aptamer pool was counter selected against Pseudomonas aeruginosa. We are currently identifying the resulting aptamers by sequencing.

Conclusion: Optimal cell-SELEX conditions were determined to produce highly specific and sensitive aptamers to detect Legionella.

Energy Efficiency and Hygiene in Drinking Water Installations

Authors Petzold M., Koshkolda T., Löser J., Hoppe S., Lück C., Rühling K.

Since 2011, routine testing for Legionella spp. is obligatory in German buildings with large volumes of potable hot drinking water (PWH). Current regulations to prevent contamination of such systems by Legionella spp. are 60°C at the hot water outlet of the heater and 55°C for the recirculation circuit (PWH-C). One aim of this study was to investigate the correlation between energy consumption (i.e. water temperature) and the occurrence of Legionella. Methods A test rig was designed and constructed representing the complete hot and cold-water plumbing installation of a six party building. The PWH-part of the test rig is continuously driven by the European tapping cycles representing different household sizes. Different temperatures (45, 50, 55, and 60°C) within the PWH-system were tested using different methods for hydraulic balancing of the PWH-C-system. The complete test rig, potable water cold and hot, was monitored for Legionella spp. In addition, extensive thermo-hydraulic measurements were performed during all experiments. Results Microbiological and thermo-hydraulic monitoring revealed that a well harmonized PWH system inhibit growth of Legionella at 50, 55, and 60°C. However, when lowering the temperature to 45°C Legionella growth occurred after 2 weeks. After rising the temperature to 55°C the viable Legionella disappeared. The isolated Legionella strains revealed specific temperature preferences for optimal growth, which correlates with the time point and site of sampling. Conclusions This is the first description of a test rig that simulates the complete potable water installation of a six party building. We established different regimes for PWH-systems in buildings (e.g. temperature, circulation, flushing). Our results strongly suggest that we will identify a regime for PWH-systems in buildings (e.g. temperature, circulation, flushing) which is energy saving compared to the current German standards without increasing the risk of Legionella growth

MaxImpact of temperature, copper and silver exposure on the viability and recovery of clinical and environmental strains of *Legionella pneumophila*

Authors Prévost M., Doberva M., Faucher S., Allegra S., Bédard E.

Disinfection procedures in large building water systems can promote a viable but non culturable (VBNC) state of Legionella pneumophila (Lp) undetected by culture. Environmental strains in premise plumbing are likely to develop resistance over time to stressors from treatment. The main objective was to evaluate the impact of copper, silver, chlorine and temperature on the viability and culturability of various Lp strains using viability flow cytometry, culture and qPCR. 1 / 2 inserimenti A total of 13 strains, 3 reference, 2 clinical, and 8 environmental (sg.1&5) isolated from hot water systems (biofilm &water) and cooling towers were studied. After starving, strains were exposed to Cu (300 to 5000µg/L), Cu/Ag ions (300/30 to 800/80µg/L), chlorine (3 & 20ppm) and temperatures (45 to 70°C). Lp was monitored over 1 week of contact time by culture (AFNORNFT90-431), flow cytometry (Thiazol Orange™ & PI) and qPCR. Intracellular multiplication after treatment was evaluated in Acanthamoeba castellanii. The exposure (dose/temperature X contact time) leading to V varied considerably depending on the strain. For example, an exposure of >3d at 5000 µgCu2+/L were required to inhibit culturability of the biofilmisolated strain vs. 6 hours for the water-isolated strain. For chlorine, a gradual shift to VBNC was observed and the ability to recover viability and growth after 1-week was inversely correlated with the dose, showing the benefits of intensive shock chlorination. Only the most stringent stressor conditions prevented regrowth. Findings show that monitoring Lp in building water systems by culture may underestimate their presence, especially when subjected to stressors such as copper, silver or high temperature. These may induce a VBNC state, with a potential to regain infectivity following internalization or suppression of stress conditions. Treatment conditions should be set to ensure the elimination of VBNCs to lower the potential exposure when more favorable growth conditions are present.

Bacterias From *Legionella* Group In Thermal Water Used In Swimming Pools

Authors Mika A., Kmiecik E., Wątor K.

There is known about 57 species of Legionella. Some of them are considered as potential human pathogens. These bacteria can cause Legionnaire disease (LD) – hard pneumonia with the very high mortality rate 10-15%. Other, milder form of Legionella's infection is Pontiac fever. The main cause of infection is an inhalation of the aerosol infected by the bacteria. Legionella occurs in natural and artificial water environment. The preliminary research were performed. The main purpose was verification of bacteria from Legionella group occurrence in thermal water used in the swimming pools and the assessment of the influence of way of usage of water on the obtained results. Methods In this experiment, samples

Evaluation of Laboratory kits for *Legionella* detection by Real Time PCR

Authors Mucci N., Cianfanelli C., Braconcini M., Gianfranceschi G., Santucci S., Valeriani F.

Introduction Environmental monitoring and research development are fundamental for prevention and control of Legionellosis. New advances in molecular methods such as Real Time PCR (RTPCR) have opened new perspectives to detect potential sources of infection and to check the effectiveness of corrective actions. Recent regulations and guidelines suggest innovative measures and tools, mainly biomolecular, for surveillance of Legionellosis, In order to define the essential steps for the integration of these methods in monitoring programs, commercial kits for Legionella quantitative identification were tested. 1 / 2 inserimenti Materials and methods. Different RT-PCR detection kits (n=4) were compared by standard laboratory strains (L. pneumophyla sg 1, sg 10, sg 14 and L. spp.). The RT-PCR assays were performed using two different instruments (ABI 7000-Applied Biosystem and CFX96-Biorad). The key points used to evaluate the performance of the different kits were: sampling procedures, DNA extraction, quantitative amplification, interpretation of results. In addition, the evaluation in field was performed on environmental water samples collected from residential buildings in the area of Rome. Detection of Legionella was carried out using both methods, the traditional ISO 11731:1998 and the ISO/TS12869:2012. Results All the tested kits showed efficacy and considerable concordance between the different systems in every key point, suggesting a reasonable applicability to environmental monitoring. Compared to the traditional culture methods, the molecular RT-PCR test presented a significant reduction of running times (from 10-15 days to 1-2). Some critic points emerged, such as the impossibility to recover the isolated strains for further typing studies and the inability to discriminate the serogroups. Conclusions Despite some limits, RT-PCR procedures are extremely applicable and useful when a fast response is required, such as in risk and management assessment, community case studies or environmental monitoring after a remediation.

Air-bubbles, a “green” new alternative for biofilm removal

Authors Navalón P., Martínez J., Yáñez M. A., Creeswijk S., Catalán V.

Background The formation of a biofilm on the surface of pipes is generally believed to be responsible for the deterioration of microbial water quality and the onset of disease via pathogen release. Legionella spp is one opportunistic pathogen that can be growth together with other 1 / 2 inserimenti microorganism to form biofilms and can be a concern in the safety of tap water. National regulations usually propose disinfection treatments based on overheating and/ or chlorination, although there is no scientific evidence of the long-term effectiveness of these treatments on biofilm and Legionella removal. This study evaluates Air Bubbles, a patented technology designed for biofilm and Legionella removal, against the traditional methods used for disinfection. This system employs overheating water (80 °C), followed by a treatment with Air Bubbles that generates sudden local oscillations in pressure and temperature. Materials and Methods To validate the Air bubble technology, a pilot pipe system was constructed. Legionella pneumophila and Escherichia coli were grown in a hot water pilot plant with copper (Cu) and polypropylene (PP) pipes. Tap water at 25-35 °C was recirculated 13 min/day for 75 days. Once the formation of the biofilm was verified, conventional and Air bubble treatments were performed in both pipes and 5 cm2 surface sample were collected from each pipe after the treatment (following the ISO 18593:2004). The analyzed parameters were Total Viable Counts (TVCs) at 37°C (ISO6222:1999) and Legionella spp. (ISO11731:2007). Results The chlorination efficiency was 34.6% for copper pipe and 72.8% for PP pipe for Legionella counts, and in the case of TVCs, the efficiency was 98.1% and 99.7% respectively. By contrast, the Air Bubbles efficiency was 73.3 % in cooper and 100 % in PP for Legionella spp and 99.9 % and 100 % respectively for TVCs. Conclusions According to the obtained results, Air Bubbles Technology could be used as a “green” alternative method for the removal of biofilms and Legionella spp. that uses less chemicals than conventional disinfection processes and showing higher efficiency rates to biofilm and Legionella spp. removal. Moreover, this patented technology has also been tested in real facilities with excellent results in Netherlands, Germany and Spain.

Hospital-acquired *legionella* infection: addressing new challenges and critical issues in the application of the Water Safety Plan.

Authors Pierobon A., Lorenzoni M., Berti C., Rosato L., Baldovin T.

In accordance with the national guidelines for the prevention and control of Legionnaires' disease, we report our experience with the application of a plan for the prevention and risk management of hospital-acquired legionellosis in two acute care hospitals in northeastern Italy. METHODS: A mapping of all the water samples was carried out between 2010 and the beginning of 2017 both in clinical and technical areas. The departments were 1 / 2 inserimenti divided according to the risk associated with clinical procedures, the setting where patients have a higher risk of contracting the disease with a fatal outcome and the areas with a previous history of contamination. Technical staff carefully assessed the environmental risk, concurrently mapping the water distribution systems. RESULTS: A multidisciplinary team developed the Water Safety Plan which integrates clinical and environmental risk assessment, including the workers' one, with a plan of periodic environmental sampling and a risk management plan. The most critical points were birthing tanks, surgical sinks in the operating rooms, dental units and areas with immunosuppressed patients. Different control measures to prevent contaminations have been implemented, depending on criticality. Point-of-use filters have been applied in areas with high risk patients and filters are also being evaluated for birthing tanks. Pipes and taps of surgical sinks were replaced in the operating rooms. A weekly disinfection with 1,41% hydrogen peroxide was applied to dental units. In general, water temperature, flushing and residual disinfectant concentrations of chlorine based biocides were used on a regular basis and were subjected to regular controls. CONCLUSIONS: Patient characteristics, clinical procedures and the quality of water system are the main risk factors for hospital-acquired Legionella infections. A Water Safety Plan along with a locally adapted approach should be applied to manage the risk of Legionella infections.

Does storage time of samples influence the recovery of *Legionella* in waters? A study in real environmental water samples.

Authors Poznanski E., Romanin E., Freguglia M., Stenico A.

Microbial analysis of water samples for detecting Legionella in sensible structures as hospitals and retirement homes is critical in managing Legionella risk, as foreseen in the Italian guidelines published in 2015, which recommend not exceeding 4 days of storage of water samples before analysis. Aim of this work was to evaluate the effect of prolonged storage times on the recovery of Legionella in real water samples collected in buildings where the presence of these pathogens is critical. Methods Thirty-two 10-L samples of hot water were collected from eight different structures critical for presence of environmental Legionella. Each of the original samples was split into 2 subsamples, stored according to the guidelines and plated after 1, 2, 3, 4 and 7 days on culture media for microbial analysis. Plating and incubation followed according to ISO 11731:1998 (by concentration) and ISO 11731-2:2004 (by membrane filtration), both methods are accredited by Accredia. 1L of the second subsample was subjected to molecular analysis (qPCR) for the detection of L. spp. and L. pneumophila. Results A total of 160 subsamples were analysed along the whole holding time, leading to 640 plate counts and 320 real-time PCR analyses. Count results were not always of easy interpretation, because no clear trend was shown, being the data along the storage period only in a small percentage 1 / 2 inserimenti concordant with data produced plating 24h after sampling. The molecular analysis revealed the presence of Legionella even in samples where the classical method showed their absence. Conclusion The holding time seems to affect the recovery of Legionella from samples stored according to the guidelines: microbial counts of samples analysed 24h after sampling were in concordance with those of the samples analysed after 48h the in 34% of the cases, in 26% after 72h and only in 15% after 96h and 7 days. It is therefore of big importance to analyse the samples within the 24h after sampling.

Legionella in waterworks: effectiveness evaluation of an innovative method of prevention and control

Authors Rama A., Boschetto G., Brioni A., Bertoncello C., Baldovin T.

Background Aerosol inhalation containing a quantity of 10^3 Legionella by susceptible subjects increases the risk of contracting a severe type of pneumonia, called “Legionnaires’ disease” or “Legionellosis”.

Materials/Methods The implemented “Aclorbosa” method consists in two main phases: the first step is the biofilm removal, which allows to clean the water systems followed by a disinfection phase, obtained by an articulated procedure of improved hyperchlorination shock. To evaluate the effectiveness it has been used this formula: ?%= (number of positive points pretreatment)-(number of positive points post-treatment)/(number of positive points pre-treatment). The results have been compared with prevention and control methods utilized in different tourist accommodations.

RESULTS Structure 1, built in 2012, has never been reported for cases of Legionella related to travellers. In 2015 the analysis of water samples resulted positive to Legionella spp. 10^3 UFC/L concentrations. The disinfection made utilizing “Aclorbosa” method showed that the positiveness was eliminated with 100% effectiveness. Structure 2, situated in an ancient building, in 2007 was indicated by Ministry of Health as a possible source of cases of legionella. From September 2007 to February 2009 the analysis of water samples resulted positive to Legionella spp. 10^3 UFC/L concentrations. In June 2009 was made the drainage and disinfection of the water system using the “Aclorbosa” method and the positiveness was eliminated with 100% effectiveness.

CONCLUSIONS The results obtained from the survey of the different installations in the examined buildings, showed the effectiveness of methods “Aclorbosa” as a procedure to remove factors that reduce time effectiveness of traditional methods to prevent and control the risk of Legionella. Moreover, the procedure of improved hyperchlorination shock, resulted more effective than the traditional ones.

Surveillance and containment of *Legionella pneumophila* in water plumbing systems of the Hospital of Padua, Italy

Authors Richter S. N., Sciro M., Vanuzzo M. C., Narne E., Rossi L., Palù G.

Legionella pneumophila has been detected in water plumbing systems, possibly due to its ability to form biofilm and to grow in phagocytic amoeba that could provide insulation from high concentrations of disinfectants. The presence of Legionella pneumophila in plumbing systems of hospitals is of particular concern for the presence of immunocompromised patients. 1 / 2 inserimenti Materials/Methods Detection of Legionella pneumophila according to the guidelines of the document 04/04/2000 of the Official Gazette 103, attachments 2 and 3, and genotypic characterization by the Legionella latex test of Oxoid Diagnostic reagents. The presence and genotype of Legionella pneumophila has been followed at the Hospital of Padua from 2013 to 2016. Results Between 2013 and 2016 we have tested 200-320 samples per year. Samples were taken from showerheads and taps of all hospital units. In 2013, 33% of samples were positive. Plumbing system restoration works have steadily decreased the presence of positive samples to reach 19% in 2016. In addition, while in 2013 94% of Legionella pneumophila belonged to serotypes 2-14, in 2016 46% were serotypes 2-14 and 44% serotype 1. Conclusions We have shown that the surveillance coupled to containment measures have greatly decreased the presence of Legionella pneumophila in water plumbing systems of the Hospital of Padua.

Validation and effectiveness of a rapid method for Legionella detection (legipid®) in Health Centers.

Authors Saa-Casal A., Muñoz-Miguel J., Salvador-Aguilá M., Calvo-Valencia M., Ortega-Llavador B., González Steinbauer C., Ortí-Lucas R. M.

Legionella detection in sanitary water precedes both isolated cases and Legionella outbreaks and requires a fast preventive approach. Although culture methods are considered the method of choice, it remains a slow process with the consequent delay in results which generates uncertainty in decision making. The objective of this study is to analyze the validity and effectiveness of legipid® for the detection of Legionella in hospital water systems. Materials/Methods: 58 water samples were analyzed using Legipid® and 87 using a traditional culture method. Samples were gathered by 3 independent laboratories from 29 sample points in the hospital 1 / 2 inserimenti water system. Samples were considered positive when CFU count exceeded 100 colonies. In order to evaluate the test, false positives (%FP) and negatives (%FN), sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV) were calculated. Legipid® was compared to 3 different Gold Standards: IGS: Individual culture, CGS: A combination of 3 cultures from the sample point and AGS: a gold standard reached through consensus among 3 microbiologists considering culture results, Legipid® and PCR. Results: 19.5% of cultures (20.8% in sanitary hot water) were positive for Legionella. Prevalence reached 31% with combined cultures and 41,4% with the AGS. When compared to the IGS, Legipid® presented Se= 71.4%, Sp= 50% and FP= 38%. Due to the variability among laboratories, comparison with the CGS was prioritized. Thus, although specificity was lost (Sp= 52.9% vs. 100%) and the NPV were similar (69,2% vs 71,8%), Legipid® was more sensitive (44% vs 66,7%) and presented less FN (14% vs 21%). Conclusion: Variability of the results and the diagnosis delay questions culture methods as the technique of choice. Although more studies are required, higher sensitivity and availability of results in 3 hours make Legipid® an alternative, at least for screening purposes, to be considered by Preventive Medicine units.

A successful experience in controlling hospital acquired *Legionella* infections: a 6 year experience in a University Hospital in North Italy

Authors Cutti S., Muzzi A., Corbella M., Lodola L., Monzillo E., Lanave M., Bonadeo E., Marena C.

San Matteo Hospital of Pavia is an acute care university hospital, comprising 27 historic buildings, a new 60,000 m2 facility (2013) and another facility far from the main hospital (over 200,000 m2 , with more than 4.5 Km of waterline systems). Since 2000, different disinfection methods have been adopted to reduce and prevent contamination of Legionella pneumophila (Lp), biofilms and other waterborne microorganisms: hyperchlorination and thermal shock (2000-2005; A), copper silver ionization (2005-Aug 2010; B) and chlorine dioxide (ClO2) (Sept 2010-today; C). The objective of this study is to describe our positive experience of achieving zero nosocomial cases during the last six years using the ClO2 method. Methods In August 2010, we organized a multidisciplinary group to manage Lp surveillance and employed an integrated disinfection-filtration ClO2 system to reduce Lp water contamination: an 1 / 2 inserimenti oxidizing disinfectant agent, produced in situ, able to reduce biofilm without causing organic compounds, and which minimizes pipe corrosion. According to multidisciplinary group, we performed 15 water samples/month and implemented our clinical surveillance using Legionella urinary antigen. Results Only 3 cases of hospital-acquired Legionellosis occurred in period C, compared to 12 cases recorded using other disinfection methods (A-B period). Since August 2011, no more hospitalacquired cases have been reported. In period C, 1,111 samples were collected: 217 (19.5%) ?100 UFC/L and 129 (11.61%) ?1,000 UFC/L. In 80.65% of contaminated samples serogroups 2-14 were isolated. ROC curves showed that a concentration of 0.17 ClO2 ppm was effective in maintaining Lp growth 1,000 UC/L (AUC 0.77, IC 95% 0.72-0.82). Conclusions Our data show that a multidisciplinary approach to Lp surveillance management and use of ClO2 water disinfection, is more effective than other conventional treatments in reducing water colonization and preventing new hospital-acquired legionella infections.

ISO methods may significantly underestimate *Legionella* concentration

Authors Shelton B. G., Flanders W. D., Sauerborn Klobučar R.

The ISO (International Organization for Standardization) Standard for Legionella detection from environmental water samples, specifically ISO 11731:1998 (a three plate method often used for non-potable water) and ISO 11731-2: 2004 (a single plate method often used for potable water where low Legionella and other contaminant bacterial counts are assumed) are often dictated by health departments as the method for microbiology laboratories to follow for detecting Legionella in environmental samples. To assess the reliability of these methods which use relatively few plates for Legionella analysis, we compared the sensitivity of the ISO methods compared to a more extensive method using 13 plates for every sample, each with additional treatments. This eliminates the need to assume whether Legionella counts or contaminants would be high or low in any particular sample before analysis. All samples were processed using one of two ISO methods (ISO 11731:1998 for non-potable samples, and ISO 11731-2:2004 for potable samples). The ISO methods are included as subsets of the extensive method which incorporates an additional 10 plates. 0 0 1 311 1779 PathCon Laboratories EU 14 4 2086 14.0 Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:”Table Normal”; mso-1 / 2 inserimenti tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:””; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-paramargin-right:0cm; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0cm; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minorlatin; mso-ansi-language:EN-US;} A total of 3204 samples were analyzed of which 283 (approximately 9%) were positive for Legionella, 74 non-potable samples, and 208 potable samples. Of the 283 positive samples, only 138 ISO samples yielded either the same result or concentrations within 1 log of the extensive method; 122 samples were positive only by the extensive method and were negative by ISO; and 72 samples using the ISO method were more than 1 log lower than the extensive method, while 23 ISO samples were more than two logs lower than the extensive method. These findings suggest that it might be misleading to rely on results of these ISO standards. However, perhaps the results should not be too surprising given how few plates and treatments are applied to a sample processed using the ISO methods. These results are particularly important given the number of public health agencies globally that use or specify the ISO methods as a requirement for accreditation.

Influence of water temperature on the growth of *Legionella* in real environmental samples: Surveillance in South Tyrol 2011-2016

Authors Stenico A., Seeber M., Romanin E., Prast A. M., Mupo M. R., Blasior P., Oberlechner A., Sigmund T., Ties N., Koch E., Poznanski E.

Surveillance of water samples for monitoring the presence of Legionella in sensible structures is critical in managing Legionella risk, as foreseen in the Italian guidelines published in 2015. The Biological Laboratory of APPA Bolzano is the reference laboratory for the Province, collects all results of the programmed and extraordinary controls in case of illness, as well as data of self-monitoring representing therefore a privileged observatory with a huge database of real data. We present here the results of data collected in the last 6 years (2011-2016) in ca. 400 structures spread in South Tyrol with focus on the temperature of water samples. Methods All samples are supposed to be accepted by the laboratory only if accompanied by supplementary information about kind, mode and date of last plant treatment, water temperature, notification of a case of illness. Samples are analysed by accredited ISO 11731. Results A total of 5784 samples were analysed in 6 years. 61% of the samples were Legionella-free and 10% contained 50-100 cfu/L, which are values below the attention threshold indicated in the Italian guidelines. In the remaining 29% of samples Legionella was found. The analysis of supplementary information showed that for most of the positive samples (65%) the temperature of water was in the range 20-50°C. Nevertheless, a high amount of positive samples (29%) were collected with temperatures >50°C. A different range of water temperature 1 / 2 inserimenti (25-55°C) comprehends the 85% of positive samples, while at T>55°C only 5% of samples were positive. Conclusion The guidelines indicate the temperature range 20-50°C as ideal for Legionella growth. We have shown that a not negligible amount of Legionella can be recovered even at higher temperatures. It should be therefore taken into account to change the temperatures proposed in the guidelines, according to UNI EN 806-2:2008 that recommend keeping cold water below 25°C and hot water above 55°C.

Comparison of chlorine dioxide and anolyte for a continuous hot water disinfection in a nursing home

Authors Totaro M., Casini B., Valentini P., Frendo L., Porretta A., Privitera G., Baggiani A.

Drinking water is a recognized source for infections and Legionella control is a critical issue in healthcare settings. Continuous disinfection is a control measure needs to be fine-tuned to obtain satisfactory results in individual hospitals over prolonged time periods. We compared the effect of anolyte and chlorine dioxide, applied in two different hot water networks of a nursing home to manage Legionella risk. Materials/Methods: Nursing home has two buildings (A with 39 beds; B with 42 beds), with the same point of aqueduct water entrance. Following a shock chlorination (50 mg/L;1 h), aimed to remove Legionella colonization, from June 2016 the continuous disinfections with chlorine dioxide (0,33±0,04 mg/L) and anolyte (0,23±0,04 mg/L) were applied in hot networks of building A and B, respectively. From each building hot water was sampled at central heating 1 / 2 inserimenti system (recirculation; boiler) and at two points of use as suggested by water safety plan. Legionella research (ISO11731:1998) was performed with a monthly basis while chemical tests of iron ions (Fe), manganese ions (Mn), zinc ions (Zn) and trihalomethanes (THM) were fulfilled with a quarterly monthly. Results: Before shock chlorination Legionella pneumophila sg1 was recovered in all buildings from 2x102 to 3,8x104CFU/L, while chemical compounds concentrations were within the limits provided by Directive 98/83/EC. After the application of the continuous disinfections, Legionella was not recovered in water samples and physical-chemical data were comparable between both buildings. From water samples treated with chlorine dioxide we obtained 22,8±3,6µg/L of Fe; 11,8±3,3µg/L of Mn; 106,4±12µg/L of Zn; 13,2±1,9µg/L of THM. From water samples treated with anolyte we detected 54±7,7µg/L of Fe; 1,7±0,3µg/L of Mn; 85,7±9,6µg/L of Zn; 1,7±0,9µg/L of THM. Conclusion: Both disinfectant appears effective against Legionella growth in water network, but anolyte ensure a lower disinfection byproducts release.

Contextualization of Italian National Guidelines for the prevention and control of Legionellosis in a large teaching hospital in Rome.

Authors Vincenti S., Boccia S., Berloco F., Cambieri A., Laurenti P.

In Italy the case-fatality for hospital-acquired cases of Legionnaires' disease is 44.2% in 2015. Considering this, it is fundamental to elaborate a safety plan for the prevention and control of Legionellosis in this settings. We elaborated an internal procedure based on the recently published Italian guidelines (GLs) on the prevention and control of Legionellosis. 1 / 2 inserimenti Materials/ Methods GLs have been contextualized in a large hospital with 1,500 patient beds, made up of several interconnected buildings. The hot water system is disinfected in continuous with chlorine dioxide. The internal procedure was developed using a triple approach: i) discussion of the latest GLs in a multidisciplinary context; ii) reviewing the literature on the effectiveness of the different corrective actions in case of Legionella spp positivity, using MEDLINE and ISI Web of Science databases; iii) data analysis of the historical surveillance system of the hospital. Results We identified 12 buildings, each one considered as a single hospital when applying the national GLs. We classified 7 wards as high risk (HR) that included Intensive Care Units and 17 augmented risk (AR) that included 10 other wards not explicitly mentioned in the GLs. We scheduled environmental sampling every 3 months in the HR wards and every 6 or 12 months in the AR ones, based both on historical data. We decided to collect a minimum of 6 samples (heaters, return loop, and at least 4 distal outlets) at each samplings. For contaminated samples we had foreseen many decontamination steps. Consequently, our program expects to analyse 60 samples from 7 HR ward, 60 samples from AR wards and 70 samples from heaters, yearly. Conclusion The development of the internal procedure for the control of Legionella spp contamination in a hospital setting has allowed to set up an accurate action plan which includes planned environmental monitoring and, in case of contaminated samples, the application "step by step" of specific actions.

Five-year follow-up of a multifaceted response to an outbreak of Legionnaire's Disease (LD) at a U.S. Veterans Affairs (VA) healthcare system

Authors Decker B. K., Harris P. L., Muder R. R., Sonel A. F., Clancy C. J.

Investigation of a 2011-12 LD outbreak involving 22 patients (pts) at VAPHS identified failure of copper-silver biocide, inconsistent communication between departments, construction, and non-systematic testing for Legionella as possible contributing factors to a control-system failure. A multifaceted response was initiated in Nov 2012. Our objective is to report 5-year clinical and environmental follow-up data. Methods. We retrospectively reviewed clinical, epidemiologic, and water system (WS) surveillance data from December 2012-present. Results. The institutional response consisted of switching from copper-silver to hyperchlorination WS treatment, and instituting multiple analyzer-chlorination units, daily random biocide level sampling, active monthly WS Legionella surveillance, zero-tolerance WS remediation, mandatory ID consults and Legionella testing for all pts with pneumonia, and a rigorous genomic epidemiology program. Post-outbreak, 21 cases of LD have been diagnosed, including 12 community and 9 possible healthcare-associated (See Figure 1). Etiologies 1 / 2 inserimenti included L. pneumophila (Lp) serogroups 1 (n=17) and 2-14 (n=4). No cases of definite hospital associated LD have been documented by classical or genomic epidemiology post-outbreak, and the outbreak-associated Lp subsp pascullei strain has not been recovered. WS positivity for Legionella has decreased from 15.5% during the outbreak period, to 1.8% post-outbreak. Conclusions. A rigorous, multi-faceted institutional Legionella surveillance and prevention program at a VA system in an endemic area ended an LD outbreak, eliminated subsequent nosocomial cases, and significantly reduced WS Legionella positivity. Results have been sustained for 5 years.

A Comprehensive System for the Prevention of Legionellosis in A Hospital

Authors Gimigliano M., Talarico F., Minchella P.

This article aims to demonstrate that a comprehensive prevention system is able to diminish risks of Legionella Pneumophila spreading and infection. Risk mapping was the first intervention, then control of critical points and, in particular, a plant of water treatment have been developed. Monitoring of possible bacterial contamination has been performed through laboratory analysis, as it is prescribed by ISS (Istituto Superiore di Sanità) guidelines. Site of entrance of cold water in the hospital was the first critical point to control by a disinfection treatment. Furthermore, maintenance has been improved providing for specific instructions to the maintenance companies. 1 / 2 inserimenti MATERIALS/METHODS Pugliese-Ciaccio is a general hospital with 450 beds. Firstly, a protocol for prevention of Legionellosis was adopted by the hospital. Water plant and distribution were analyzed in detail. Water has periodically been sampled in a series of specific control points along the distribution system. A UV lamp with a UVC probe and a chemical disinfection including H2O2 , Ag+ ions and an anti-corrosion product (based on phosphorus silicate) have been provided at the entrance of the water supply. Besides, a further UV lamp was installed in the point of return of warm water before entering the boilers. Finally, possible presence of Legionella had been monitored for six months. RESULTS The concentration of Legionella in the samples of water was always found below the threshold considered at risk. After some months of use of disinfection treatment, the concentration of Legionella was found significantly decreased along the entire water distribution system. CONCLUSION A combined approach using physical and chemical disinfection has achieved the result of reducing the likelihood of Legionella infection both by decreasing the preexisting microbial contamination and by impeding new contamination

Breakpoint Chlorination as Control of *Legionella* in Bath Water using flow cytometry

Authors Taguri T., Cai G., Ebisu-Ojim H., Amemura-Maekawa J., Kura F.

Controlling the risk of Legionella contamination (LC) in bath water (BW) by free chlorine disinfection is challenging because of some components in hot springs, and the presence of skin debris and/or biofilms. We previously developed a rapid detection method (RDM) using flow cytometry to monitor the risk of LC in BW (Taguri T. et al, J Microbiol Methods, 2011). In this study, we hypothesized and examined that RDM-tested cleaned BW satisfies the breakpoint (BP) on chlorine demand (CD). Materials/Methods Five types of BW samples including hot springs were used for testing. CD detected by N, N-diethylparaphenylenediamine (DPD) was performed following the standard method slightly modified. Three bacterial species were used in RDM spike testing in the same condition as the CD test using DPD. A cell count Powered by TCPDF (www.tcpdf.org) 1 /

Legionella control without *Legionella* testing is guessing

Authors Shelton B. G., Kirkland K. H., Flanders W. D.

Outbreaks and large numbers of sporadic cases of Legionnaires disease continue to be reported world-wide, underscoring the need to re-evaluate prevention recommendations. Although professional guidelines and industry generated standards have been published, there is little scientific peer-reviewed information on the beneficial effect of any of these various and differing approaches, which could explain the lack of any consistent international approach. In this study, we prospectively tracked Legionella colonization in a large number of cooling towers over time with ongoing treatment maintenance consistent with the ASHRAE 188 Standard. If 1 / 2 inserimenti Legionella growth was detected and counts were considered elevated, we determined the change in counts following the notification of the operator and disinfection shock treatment. Some cooling towers remained elevated and needed to be shock disinfected multiple times before Legionella counts were below the test's detection limit. Results of this study cast doubt on the ability to reliably control Legionella colonization without routine periodic feedback from Legionella culture results. Merely assuming routine or shock disinfection reliably eradicates Legionella can lead to a false sense of security.

Evaluation of *Legionella pneumophila* cellular viability and nucleic acids stability using copan srk^{tm} evironmental collection kit

Martinelli M., Mesumeci R., Calaresu E., Perdoni F., Baisotti V., Zanellato E., et al.

Dental unit waterlines: *Legionella* monitoring and disinfection.

Authors Franzin., Demarie V., Mussano P., Arrigoni C., Tealdi R., Mainardi G., Caldarola F., Prandi C., Tegani E., Romano C., Avanzini

Dental unit waterlines (DUW) contamination is an important potential source of cross-infection. Water quality is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from DUW. Aim of the study was to evaluate microbiological quality of DUW, Legionella colonization and disinfection procedures. 1 / 2 inserimenti Materials/Methods: 212 water samples were examined: 181 collected from cup filler, microengine, turbine, air-water syringe, ablator of 16 DU, 31 from washbasins nearby. Culture of Legionella, free-living Amoeba, heterotrophic bacteria, Pseudomonas, Coliforms and Escherichia coli, Enterococci was performed. After 1 L water filtration, Legionella was quantitative detected on BCYE, BMPA, MWY, directly and after acid and heat treatment. Quantitative detection of Legionella spp. and L. pneumophila was performed by real-time PCR. Amoeba was detected by MPN at 25°C and 37°C. Aerobic bacteria count (TVC) was performed by serial dilutions at 30°C. Temperature, free chlorine and pH were determined. Disinfection was performed in 12 instances (8 with H2O2 different concentrations, 4 with organic peroxides mix). Results: Legionella was positive in 32/154 (20.8%) DU water at 102 -104 cfu/L. Isolates were L. pneumophila 3, L. anisa and other slow-growing Legionella species. Legionella was positive by qPCR in 99.3% of samples, L. pneumophila in 49%. Amoeba was isolated from 25% of samples. TVC exceeded CDC threshold values (500 cfu/mL) in 32.4% of samples, P. aeruginosa was positive in 7.9%, the other bacteria were not found. Temperature was 18°C-35°C. H 2O2 was less efficient for disinfection than organic peroxides especially against slow-growing Legionella spp. Conclusion: Legionella detected by culture was underestimated as shown by PCR. As some DUWs showed high contamination, Legionella associated risk was reduced and water quality was improved by proper disinfection products that were efficient after strict protocol use.

Assessment of flow cytometry for monitoring microbial water quality monitoring in cooling tower water and oxidizing biocide treatment efficiency in cooling tower

Authors Helmi K., David F., Di Martino P., Ingrand V.

Legionella proliferation in cooling tower water circuits is monitored using plate culture method before and after disinfection has major weaknesses: time to result up to 10 days, and absence of detection of viable but not culturable bacteria. Thus, for operational monitoring, an alternative approach using flow cytometry was tested to monitor water contamination and disinfection treatment efficiency on bacterial cells. Material/Methods: FACSCanto™ II and Accuri™ C6 flow cytometric systems and fluorogenic dyes were used in order to assess nucleic acid injury (SYBR® Green II), cell integrity (propidium iodide) and metabolism activity (ChemChrome V6) through in vitro bacteria inactivation measurements in the presence of 3 different types of oxidizing biocides commonly used for cooling tower disinfection, including HOBr, NaClO and NaBr. Besides, a total of 27 cooling tower water samples were analyzed with usual methods (including ATP measurement) and flow cytometry in order to assess water contamination levels regarding viable population. 1 / 2 inserimenti Results: Flow cytometry and plate counts methods showed a significant correlation for changes in concentrations despite a 1 to 2-log difference regarding absolute quantification, namely concentration levels near to 6 and 4 log for active and culturable bacterial cells, respectively. Regarding physiological states after the biocidal treatment, flow cytometric analyzes highlighted cell membrane injury and impact on metabolism activity, confirming the loss of ability to cultivate. Conclusion: Flow cytometry enabled to obtain within 1 hour an accurate assessment related to quantification and accurate biocide impact on bacterial cells and thus appears as a powerful onfield tool for biocide treatment efficiency monitoring in cooling towers.

ATPmetry - a rapid microbial risk assessment tool for investigation in a suspect Legionnaires' case

Authors Neyrat L., Belotti L., Hernandez C., Foegle J., Deboscker S., Boulay C., Raymond M., Lavigne T.

A case of legionellosis has been diagnosed in a medical retiring home related to the Strasbourg University Hospital. The investigation carried out didn't permit to identify real exposure factors. Rapid correctives and curatives measures have to be done but takes one week to get results of Legionella by culture method (NF-T 90-431). The aim of this investigation is to evaluate a rapid microbial risk assessment based on ATP 2G in order to have a quick overview of all water systems as hot and cold domestic water systems, to identify critical points and allows immediate implementation of corrective with validation of the efficiency of treatment. Method In addition to Legionella culture method (NF-T 90-431), a biomass measure by using QGA TM kit (LuminUltra - canada) by ATP 2G has been carried out by aqua-tools. Results of the investigation -1st measurement campaign The investigation carried out on site showed that cold water network has a low level of ATP at 30 pg/ml comparing 0.7 pg for cold water. This increasing ATP level by 42 time higher in hot water demonstrates that the whole hot water 1 / 2 inserimenti systems was contaminated by total flora which indicates risk to have legionella proliferation. Corrective measures have been engaged: intensive flush in the building A, increasing hot water temperature > at 60°C. -2nd measurement campaign ATP results on cold water showed same level of microbial content .Level of ATP in hot water came back at the same level as cold water that indicates a clear improvement of hot water compared to the first campaign measurement. Daily flush and temperature were efficient in order to maintain a low level of circulating biofilm. Conclusion QGA kit based on ATP-metry second generation TM proved to be a cost-effective rapid method and real-time investigation tool that gave an orientation of corrective action.

Cultural and RT PCR method comparison: the Udine ARPA FVG Laboratory expertise

Authors Franchi M., Felice A., Pillinini D.

Background: The objective of this study is to compare "classical" cultural method and the PCR Real Time method, both applied to the Legionella pneumophila identification in drinking water. Those samples are taken from structure in which we had Legionnaires'disease.
The PCR technique was tested as complementary method according to the "Allegato 6 - Guidelines for Legionellosis prevention and control" 79/CSR 2015 (issued by "Istituto Superiore di Sanità").
Results from 2014-2016 sampling period has been used for comparison in the ARPA FVG Udine Lab, which is the Regional Reference Center for the Environmental Legionellosis Diagnosis. Both classical legislation compliant method and the qualitative PCR Real time have been applied. A correlation between the results of the two methods has been evaluated to demonstrate CFU and GU equivalence.

Materials/Methods: The Udine Laboratory is certified ISO 17025:2005 for Legionella pneumophila identification in drinking water samples and the ISO 11731:2008 " Water quality enumeration of Legionella." material and methods have been used throughout this work.
The PCR Real time method is reported in the guideline "Allegato 6, 79/CSR/2015". The method consisting in different phases: sample concentration by filtration, DNA extraction, DNA purification and amplification by qualitative PCR Real Time.
Quantification of DNA has been made according to ISO/TS 12869 : 2012 "Water quality- Detection and quantification of L. peumophila by concentration and amplification by qPCR".
Correlation was checked by means of linear regression computed positive results expressed in CFU/1000 ml e GU/1000 ml units from 16 samples.

Results: In the whole period 2014 -2016, 308 samples have been analyzed applying the two methods. (46 samples in 2014, 159 samples in 2015 and 106 samples in 2016). The Legionella pneumophila was found to be positive for 25% by cultural method and 42% by PCR method for all samples.
Details for year 2014 are positive samples 25 % (Cultural method) and 39% (PCR method),year 2015, 32 % (Cultural method) and 41% (PCR method) , year 2016,16 % (Cultural method) and 46% (PCR method). Correlation coefficient r is 0,52 .

Conclusion: The results confirmed that PCR is more sensitive and specific than the conventional method for the L.pn. detection, but the PCR detects living and dead cells. This techniques can be used for preventive screening and complementary method because permit to analyze a significant numbers of samples in a short time.
The correlation analysis did not reveal any significant relationship between the two methods, as reported in others studies.

Example of management of two groups of dental units in the water safety plan for hospitalacquired *Legionella* prevention

Authors Lorenzoni M., Pierobon A., Rosato L., Berti C.

The national guidelines for the prevention of legionellosis highlight the importance of control measures to reduce the possible contamination in dental units. METHODS: A technical and health check was carried out of dental units that were installed in two hospitals of northeast Italy. There was 17 dental units in one hospital (group A) and 6 in the other one (group B). Of each dental unit, water supply, ordinary and extraordinary sanitation were evaluated. Since no environmental control had ever been carried out, water sampling was conducted in all dental units. Positive results were found 1 / 2 inserimenti in both groups and different technical works and disinfection interventions were performed in the two groups until sample negativization. RESULTS: All dental units were feeding from city water network; group A was monthly sanitized with 1.41% hydrogen peroxide, while in group B a disinfectant based on sodium percarbonate, silver nitrate and cationic surfactants was routinely used. In group A, water dispensing points of cuspidor were positive for Legionella pneumophila serogroup 1 in two dental units; in one of these, also the delivery unit was positive. In group B, delivery units were positive for Legionella pneumophila serogroup 2-15 in two dental units. The subsequent extraordinary disinfection took place with 1% poly-esamethyl-biguanide chlorohydrate and 5% 2-bromo-2-nitro-1,3-propane in group A; a 0.05-micron filter was also placed at the water entry point. In group B, extraordinary disinfection was performed with 4% hydrogen peroxide. Positive points in group B returned negative after the first chemical disinfection; in group A, a second disinfection with 4% hydrogen peroxide was necessary. CONCLUSIONS: It was considered necessary to implement disinfection of all dental units weekly with 1,41% hydrogen peroxide. Since the dental units in group B are equipped with a water tank, the use of deionized and osmotized water instead of water network is under evaluation.

Genotyping approach on environmental monitoring of *Legionella spp*. in a Hospital hot water network

Authors Mancini B., Iervolino M., Pellati T., Cristino S.

Legionella isolation was performed by cultural method, but different molecular techniques were implemented to identify Legionella strains in order to undertake the epidemiological investigation and establish the link with the source of infection. Our study was focused on a genotypic approach to evaluate Legionella contamination of a hospital hot water network (HWN) in association with the ISO11731-1998. The phylogenetic relationship between strains permitted to elaborate an environmental risk map. Materials/Methods: From October 2013 to July 2016, 547 hot water samples were analyzed to detect and enumerate Legionella in a HWN treated by a new disinfectant (WTP828) based on hydrogen peroxide (H2O2) + silver salts (Ag+). Isolates were serotyped using agglutination test and genotyped by mip gene sequencing and Sequence-Based Typing (SBT). Phylogenetic analysis was performed on the Phylogeny.fr platform and the tree was reconstructed by the maximum likelihood method. Results: Culture method permitted to identify 37% of positive samples belonging to L. pneumophila SG1 (60%) and L. species (40%). SBT assigned ST1 and ST104 to 123 samples of L. pneumophila. The mip gene sequencing identified inner L. species, L. anisa and L. rubrilucens in 82 positive samples. Phylogenetic analysis performed on strains of L. rubrilucens 1 / 2 inserimenti and L. anisa confirmed the presence of two separate clusters. The 100% of homology in 7 genes investigated in L. pneumophila strains did not permit to elaborate phylogenetic correlation. Conclusion: The genotypic approach used during environmental monitoring in a HWN under disinfection treatment might support the study of Legionella colonization. The elaboration of a phylogenetic map of isolates can help to correlate strains in response to the disinfection treatment. These knowledges might support the Health Authority to rapidly undertake the epidemiological investigation and preventives measures.

Industrial cooling tower, treatment and control measures to prevent *Legionella spp.*

Authors Iervolino M., Mancini B., Cristino S.

Contamination of industrial cooling towers has been identified as the cause of sporadic cases and outbreaks of legionellosis. The epidemiological data showed as the risk of Legionella infection coming from cooling tower is underestimated. The aim of the study was to assess the effectiveness of different disinfection treatments on Legionella colonization, performed within an industrial Cooling Tower System (CTS) starting from opening to closing season. Materials/Methods: Cold water samples were collected monthly from May to October 2016, during an environmental monitoring of industrial CTS nearby Bologna, Italy. Legionella culture was carried out according to ISO11731-1998. Two disinfection treatments were subsequently performed in relation to microbiological levels found. The first performed for 7 days based on hydrogen peroxide (H2O2)/silver salts (Ag+) at shock concentration of 100 mg L-1 in synergic association to an antialgal treatment and the second performed by sodium hypochlorite at 50 mg L-1 for 15 days. 1 / 2 inserimenti Results: Our data show the presence of L. pneumophila SG8 at a level of 5,06 log10 UFC L -1 after the water CTS filling, that required a shock treatment performed for one week by H2O2 /Ag+ . This treatment increases a Legionella contamination level until 6,14 log10 UFC L-1 . These data required a stop of CTS activity, washing of circuit by peracetic acid and two subsequently shock hyperchlorination. After 15 days we observed a decrease of Legionella level to 1,90 log10 UFC L-1. maintained for all tested period. Conclusions: Our data confirm the role of routinely environmental monitoring before and during CTS operating conditions. The introduction of preliminary procedures before the disinfection treatment as anti-scale treatment, washing of CTS, measures of physical-chemical parameters and control of microbiological contaminants, permit a good performance of CTS, reducing the risk for the stakeholders and public health.

Legionellosis in Campania: report of the year 2016 and fifteen years of activity of Legionella Reference Laboratory

Authors Rossi A. M., Di Leo F., Lucibello T., Pagano M., Coppola A., Petrosino A., Siani M., Lambiase E., Frusciante M.

Since 2001 Regional Agency Environmental Protection (ARPAC) is responsible for environmental surveillance for legionnaire's disease (L.D.) in Campania, by means the Regional Reference Laboratory for Legionella (LRL), integrate the National Surveillance Programma. Methods: In 2016, 88 cases notified of L.D. were correlated to Campania Region: 38 were travelassociated, of which 29 notified by ELDSNet, 16 hospitals, 1 offices. A total of 1456 samples 1 / 2 inserimenti (air, water, biofilm) were taken from several sites (tap, shower, swimming-pools, thermal springs, climatisation system and other). Legionella was detected by culture methods (ISO 11731:1998, 11731-2:2004). Results: 12/36 hotels resulted contaminated by Legionella spp. with 67% of positive samples showing Legionella pneumophila sg1 and in lower concentration appeared other strains: 4% Lp 10, 8% Lp6, 3% Lp3, 10% Lp8 and 8% Legionella non pneumophila. 4/10 health-care facilities were positive with 55% of positive samples showing Lp1, 45% Lp10. 1/15 workplaces showed presence of Legionella with 100% Lp1 in positive samples. Private houses no showed presence of Legionella pneumophila. Conclusion: In past 15 years in Campania the clinical diagnosis of L.D. has largely increased. In the mean time special efforts has had legionella enviromental surveillance by means synergistic action of Health - Enviromental Authorities. Nevertheless, our results supports the need to improve preventive measures to reduce building contamination and, therefore, to better manage the risk.

International return of experience of the application of a comprehensive *Legionella* Risk Management scheme on cooling towers and domestic hot water systems in health care facilities

Authors La Mura S., Hercule-Bobroff S., Mathiot J. M.

Veolia operates more than 2.000 cooling towers circuits and domestic water systems in health care facilities over 35 countries. Effective management of Legionella risk has been a priority issue for several decades. Veolia has thus implemented a comprehensive scheme to assess and manage Legionella in those water systems, from risk analysis to the regular review in a continuous improvement process, in line with WHO recommendations. The regular update of this risk prevention plan is performed by a working group of internal experts from different geographies (France, Italy, Benelux, Spain, USA, China and United Arab Emirates) and business activities. 1 / 2 inserimenti Material and methods: To implement this risk management scheme, comprehensive developments have been performed over the years: · A range of treatment products and services for effective control of Legionella. · Best practices on design, operation, maintenance and crisis management · Risk assessment and audit grids to verify that mandatory rules are in place to secure the system · Training material for staff and contractors. R&D studies have been carried out to support this prevention plan: improvement of disinfection, hydraulics, corrosion control and surveillance (e.g. PCR development for Legionella and amoeba). Finally, key performance indicators are set to verify the efficiency of this risk management scheme. Results: This framework is successfully applied worldwide on sites operated by Veolia, taking into account the local regulation. From 2004 to 2015, the percentage of compliant facilities to this framework has increased of +50% and +150 % for hot water and cooling systems, respectively. From the first years of its implementation, this prevention plan has shown its efficiency with a decrease in the percentage of alert threshold exceeding of -200% in 5 years. A case-study presenting its application in Italy will be detailed in the presentation. Conclusion: As this voluntary approach has been strengthened and systematized, the number of alerts has decreased on sites operated by Veolia. The application of such risk management scheme thus ensures the prevention of occupational and health risks, enhances facility safety, and contributes to the reduction of non-compliances and health crisis.

Assessment of new terminal antibacteric filters sbs model wf series for water network applying to network points of sanitary structures for prevention of legionellosis

Authors Sensoli E., Mariotti E.

Normal 0 14 false false false IT JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:”Tabella normale”; mso-tstylerowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; msostyle-parent:””; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-marginbottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria;} The research is aimed to evaluate the efficacy and efficiency of the application of new models of triple-layered antibacterial filters (internal pre-filter: 1 m; membrane: 0.2 m and 0.1 m), plastic “second Life” with bacteriostatic additive to protect retrograde contamination, located at the 1 / 2 inserimenti water network terminal points in the New Hospital of Pistoia. Results were studied over a 62 days period of use. Introduction The bacterial contamination of the water network is one of the possible causes of infections not easy to solve. The water treatment issue and dispensing is particularly complex, even for different standards regarding the use of materials and products with less environmental impact (GPP). Antibacterial terminal filters prevent infections caused by microorganisms, primarily Legionella, as recommended in the Ministerial Guidelines. Materials and methods The collection of data was took place in 5 water points. During the 3 sampling sessions (time -15 days, -7 days and 0), a water test (M.U. 1037: 14) and a Tampon Test on Surface (ISO 11731: 1998) were performed. After the installation of filters, the samples were collected at time 0, 15, 31, 45, 62, 75. At last, a sample (water and tampon) was collected without a control filter. Results During the observation period, the efficacy of the antibacterial filtration of the devices was demonstrated and no significant deviation was found about the pre-filter contamination before and after the filter’s positioning. Conclusion The data collected confirms that the method is effective for application in healthcare facilities in compliance with current regulations, including the use of eco-compatible materials. The microbiological analysis confirms the maintenance of declared performance (filter efficacy for 62 days).

Poseidon Project: Photonics against *Legionella*

Authors Pierobon R., Bellò B., Vicini I., Rossi G.

Background:

The detection of Legionella pneumophila is actually relied on time-consuming protocols based on in-vitro selective bacteria culture methods needing specialized personnel in laboratory. Recognition and investigation of viruses, bacteria and eukaryotic cells is nowadays becoming a field in Surface Plasmon Resonance biosensing (SPR) , but it was only achieved in lab settings. POSEIDON Project (www.poseidonproject.eu) targets to change the approach in bacteriological environmental monitoring and in infection risk management, improving knowledge towards real applications.

Materials and Methods:

Several custom components have been designed and integrated from the handling of the air/water sample in preconditioning unit to the delivery in a microfluidic device through which whole bacteria cells are transported to the sensing plasmonic surface. Sensors based on Grating Coupled SPR in azimuthally rotating configuration have been used for detection of pathogens. Specificity has been ensured by immuno-based functionalization of grating surfaces and system sensitivity has been granted by the optimization of the optical detection system architecture.

Results:

A prototype system has been designed: several units have been realized and already tested, others are currently under test. Procedure of water sampling and preconditioning has been validated comparing with culture results (ISO 11731). Air samples have been checked with reference to a commercial microbiological impactor. Status of the project activities and progression of findings will be presented.

Conclusions:

An innovative sensing device architecture is under test to yield reliable measurement readouts of pathogenic presence. The prototype implements water and air sampling, sequential concentration, injection into microfluidic system and delivery to the SPR sensor for analysis. The system is designed for its future integration in HVAC plants for prevention of L. pneumophila outbreaks.

Legionella detection in Jordan: preliminary data on accommodation sites and water systems

Authors Said R., Laboratories Directorate, Water Authority, Amman-Jordan

Background Legionnaire's diseases are still not a notified disease in Jordan. However guidelines have been published, indicating action level should be implemented in a water system when 1000CFU/L of Legionella is detected. Following the concern of the World Health Organization (WHO) about worldwide increasing incidence of this severe disease the Jordanian Ministry of Health (MOH) has increased the attention on the capacity of Jordanian laboratories in Legionella testing. To this aim, the Water Authority of Jordan (WAJ) has implemented Legionella detection and enumeration in environmental samples according to APHA international method. In the last five years37 samples were collected and analyzed, 25 cases have been associated to hotels, 8 samples from accommodation sites (building water systems) and 4 samples from water systems.

Results Among the 37 samples analyzed, we found 8 positive and 29 negative samples for Legionella detection. A concentration of Legionella pneumophila Serogroup1ranged from 2200-3700CFU/L for building water systems, which was found in 8% of samples, whereas 660-4666 CFU/L Legionella species which was found in 6% of samples and 620-20600 CFU/L Legionella species(2-14) were detected in hotels and contributed 8% of samples.
This method is validated through Applying the application of quality assurance and validation requirements, where their results were acceptable, therefore validation and quality control samples have proved that the method is fit for the purpose.

Conclusions Awareness should be implemented in prevention and control of legionella contamination of building water systems (hotels, hospitals, nursery homes, etc) and cooling towers and evaporative condensing water systems, in order to control the occurrence of legionnaire's disease cases.

Quantification Of *Legionella* In Sanitary Water Samples By A Qpcr Method

Authors Ceppetelli V., Omiccioli E., Amagliani G., Grottoli A., Barbadoro P., Ponzio E., Napolitano L., Savini S, Magnani M., Brandi G., D'Errico M.

Background: In 2014, about 7,000 cases of Legionnaires' disease were reported in the European Union and the number of unreported infections is probably much higher. Hospitals are the most likely places to contract the disease for immunocompromised patients and the elderly. Monitoring programs must be conducted to assess the infectious risks in sanitary water networks. Preventive measures include the surveillance and control of water networks and the improvement of the techniques for Legionella detection. Indeed, drawbacks of culture method can have great impact on the results, which can lead to underestimation of the risk. qPCR offers sensitive and specific detection of Legionella, especially in high risk units where the application of timely measures is needed. The aim of this study was to evaluate the efficacy of a molecular method to monitor the presence of Legionella in sanitary water samples.

Methods: Hot and cold sanitary water samples were collected from cooling towers and substations from the Ospedali Riuniti of Ancona, Italy. The samples were artificially inoculated with 100,000 GU/sample of L. pneumophila ATCC33152 and the robustness of the method was evaluated according to ISO/TS 12869. Briefly, after sample filtration DNA was extracted from filter and quantified with "New Legionella spp. Quantitative kit" (Diatheva, Italy).

Results: Results were expressed as recovery rates, calculated as percentages of Legionella GU in the spiked samples respect to the contaminating suspension. Samples from the cooling towers showed values ranging from 33 to 19% (average 29±6.7%). Samples of the substations showed a recovery from 27 to 35% (average 29.5±3.7%).

Conclusion: Preliminary results showed recovery rates >25% in almost all samples in compliance with the ISO/TS 12869. The method is suitable for the tested water samples in which high concentrations of inhibitory substances may be expected. Further improvement will include a viability PCR protocol for live/dead cells differentiation.

Frequency of free-living amoebae in Italian man-made water environments

Authors Briancesco R., Bonadonna L.

Background: Free living amoebae (FLA) can support the growth, survival, virulence and dissemination of several waterborne pathogens. Numerous bacteria, including Mycobacterium and Pseudomonas and especially Legionella, survive inside free-living protozoa and establish an endosymbiontic or a parasitic relationship with these hosts. For these reasons these protozoa represent a primary mechanism for survival and proliferation of pathogenic amoebic resistant microorganisms in water systems. For their ubiquity in the environment, FLA are easily present into man-made water systems becoming vehicle of pathogens. It is thus important to investigate the distribution of FLA in water systems and to correlate their occurrence with the microbial water quality. Accordingly, a monitoring study was performed on different types of water with the aim to evaluate the incidence of these organisms.

Methods: Eighty six water samples from public water supplies at the water meter, of private houses, hospitals, swimming pools and spa were tested for the presence of FLA; heterotrophic plate count (HPC) was also quantified.
FLA were detected by a cultural method (NN agar added with heat killed E. coli). After a microscopic examination, PCR and sequencing were applied for the identification at genus level. HPC was detected with the standard EN ISO 6222.

Results: FLA were found in 53% of the samples, with the highest counts in the tap waters of hospitals (82%) and houses (68%), where HPC had a maximum value of 5 x 102 cfu/ml. No water samples collected at the water meter showed FLA. Swimming pools showed 40% of positive samples and even if their bacteriological quality complied with the Italian specific legislation. Higher microbial counts were detected in water spa samples where FLA had a prevalence of 44%. Acanthamoeba spp. was the more frequent detected genus.

Conclusions. The investigation showed a wide distribution of FLA in man-made water environments also with a good water microbial quality.

EMA-qPCR: a rapid technique for managing the risk of *Legionella spp* in waters subjected to disinfection treatments

Authors Mansi A., Marchesi I., Amori I., Marcelloni A. M., Proietto A. R., Bargellini A., Paduano S., Borella P.

Background. The development of rapid and sensitive methods for the detection and quantification of Legionella viable cells is essential to prevent Legionella infections. A technique named "EMA-qPCR" has been proposed over the last years; it is able to discriminate between viable and dead cells using a DNA intercalating dye (Ethidium Monoazide Bromide, EMA) in combination with qPCR. The main aim of this study is to evaluate the advantages of this method in evaluating the presence of Legionella spp in water compared with both culture and qPCR.

Methods. One hundred and fifty-eight hot water samples collected in different hospital distribution systems either untreated or treated with disinfection procedures (monochloramine, chlorine dioxide, hydrogen peroxide or heat) were simultaneously analysed by culture (ISO 11731), qPCR and EMA-qPCR. Real Time PCR for the quantification of Legionella spp was carried out using the "New Legionella spp Quantitative kit" (Diatheva, Italy) validated in agreement to ISO/TS 12869:2012.

Results. Thirty-six percent of the samples (57/158) were positive for Legionella spp with all three methods (culture, qPCR and EMA-qPCR). This percentage increased up to 46% by using EMA-qPCR, and up to 70% with qPCR. Considering the culture as the reference method, sensitivity and specificity were 100% and 47% (PPV 52%) for qPCR and 100% and 84% (PPV 78%) for EMA-qPCR. Interestingly, the specificity of EMA-qPCR was 100% in waters treated with monochloramine, chlorine dioxide, and heat between 50° and 58°C.

Conclusion. EMA-qPCR provides rapid information about the presence of still viable legionellae, and we suggest that it can be suitable for monitoring the effectiveness of control measures adopted for reducing the Legionella contamination. Further investigations are in course to confirm these data and assess the applicability of the EMA-qPCR in waters subjected to other disinfection procedures.

Association between sporadic legionellosis and river systems in Connecticut

Cassell K., Gacek P., Warren J. L.,
Raymond P. A., Cartter M., Weinberger D. M.

Environmental and molecular investigation of an nosocomial Legionnaire's disease

Authors Valero N., Gallés P., Simeon J., Torrens A., Gonzalez R., Manzanares-Laya S., De Andrés A., Gómez A.

Background In Spain 925 cases of Legionnaire's Disease (LD) were reported in 2014, of which 5% were healthcare-associated. Only 5% of the reported cases were confirmed by culture. Stagnation and temperature are factors commonly associated with Legionella in hot water systems. A 66-year-old female began to show symptoms compatible with LD on January 23, 2017.
Diagnose of LD was confirmed by a urinary antigen test and L. pneumophila serogroup (sg) 1 was isolated in a respiratory sample. An environmental investigation with Legionella and molecular subtyping was conducted to determine the source of exposure in the hospital visited by the patient during the incubation period.

Methods Samples of the first 2000 mL of water were collected from the faucets in the hot water system of the hospital. Water temperature at 1 minute was measured to determine the circulation of the water. Counts were obtained of L. pneumophila sg 1 and sg 2-15. Molecular typing of the strains of L. pneumophila sg 1 isolated in the environmental and clinical samples was performed via DNA macrorestriction and subsequent pulsed field electrophoresis.

Results 27% (3/11) of distal sites were positive for L. pneumophila. In the 3 faucets where L. pneumophila was detected, water temperature at 1 minute was under 55ºC. Counts of 600 cfu/L of L. pneumophila sg 1 were detected in a shower of the emergency room and L. pneumophila sg 2-15 was detected in two faucets of the same line of the water system.

ABSTRACT BOOK

The 9th International Conference on

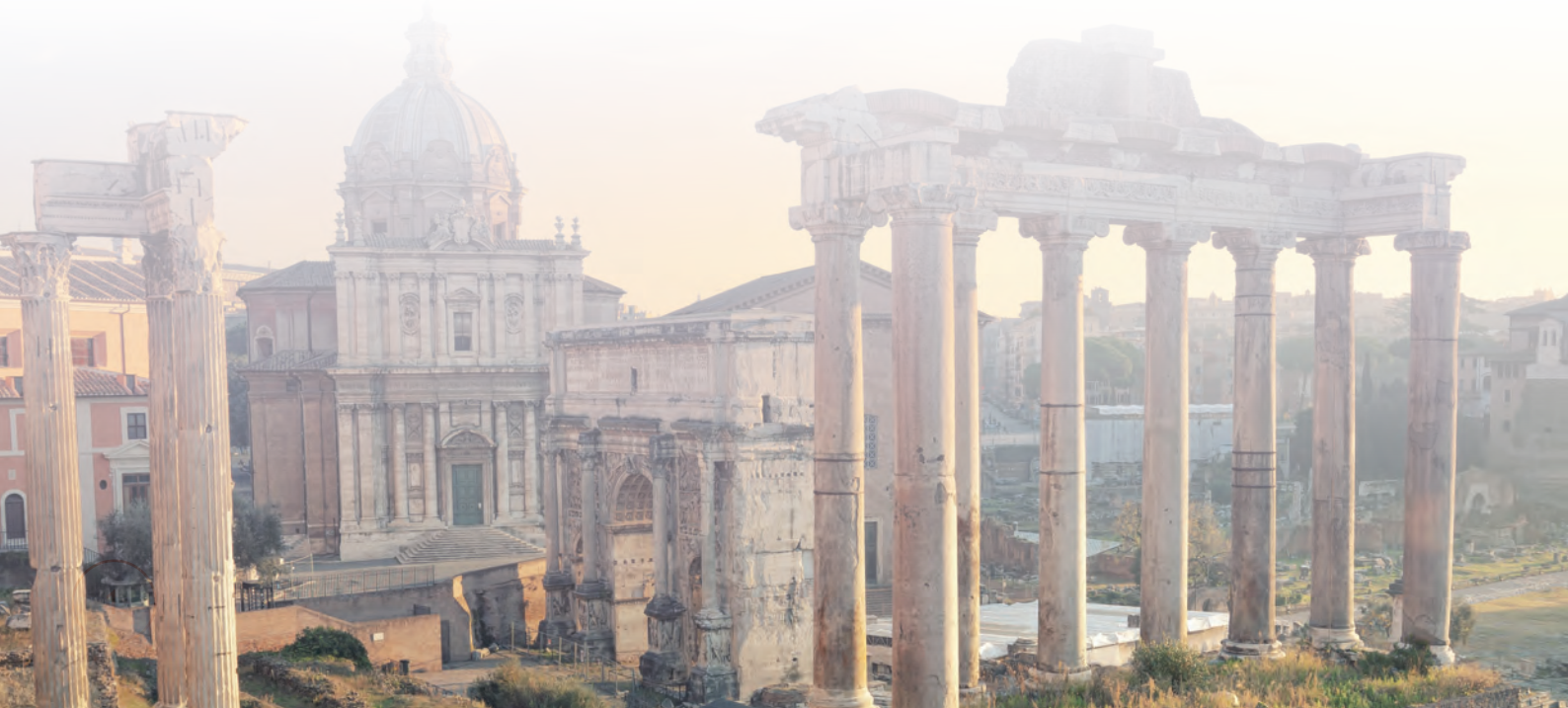
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

TUESDAY, SEPTEMBER 26TH, 2017

17:00-18:30 **SESSION 1 - GLOBAL TREND OF LEGIONNAIRES' DISEASE**



Legionnaires' Disease, A Brief History

Author Paul H. Edelstein

The history of Legionnaires' disease (LD) includes the 1976 epidemic in Philadelphia, as well as several LD epidemics that occurred well before 1976. Many people played important roles in defining the epidemiology of the disease as well as determining the etiologic agent.

Important contributions to the mechanisms of nosocomial infection, the environmental ecology of Legionella spp and its interactions with other microbiota, and the discovery of novel Legionella spp will be discussed

Legionnaires' disease from a European perspective

Author Birgitta de Jong

Background Epidemiological trends in Legionnaires' disease (LD) in Europe are monitored by ECDC together with the EU Member States through annual enhanced surveillance of the disease.
In addition, through the European Legionnaires' Disease Surveillance Network (ELDSNet) realtime daily surveillance of travel-associated Legionnaires' disease (TALD) is undertaken for targeted response action by countries. The detection of clusters associated with travel accommodation sites triggers local control actions that play an essential role in minimising the risk for travel related LD.

Method Data collated by nominated ELDSNet members from each EU/EEA country are electronically reported to The European Surveillance System (TESSy) database at ECDC on the basis of the EU case definition. For TALD cases, data are entered on a real-time daily basis to TESSy. An annual retrospective data call was undertaken April 2017 for all 2016 LD cases. The two data sets were analysed separately.
Results The number of reported cases, both TALD and LD cases shows an increasing trend which will be discussed in the presentation.

Conclusion The added value of European surveillance is demonstrated by the TALD cluster detection where a large number of these clusters would not have been detected by any single country.

A Watershed Moment: The Increasing Challenge of Legionnaires' Disease in the United States

Author Laura A. Cooley

Background: Legionnaires' disease (LD), a severe pneumonia, is typically acquired through inhalation of aerosolized water containing Legionella bacteria. During 2000–2015, the rate of reported cases of legionellosis, both LD and Pontiac fever, increased 350%, from 0.42 to 1.89 cases per 100,000 persons in the United States. In 2015, 6,079 legionellosis cases were reported, although this is likely an underestimate. Cases and outbreaks are often caused by problems that could be prevented with more effective water management.

Methods: Using national surveillance data from 52 jurisdictions, LD cases were characterized from the 21 jurisdictions (20 U.S. states and one large metropolitan area) that reported supplemental epidemiologic information, including health care, travel, and assisted living exposures in the 10 days prior to onset of symptoms, for ≥90% of 2015 legionellosis cases. Descriptive statistics were generated.

Results: Supplemental epidemiologic information was reported by the 21 jurisdictions for 2,809 LD cases. Median patient age was 61 years and 61% were male; the overall case fatality rate (CFR) was 7%.
Health care exposure was reported for 553 cases (20%), travel during the 10 days prior to onset was reported for 367 cases (13%), and exposure to assisted living facilities was reported for 76 cases (3%). CFRs associated with these exposures were 12%, 4%, and 9% respectively. No health care, travel, or assisted living exposure was reported for a majority of cases (1,897; 68%).
Conclusions: Reported cases of LD continue to increase in the United States and disease is associated with substantial mortality. Improved investigation and reporting of exposures is needed to gain a more comprehensive understanding of exposures to guide specific prevention interventions. In buildings at increased risk for Legionella growth and transmission, water management programs should be implemented to help prevent cases and outbreaks.

ABSTRACT BOOK

The 9th International Conference on

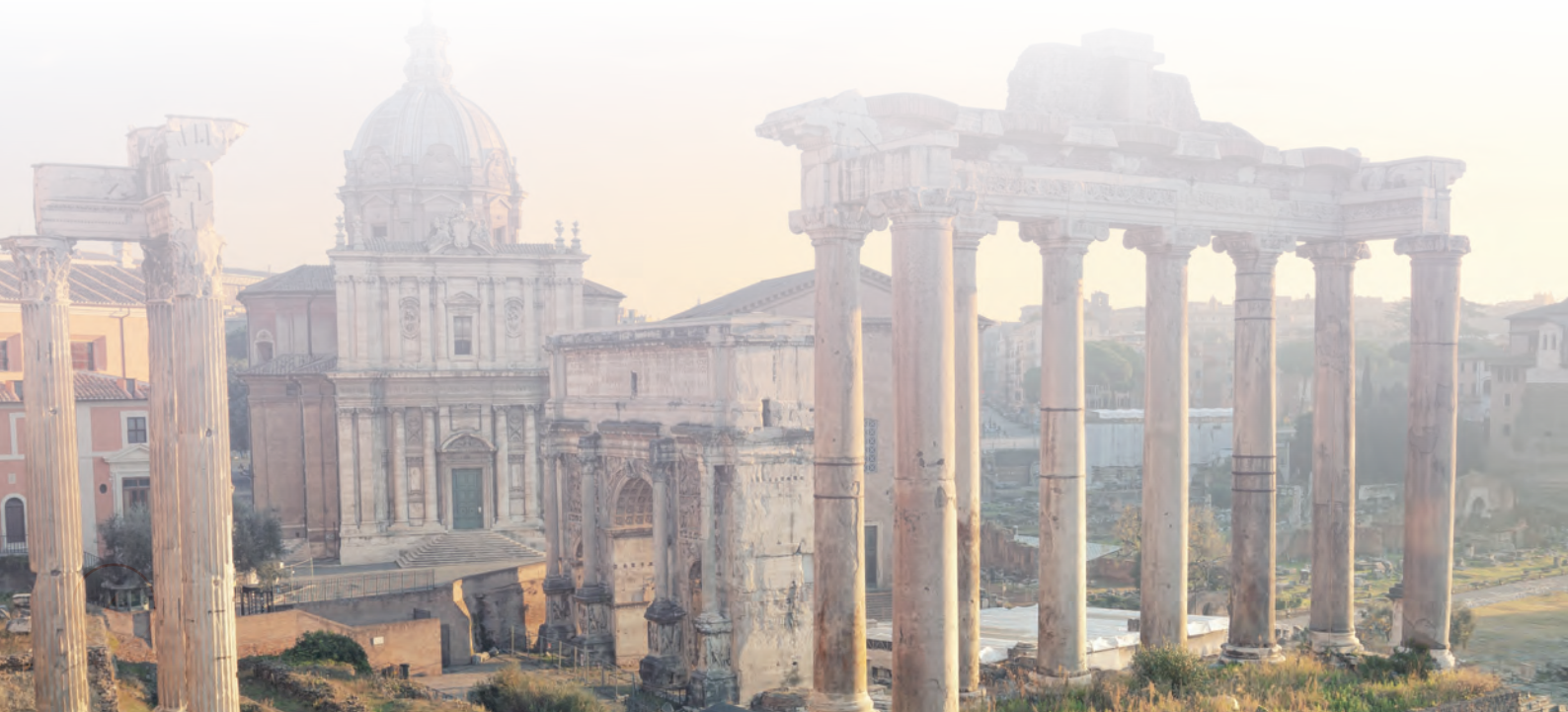
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

WEDNESDAY, SEPTEMBER 27TH, 2017

08:30-10:30 **SESSION 2 - PATHOGENESIS, IMMUNOLOGY AND HOST CELL INTERACTIONS**



Modulation of membrane dynamics and cell migration by *Legionella*

Author Hilbi H.

The facultative intracellular bacterium Legionella pneumophila grows in free-living amoeba and macrophages within a specific membrane-bound compartment, the Legionella-containing vacuole (LCV). LCV formation is a robust and complex process, during which the pathogen vacuole interacts with the endosomal, secretory and retrograde vesicle trafficking pathways, and tightly associates with the endoplasmic reticulum (1). Proteomics analysis of intact purified
LCVs revealed a number of host factors functionally relevant for LCV formation, including small and large GTPases.

The L. pneumophila Icm/Dot type IV secretion system (T4SS) is essential for LCV formation and translocates the astonishing number of about 300 different “effector proteins” into host cells (2). In eukaryotic cells, these effectors subvert cytoskeleton dynamics, vesicle trafficking and signal transduction. Some Icm/Dot substrates promote intracellular bacterial replication by targeting phytate (inositol hexakisphosphate), phosphoinositide lipids, small GTPases, the retromer complex, or other host cell components. L. pneumophila also modulates the motility of eukaryotic cells through Icm/Dot-translocated effectors and the small signaling molecule LAI-1 (3-hydroxypentadecane-4-one) (3).

Recent findings will be presented about how L. pneumophila Icm/Dot substrates subvert small and large GTPases or the retromer complex to govern pathogen-host interactions and intracellular bacterial replication.

A new approach to identify *Legionella pneumophila* effector mutants with distinct virulence phenotypes.

Authors: Stephanie R. Shames, James C. Havey, Whitman B. Schofield, Andrew L. Goodman, and Craig R. Roy

Background: Intracellular bacterial pathogens such as Legionella pneumophila cause disease by delivering effector proteins into host cells to manipulate biological pathways. A single strain of L. pneumophila encodes a repertoire of over 300 different effector proteins, which are delivered into host cells by the Dot/Icm type IV secretion system. The large number of L. pneumophila effectors has been a limiting factor in assessing the importance of individual effectors for virulence.

Materials and methods: Insertion sequencing (INSeq) was used to reveal the contribution of multiple previously uncharacterized effector genes to L.
pneumophila virulence. Replication of hundreds of targeted effector mutants was measured in parallel in both a mouse model of infection and in host cells cultured ex vivo.

Results: This screen identified 53 novel phenotypes resulting from loss-of-function mutations in several effector genes, including a subset of effectors that appear to enhance clearance of L. pneumophila from the mouse lung. Distinct virulence phenotypes displayed by L. pneumophila mutants deficient in either the effector protein LegC4, RavY, or Lpg2505 were validated using a mouse model of infection.
In the absence of LegC4, L. pneumophila virulence in the lung is enhanced, whereas
RavY and Lpg2505 are important for virulence in all hosts examined. It was found that Lpg2505 functions as a metaeffector that counteracts the activity of the effector protein SidI.
Conclusions: This study has identified an important subset of effectors that contribute to virulence, and demonstrates that regulation of effector protein activities by cognate metaeffectors is critical for host pathogenesis.

The Dot/Icm type IV secretion system effector LtpM defines a new family of modular Glycosyltransferases

Authors Mattheis C., Levanova N., Carson D., To K, So EC., Aktories K., Frankel G., Jank T., Schroeder G. N.

Background: The virulence of Legionella spp. depends on the Dot/Icm type IVB secretion system (T4SS). Genomics and bioinformatics identified several thousand potential Dot/Icm effectors. The vast majority of these effectors remains uncharacterised, but it has become clear that many contain multiple functional domains, which often share high similarity with eukaryotic proteins. How these multi-domain proteins arise is unknown. Comparative genomics indicate that domains might be swapped between proteins facilitating the evolution of new effectors. We investigated this hypothesis focussing on the LtpD family of effectors.
Materials/Methods: Employing bioinformatics we identified proteins, which share different domains with LtpD. Using biochemical and cell biological assays in combination with infection assays we set out to characterise the function of these modular effectors.

Results: Here, we show that one of identified proteins, named LtpM, is a new T4SS effector, which shares a C-terminal PI3P-binding domain with LtpD that targets it to endosomal compartments as well as the Legionella-containing vacuole. A homologue of LtpM from L. cincinnatiensis, LciM, which lacks the PI3P-binding domain, was also translocated, but showed a different subcellular localisation. Further analysis of LtpM revealed an N-terminal domain with weak homology to the glycosyltransferase (GT) toxin PaTox from Photorhabdus asymbiotica.
Although the characteristic, catalytically important DxD motif of GTs is not conserved in LtpM, in vitro assays showed that LtpM has glucohydrolase and auto-glycosylation activity using UDPglucose as sugar donor, confirming that this non-classical GT domain is functional. Ectopic expression of LtpM affected vesicle trafficking in mammalian cells.

Conclusions: Our data show that LtpM is the first member of a novel family of glycosyltransferase effectors, which evolve by domain swapping, and implicates LtpM in the manipulation of the endosomal system by Legionella.

New Insights Into the *Legionella* Type 1 Secretion System

Authors Hussein Kanaan, Claire Andrea, Annelise Chapalain, Patricia Doublet, Christophe Gilbert.

Background: Recent work in our team reports that 3 proteins, LssB, LssD and TolC, are the components of a type 1 secretion system (T1SS) involved in Legionella pneumophila virulence. This T1SS enables the secretion of a unique substrate, RtxA (800 kDa) from the RTX proteinfamily. (1). Similarities with LapA adhesin in Pseudomonas suggest that RtxA might be released in the extracellular space or embedded in the external membrane, depending on local c-di-GMP concentration (2). The goal of this work is to elucidate the location of RtxA protein in relation with the LapG/lapD protein complex and in relation with the T1SS functionality.

Methods: Different approaches have been set up to elucidate key points of the mechanism: (i) a biochemical study on RtxA potential cut by LapG (ii) genetic mutant strains constructions in L. pneumophila Paris to study the location of RtxA in relation to LapD/lapG functionality (iii) Bacterial two-hybrid assays in E. coli to identify potential partners in the mechanism.

Results: Our work confirms the two location of RtxA, embedded in outer membrane or released in medium via a LapG/LapD complex dependent manner. Few accessory proteins are proposed to participate to this mechanism resulting in alternatives RtxA locations during Legionella life cycle.

Conclusion: Considering the role of RtxA in virulence (1), our results suggest that RtxA may play different roles in Legionella depending on its location and further work is ongoing to link RtxA location and virulence during the infection initiation process.

Legionella pneumophila Modulates Mitochondrial Dynamics To Trigger Metabolic Repurposing of Infected Macrophages.

Authors Escoll P., Song O., Viana F., Steiner B., Lagache T., Olivo-Marin J.C, Impens F., Brodin P., Hilbi H., Buchrieser C.

The intracellular pathogen Legionella pneumophila has been suggested to recruit mitochondria to its replication vacuole (LCV), but the role and implications of this association were unknown. Here we show that L. pneumophila modulates mitochondrial dynamics during infection and shapes the host metabolic response to favour bacterial replication. We used fluorescence based dynamic imaging at the single-cell level and metabolic assays to determine the response of mitochondria to L. pneumophila infection of human monocyte derived macrophages, and show that the LCV interactions with mitochondria are

T4SS-independent and dynamic. Subsequent to these initial interactions, L. pneumophila induces mitochondrial fragmentation in a T4SS-dependent process that leads to an alteration of mitochondrial metabolism in the infected macrophage. Further analyses identified the T4SS effector MitF, a Ran GTPase activator, to be involved in the fission of the mitochondrial network that occurs through accumulation of the mitochondrial DNM1L GTPase in the absence of cell death signs. Furthermore mitochondrial respiration is abruptly halted in a T4SS-dependent manner, while T4SS-independent upregulation of cellular glycolysis remains elevated. Thus L.
pneumophila induced modulation of mitochondrial dynamics promotes a Warburg-like phenotype in the infected cell that favours bacterial replication. Hence the rewiring of cellular bioenergetics to create a replication permissive niche in host cells is a core virulence strategy of L. pneumophila.

Manipulation of Host Metabolism by *Legionella pneumophila* Effectors that Activate and Inhibit mTORC1

Authors Justin A. De Leon, Jiazhang Qiu, Christopher J. Nicolai, Rosalie E. Lawrence, Brian M. Castellano, Roberto Zoncu, Zhao-Qing Luo, and Russell E. Vance

All pathogens must acquire nutrients from their hosts. The bacterial pathogen Legionella pneumophila (L. pneumophila), the etiological agent of Legionnaires' disease, requires host amino acids for growth within cells. The mechanistic target of rapamycin complex 1 (mTORC1) is an evolutionarily conserved master regulator of host amino acid metabolism. We hypothesized that L. pneumophila targets mTORC1 in order to regulate amino acid levels in host cells. Utilizing a microscopy based effector screen, we identified two families of effector proteins that are translocated by L.pneumophila into host cells that exhibit opposing effects on mTORC1 activity. The Legionella glucosyltransferase (Lgt) effector family, previously shown to block host protein synthesis, activates mTORC1, whereas the SidE/SdeABC (SidE) family of effectors act as mTORC1 inhibitors. SidE family effectors appear to block mTORC1 by catalyzing the ubiquitylation and inactivation of RagB and RagD, small-GTPases required for mTORC1-dependent amino acid sensing. We propose that the Lgt and SidE families of effectors work in concert to liberate host amino acids for consumption by L. pneumophila.

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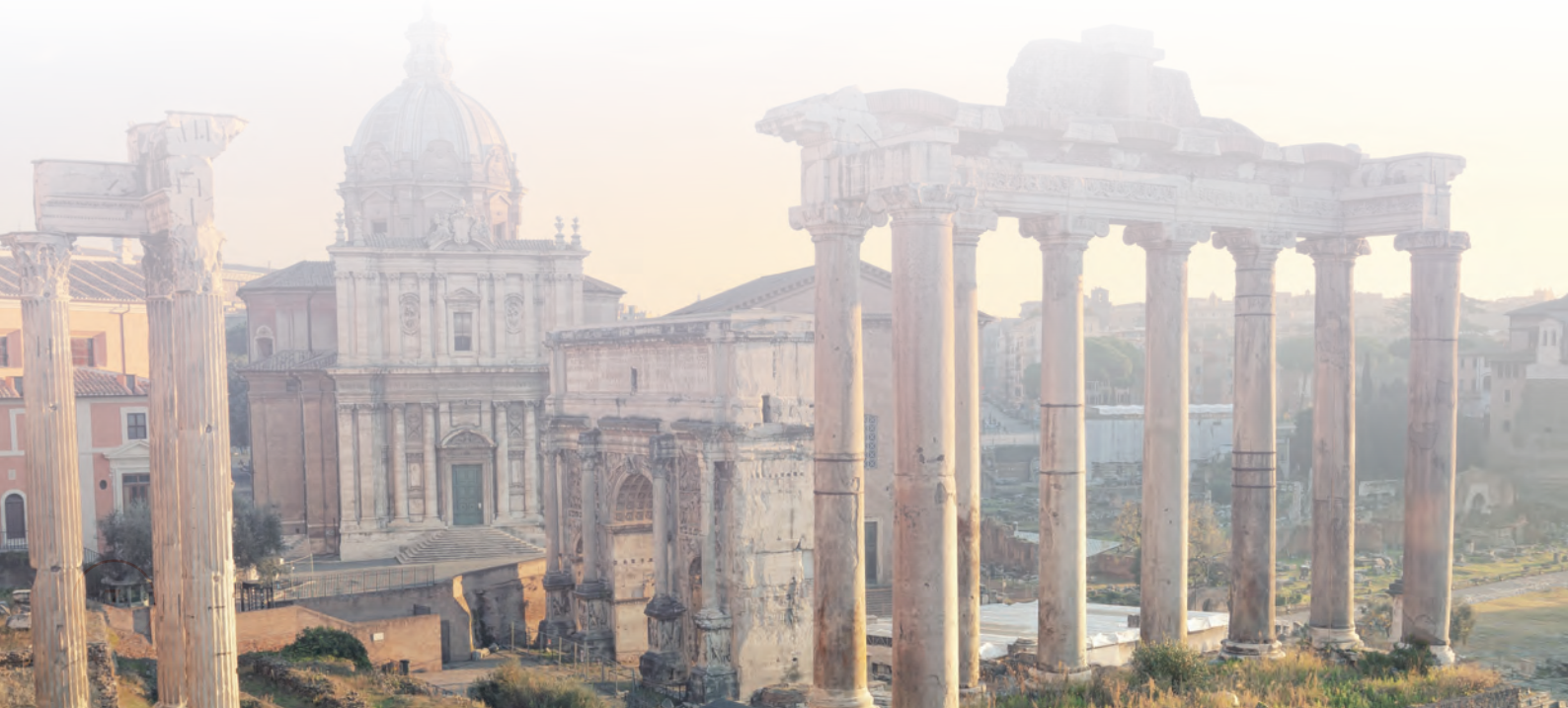
ORAL PRESENTATIONS

WEDNESDAY, SEPTEMBER 27TH, 2017

11:00-13:00 **SESSION 2 - PATHOGENESIS, IMMUNOLOGY AND HOST CELL INTERACTION (CONTINUED)**



Caspase-8 participates in the Naip5/NLRC4/ASC inflammasome that is responsible for recognition and restriction of *Legionella pneumophila* replication in macrophages.

Mascarenhas, D.p., Cerqueira, Dm., Pereira, Msf., Castanheira, Fvs., Fernandes, Td; Manin, Gz,., Cunha, Ld And Zamboni, Ds

Legionella pneumophila is a Gram-negative, flagellated bacterium that survives in phagocytes and causes Legionnaires' disease. Upon infection of mammalian macrophages, cytosolic flagellin triggers the activation of Naip/NLRC4 inflammasomes, which culminates in pyroptosis and restriction of bacterial replication. Although NLRC4 and caspase-1 participate in the same inflammasome, Nlrc4-/- mice and their macrophages are more permissive to L. pneumophila replication compared with Casp1/11-/-. This feature supports the existence of a pathway that is NLRC4-dependent and caspase-1/11-independent. Here, we identified a platform composed of NLRC4, ASC and caspase-8 that operates in response to flagellated bacteria independently of caspase-1/11. Infection of macrophages transduced with
NLRC4-GFP or ASC-GFP with flagellin-positive bacteria triggered puncta composed of
NLRC4, ASC and caspase-8. Accordingly, NLRC4 and ASC, but not caspase-1/11, were required for caspase-8 activation in response to flagellated bacteria. Silencing caspase-8 in Casp-1/11-/- cells culminated in macrophages that were as susceptible as Nlrc4-/- for the restriction of L. pneumophila replication. Accordingly, macrophages and mice deficient in Asc- /-/Casp1/11-/- were more susceptible than Casp1/11-/- and as susceptible as Nlrc4-/- for the restriction of infection by several species of Legionella. Collectively, our data reveal an inflammasome involved in flagellin sensing that is dependent on NLRC4, ASC and caspase-8 and independent of AIM2, caspase-1 and caspase-11. This platform is critical for the restriction of bacterial infection in macrophages and in vivo.

The role of lipids in *Legionella*-host interaction

Author Palusinska-Szysz M.

Background The establishment of a replicative niche and exchange of information between the pathogen (Legionella pneumophila) and its phagotrophic host (Acanthamoeba castellanii) is possible due to the efficiently functioning bacterial envelope. Three types of lipid-containing molecules are present in the Legionella envelope: phospholipids (PL), lipopolysaccharide (LPS), and lipoproteins. The unique L. pneumophila LPS structure determining specific physico-chemical properties may play a key role in the interaction between the pathogen and its host.

Materials/Methods The study was conducted on the L. pneumophila Corby strain and its mutant (TF3/1) defective in biosynthesis of the O-specific chain of LPS. The GC/MS analysis was performed to investigate changes in the fatty acid pattern. Phospholipids (PLs) were analysed using MALDI-TOF spectra. The sugar structure of LPS present directly on the surface of live cells of L. pneumophila was determined using HR-MAS NMR. FLIM microscopy was used for monitoring and analysis of the bacterial interactions with amoebal cells.

Results The fatty acid composition of PLs isolated from L. pneumophila strain Corby and mutant TF3/1 exhibited clear differences. The Corby strain synthesized nearly two-fold less 12-methyltetradecanoic acid than the mutant. The main constituents of PLs identified in both strain were PC m/z 706.46 PC[30:0 +H]+, 742.45 PC[31:0 +Na]+, 756.45 PC[32:0 +Na]+, 768.45 PC[33:1 +Na]+, and 782.46 PC[34:1 +Na]+. The HR-MAS NMR analysis of carbohydrates showed differences in acetyl residues present in the O-specific chain of LPS between the wild type and mutant strain. Monitoring of the biophysical interaction between L. pneumophila and A. castellanii using FLIM microscopy showed that the wild type strain integrated in amoebal membrane more efficiently than the mutant.

Conclusions The structure of the cell wall components of L. pneumophila, especially LPS, can determine the interaction with the host cell.

ER remodeling by the large GTPase atlastin (Sey1/Atl3) promotes vacuolar growth of *Legionella pneumophila*

Authors Steiner B., Swart A. L., Welin A., Weber S., Personnic N., Kaech A., Freyre C., Ziegler U., Klemm R. and Hilbi H.

The formation of the replication-permissive Legionella-containing vacuole (LCV) in amoeba and macrophages is governed by the Icm/Dot type IV secretion system (T4SS), which delivers ~300 different effector proteins into host cells. LCVs avoid fusion with bactericidal lysosomes, but extensively communicate with the endosomal, secretory and retrograde vesicle trafficking pathways, and eventually associate with the ER in an intimate manner. How the dynamic ER network contributes to pathogen proliferation within the nascent LCV remains elusive. Our recent proteomic analysis of purified LCVs identified the ER tubule-resident dynamin-like large GTPase atlastin3 (mammalian Atl3; Dictyostelium discoideum Sey1) and the reticulon protein Rtn4 as host components. Atlastin and reticulon proteins are crucial regulators of ER dynamics.

Using confocal fluorescence microscopy we validated that Sey1/Atl3 and Rtn4 indeed localize to early LCVs. D. discoideum Sey1 was found to show GTPase activity, similar to yeast Sey1p. Overproduction of Sey1 in D. discoideum promoted intracellular growth of L. pneumophila, whereas a dominant-negative version of the large GTPase (Sey1_K154A), or Atl3 depletion by RNA interference in mammalian cells, restricted pathogen replication. Sey1 was not required for the initial accumulation of ER to PtdIns(4)P-positive LCVs, but rather for the subsequent ER remodeling and expansion of the pathogen vacuole. We further demonstrated that GTP (but not GDP) catalyzes the Sey1-dependent aggregation of purified, ER-positive LCVs in vitro. Taken together, Sey1/Atl3-dependent ER remodeling contributes to LCV maturation and intracellular replication of L. pneumophila.

SnpL, a nucleomodulin effector protein of *Legionella pneumophila* targets host transcription elongation machinery

Authors Schuelein R., Spencer H., Webb A., Luo L., Stow J., Valero L. G., Buchrieser C., Sugimoto C., Yamagishi J., Pasricha S. and Hartland E. L.

Background: Legionella pneumophila translocates more than 300 effector proteins into the host cell via the Dot/Icm secretion system. While many effectors localize to the LCV, others traffic to remote intracellular sites. We hypothesise that some Dot/Icm effectors are nucleomodulins that traffic to the nucleus where they subvert host transcriptional responses to infection. Here we identified nuclear localised effectors and characterised a novel nucleomodulin, SnpL.

Methods: To identify L. pneumophila effectors that translocate to the host nucleus, THP-1 macrophages were infected with wild type or ΔdotA mutant L. pneumophila, nuclear fractions extracted and analysed by mass-spectrometry. Translocation was confirmed using TEM-1 ß-lactamase assays and immunofluorescence microscopy. Host-binding partners were identified using immunoprecipitation coupled with mass-spectrometry. RNA-sequencing was performed on mouse macrophages expressing the effector to elucidate its effect on host transcription.

Results: We identified a novel L. pneumophila nucleomodulin effector, SnpL. SnpL bound SUPT5H, a component of the DRB sensitivity-inducing factor complex (DSIF complex) that regulates RNA polymerase II dependent mRNA processing and transcription elongation. We found that macrophages expressing SnpL underwent widespread upregulation of host genes and cell death.

Conclusion: To date, SnpL is one of only a handful of nucleomodulin effectors described in bacteria and its activity highlights the ability of L. pneumophila to control eukaryotic processes remote from the LCV. We propose that the binding of SnpL to SUPT5H disrupts the transcriptional pausing activity of the DSIF complex.

Structural insights into *Legionella* RidL-Vps29 retromer subunit interaction reveal displacement of the regulator TBC1D5

Authors Bärlocher K., Hutter CAJ., Swart AL., Steiner B., Welin A., Hohl M., Letourneur F., Seeger M. A., and Hilbi H.

Legionella pneumophila naturally replicates in amoeba and also parasitizes macrophages in the lung, possibly leading to a severe pneumonia called “Legionnaires’ disease”. Hereby, the formation of a replication-permissive compartment, the Legionella-containing vacuole (LCV), is a crucial process. LCVs extensively communicate with the endosomal, secretory and retrograde vesicle trafficking pathways, but avoid fusion with bactericidal lysosomes. The retrograde vesicle trafficking pathway recycles cargo receptors along the endosomal route back to the Golgi apparatus and the endoplasmic reticulum (ER). A key mediator of retrograde trafficking is the retromer complex, consisting of the heterotrimeric cargo-selective subcomplex (Vps26, Vps29, Vps35) and heterodimeric membrane-deforming sorting nexins. A functional retrograde pathway restricts intracellular bacterial replication. LCV formation is dependent on the Icm/Dot type IV secretion system (T4SS), which translocates approximately 300 different “effector” proteins into the host cell, where they modulate cellular processes. Recently, we have shown that the Icm/Dot substrate RidL (Retromer interactor decorating LCVs) binds to Vps29, interferes with the retrograde vesicle trafficking pathway and is required for efficient intracellular replication of L. pneumophila. Recent data from our lab reveal that the 29 kDa N-terminal domain of RidL adopts an unprecedented foot-like fold comprising a protruding ?-hairpin at its heel. The deletion of the hydrophobic ?-hairpin, and the mutation I170E in the ?-hairpin or L152E in Vps29 abolishes the RidL-Vps29 interaction in eukaryotic cells and in vitro. RidL2-258 or RidL displaces the Rab7 GTPase activating protein (GAP) TBC1D5 from the retromer and LCVs, respectively, and TBC1D5 promotes intracellular growth of L. pneumophila. Thus, the ? hairpin of RidL is critical for binding of the L. pneumophila effector to the Vps29 retromer subunit and displacement of the regulator TBC1D5.

When life gives you lemons in the form of oxidants...: A peek into the molecular underpinnings of how *Legionella* copes with oxidative stress in water

Authors Mendis N., Trigui H., Saad M., Tsang A., Faucher S. P.

Background: As an inhabitant of man-made water systems and an intracellular parasite, it is likely that Legionella pneumophila (Lp) encounters varying levels and forms of reactive oxygen species (ROS). The oxidative stress response of many proteobacteria is regulated by the LysRtype regulator OxyR. To date, the importance of the OxyR homologue in Lp has remained elusive.

Materials/Methods: oxyR is derived from the Philadelphia-1 KS79 strain through allelic exchange. A defined freshwater medium (Fraquil) was used for water experiments.

Results: The loss of oxyR caused a striking growth defect that prevents the mutant from producing isolated colonies on CYE agar. The growth defect was corrected by supplementation with anti-oxidants, thereby suggesting ROS as the root cause. Interestingly, this phenotype was confined to solid medium since the mutant mirrored the wild-type in broth. A yet-unidentified component in commercial agar is speculated to cause oxidative stress, since the use of agarose as the solidifying agent in CYE corrected the growth defect of oxyR similarly to anti-oxidants.
Further characterizational studies revealed that OxyR conferred a modest resistance to a 10Mm hydrogen peroxide (H2O2) stress during the post-exponential phase, despite its lack of transcriptional control over anti-oxidative genes that directly target H2O2. Preliminary data reveal that OxyR is a positive regulator of the electron transport chain and efflux pumps. More significantly, OxyR was found to play a major role in resisting a 1mM H2O2 stress in water. At a concentration of108 cells/ml, ?oxyR counts declined 1000-fold faster than the wild-type.
Introduction of oxyR in trans corrected the defect of the mutant.

Conclusion: This study is the first to associate a positive physiological role for OxyR in Lp. The transcriptome of the mutant is currently being investigated to elucidate the molecule mechanisms by which OxyR exerts its protective role in the water environment

ABSTRACT BOOK

The 9th International Conference on

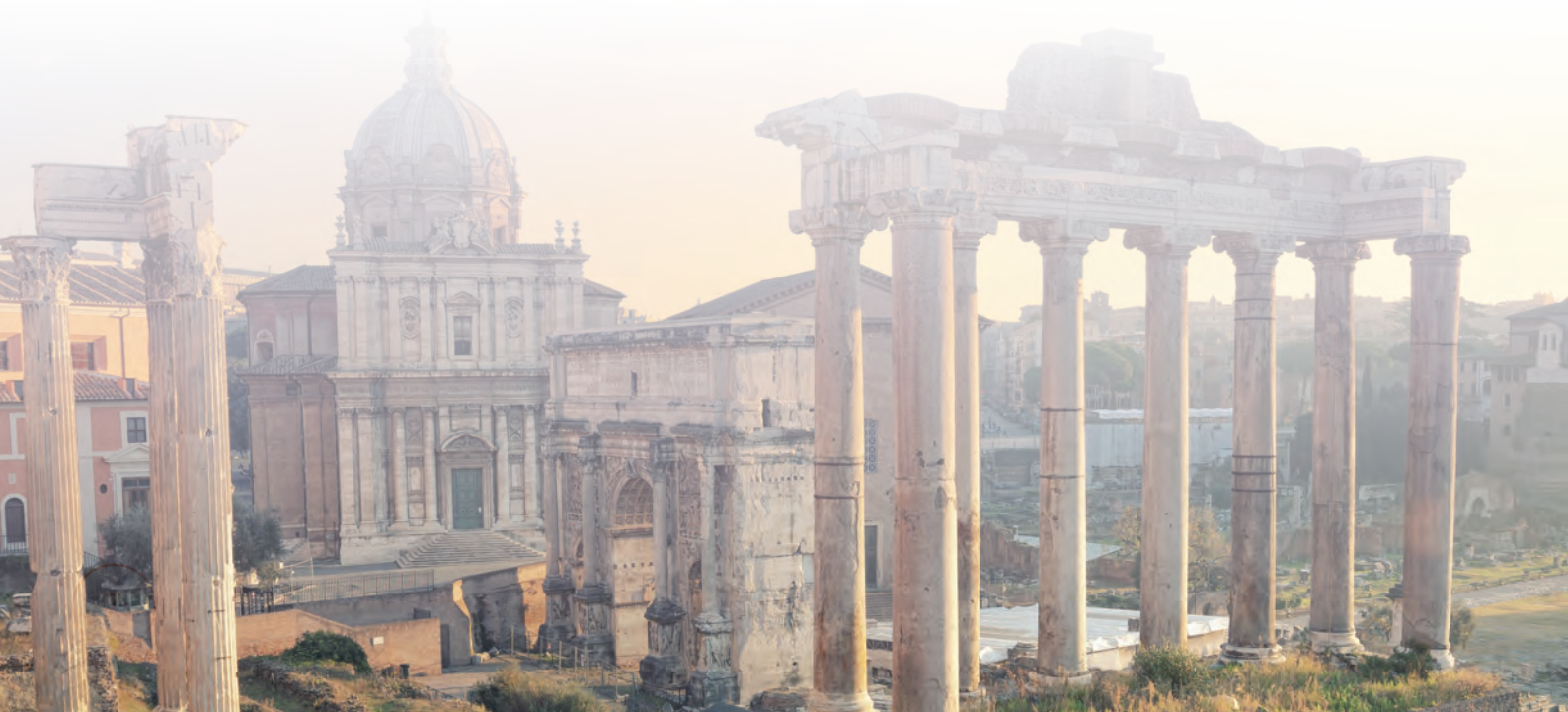
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

WEDNESDAY, SEPTEMBER 27TH, 2017

15:30-16:45 **SESSION 3 - GENETICS AND GENOMICS**



Legionella pneumophila CsrA: the key regulator of metabolism and virulence

Authors Carmen Buchrieser

L. pneumophila is able to survive and replicate in the hostile intracellular environment of a protozoan cell (or a human macrophage). Once L. pneumophila has replicated to high numbers, it is released in the water (or the lung) where low nutrient extracellular conditions are present.

To tolerate or exploit these conditions this bacterium has evolved a biphasic lifecycle wherein it alternates between a replicative and a transmissive phase. The key regulator governing this adaptation is the RNA-binding protein CsrA.

Transcriptomics, proteomics, RNA-Immunoprecipitation followed by deep sequencing (RIPseq), together with biochemical, phenotypical and molecular analyses allowed us to identify the L. pneumophila CsrA targets genome wide. By comparing a wild type and a csrA mutant strain we identified 478 RNAs with potential CsrA interaction sites and also discovered a new mode of action of CsrA that allows regulating genes comprised in the same operon, independently.

Furthermore, isotopologue profiling showed that CsrA has a determining role in substrate usage and carbon partitioning during the L. pneumophila life cycle and in regulating a substrate usage depending on the biphasic life cycle.

Retroelement-guided protein diversification in *Legionella pneumophila*

Authors Arambula D., Czornyj E., Ahuja U., Paul B., Mangul S., Ghosh P., Guo H., Miller J. F.

Background: Diversity-generating retroelements (DGRs) are pervasive within archaeal, bacterial, and viral genomes and possess an unmatched potential to generate targeted nucleotide diversity that results in accelerated protein evolution. DGRs mutagenize protein encoding genes using a copy-diversify-replace mechanism called mutagenic retrohoming (MH), which involves error prone reverse-transcription that replaces adenine residues with any DNA base. While many DGRs can generate upwards of 1019 peptide variants within proteins, adenine mutagenesis is stochastic and loss of function mutations are predicted to dominate over adaptive ones.

Materials/Methods: A combination of genetics, proteomics, next-generation sequencing, and transcriptomics were used to address how Legionella pneumophila (Lp) regulates DGRdependent mutagenesis to balance the advantages of accelerated evolution against potential loss of fitness.

Results: We observed low levels of MH in Lp cells transitioning from a replicating state to a stress-resistant transmissive state (TS). As phenotypic variation in Lp is regulated by RelA and SpoT, we hypothesized global regulatory circuits influence DGR activity. Deletion of relA and spoT resulted in increased MH, primarily during late TS. Since multiple networks control gene expression during TS, we systematically investigated the contribution of regulatory systems that work in conjunction with, or independently of, RelA/SpoT. Disruption of the lrpR/qscE two component regulatory system resulted in increased MH, similar to the relA/spoT mutant, but during a different growth phase. Simultaneous disruption of both regulatory networks resulted in a growth-phase independent increase in MH that was several orders of magnitude higher than levels observed in individual mutants. Whole transcriptome analysis suggests RelA/SpoT repress DGR encoded genes while LrpR and RelA/Spot work synergistically to control host factors essential for MH. Comparative expression profiling identified 13 candidate genes predicted to modulate MH and we demonstrated one, dhfA, antagonizes DGR activity. Lp DGRs often reside on conjugative elements, suggesting they are horizontally transferred throughout bacterial populations, so we investigated the mechanisms by which DGRs disseminate and integrate into naïve hosts.

Conclusion: Lp utilize the mutagenic potential of DGRs by integrating gene expression and host factors into multiple global regulatory system which likely allows environmentally cued cycles of MH and quiescence to allow fixation of adaptive traits.

Genetics of widely distributed natural transformability in *L. pneumophila* clinical isolates

Authors Juan P. A., Picq K. Ginevra C., Attaiech L., Charpentier X.

Background: Natural transformation results from the capture, import and integration of exogenous DNA and is a common mode of horizontal gene transfer (HGT). Natural transformation is considered the sole mechanism of HGT inherent to the species. It is under control of the recipient cell that actively acquires genetic material from its direct environment. To do so, it uses filamentous appendages related to type IV pili and a membrane-associated DNA uptake system. In L. pneumophila we recently found that expression of multiple components of the DNA uptake system is controlled at the post-transcriptional level by a non-coding RNA (RocR) and a cognate RNA chaperone (RocC). Both elements are extremely conserved in the Legionella genus with rocR displaying over 95% sequence identity.

Materials/Methods:
Here, we used a combination of transcriptional profiling, genetics, biochemistry and fluorescence microscopy to identify the components of the DNA uptake system. We investigated clinical isolates for their ability to express the DNA uptake system and undergo genetic transformation.

Results: We identified essential components of the DNA uptake system, including the major component of the transformation pilus that was previously misidentified. All components are highly conserved in L. pneumophila and natural transformability appears as a common trait in clinical isolates. However, some isolates were unable to transform and express the DNA uptake system. We identified in non-transformable strains a mobile genetic element that carries a gene that can interfere with the RocC/R silencing system to inhibit natural transformation.

Conclusion: For the first time, we provide evidence that mobile genetic elements can inhibit natural transformation by interfering with the regulatory mechanism of expression of the DNA uptake system. The evolutionary significance of this interference will be discussed.
Please express your preference for presentation: oral presentation

Evolution of host-adaptation in the bacterial order Legionellales

Authors Hugoson E., Leenheer D., Ishak H., Graells T., Larsson M., Moreno A., Guy L.

Background: Host-adapted (intracellular) bacteria are of critical importance to human health, ecology and economy, due to their medical, agronomical and biotechnological relevance.
However, they are often fastidious and obtaining whole genomes can be challenging. The order Legionellales has representatives at various stages of host-adaptation, from facultative intracellular like Legionella to obligate mutual symbionts, and is thus an excellent model to study the evolution of host-adaptation.

Methods: Twenty-seven novel metagenome-assembled genomes (MAGs) belonging to the order Legionellales were assembled or retrieved from public databases; three genomes were also sequenced. A phylogenetic tree was established from a concatenated alignment of 109 conserved marker genes, and the gene content of several key ancestors of the order was reconstructed.

Results: We confirm that Legionellales branch deep in the Gammaproteobacteria, emphasizing their early emergence, placing the last Legionellales common ancestor of (LLCA) after 1.64 Ga (Divergence Beta-/Gammaproteobacteria). Although molecular dating has to be taken with a serious pinch of salt, this dating is compatible with a scenario where LLCA appeared around the same time as eukaryotes started radiating (after 1.5 Ga). The tree confirms that the order is much more diversified than previously thought and that most of the new MAGs represent novel genera and species. A few of the MAGs display long branches and small genomes, compatible with independent events of increased dependence to the host and rapid evolution. From the ancestral reconstruction, we infer that – among others – the type four secretion system and many eukaryotic-like effector proteins were already present in LLCA.

Conclusion: The evolutionary study of the order Legionellales reveals a complex history with apparently many independent events of increased dependence on the host. Legionellales and eukaryotes might have been co-evolving since their emergence.

The 9th International Conference on

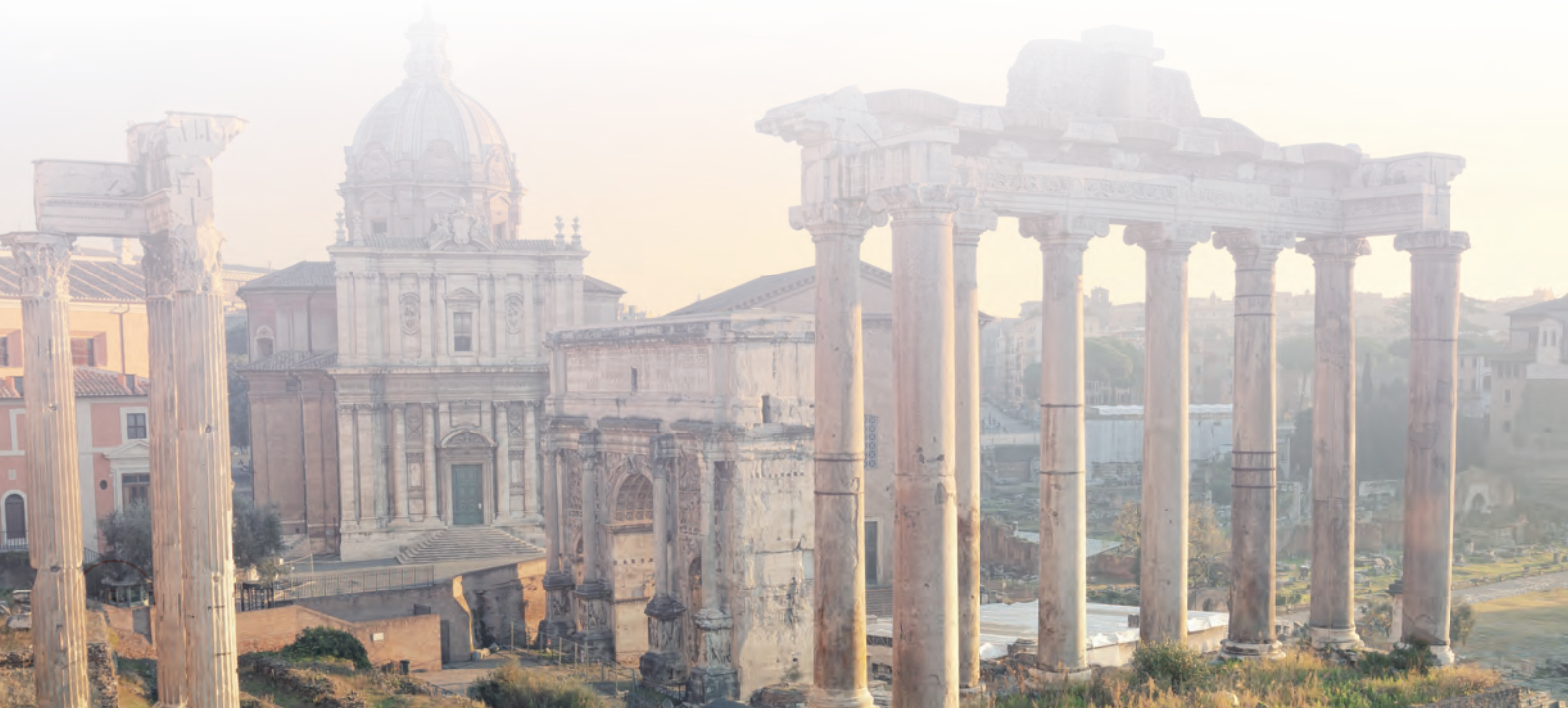
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

WEDNESDAY, SEPTEMBER 27TH, 2017

17:15-18:00 **SESSION 3 - GENETICS AND GENOMICS (CONTINUED)**



Genomic evolutionary history of Legionnaires' disease in Scotland

Authors Wee B. A., Lindsay D. L., Alves J., Loman N. J., Smith A., Fitzgerald J.R.

Background: The Scottish microbiology reference laboratory for Legionella has maintained a comprehensive collection of both clinical and environmental isolates of Legionella spp. from all over Scotland since one of the first recognised outbreaks of Legionnaires' disease in the United Kingdom in
1984. The primary aim of this study is to use whole genome sequencing to investigate relationships between clinical and environmental isolates in the context of global strains.

Methods: Comparative whole genome sequence analysis of 900 Legionella pneumophila isolates including 400 from the Scottish collection and 500 publicly available genomes was carried out. Population genomic analyses including phylogenetic reconstruction, examination of gene flow, and genome-wide association.

Results: All major outbreaks in Scotland over the last 30 years have been caused by unrelated lineages that are representative of the global diversity. Importantly, we identified clusters of related isolates that persist in specific locations over several decades with the potential to cause sporadic human infections.
Genome-wide association analyses indicate that genes linked to serogroup specificity were significantly over-represented in clinical isolates compared to environmental isolates. We also demonstrate a key role for lateral gene transfer in the evolutionary history of L. pneumophila LPS.

Conclusion: This study represents the largest population genomics study of L. pneumophila to date showing that strains responsible for Legionnaires' disease are drawn from across the species phylogeny. GWAS analysis highlights the role of the Legionella lipopolysaccharide (LPS) in human infection irrespective of clonal lineage, suggesting that specific LPS properties may enhance its ability to cause human disease.

The *Legionella* Genus Genome: a Global View of the Genus Evolution

Authors Gomez-Valero L., Rusniok C., Schroeder G., Danielle C., Mondino S., Cobas A. E., Rolando M., Reuter S., Dermitas J., Crumbach J., Descorps-Declere S., Frankel G., Jarraud S., Hartland E., and Buchrieser C.

Background Legionellosis is a severe pneumonia caused by bacteria belonging to the genus Legionella. Currently, there are 66 species/subspecies described within this genus but over 95% of Legionnaires' disease cases are caused by only two species: L. pneumophila and L. longbeachae.
The purpose of this work was to carry out the largest genomic comparison done until now at the genus level between different Legionella species/strains to decipher the genomic components that are responsible of their different capacities for human infection.

Methods With this aim, we have analyzed and compared 80 Legionella strains belonging to 58 Legionella species-subespecies. We have also developed bioinformatics approaches that allowed us to detect new eukaryotic motifs not described before and to predict new putative effectors in all studied species. Moreover we have validated for selected effectors that they are translocated via the Dot/Icm secretion system. Finally the potential virulence capacity of most of the strains has been explored using the human monocytic cell line THP-1

Results We found that species with higher clinical incidence also display enhanced infectivity in-vitro. Comparative genomics showed that presence of the Dot/Icm secretion system and of many eukaryotic like proteins are universal features of this genus. However, among the over 300 Dot/Icm effectors described for L. pneumophila, only 7 belong to the genus core genome. In addition our evolutionary analysis indicated that most of the predicted Dot/Icm effectors in the genus Legionella have been incorporated trough gene gain events and that loss events are less frequent.

Conclusion
This study provides the most complete global view of the whole Legionella genus, knowledge essential not only for Legionella research but also a step towards the study of genomic diversity at the bacterial genus level.

Whole Genome Sequence Analysis of the *Legionella* pneumophila Population Within the Water System of a Large Occupational Building

Authors Mentasti M., David S., Lai S., Vaghji L., Parkhill J., Ready D., Chalker V.

Background. The diversity of Legionella pneumophila (Lp) populations within single water systems is not well-understood, particularly in those never associated with Legionella cases. To gain insight into this matter, a collection of 239 Lp isolates from the water system of a large occupational building was obtained in 2013 by Public Health England and analysed by whole genome sequencing.

Materials/Methods. Lp isolates were obtained by culture (ISO 11731) from 28 water samples taken in 16 rooms. DNA was manually extracted (Wizard Kit, Promega) and genomes sequenced by Illumina X10. Raw sequence reads were mapped to the ATCC43290 reference genome. SNP-calling and phylogenetic analyses were performed, and sequence type
(ST) determined from the de novo assembly by an in-house script. Results. 60% of isolates were non-sg1 and 40% sg1 Lp. 235/239 isolates belonged to ST87 (32), ST27 (82) or ST68 (121), a triple-locus variant of ST27. ST87 has mostly been isolated from the environment, mainly in London, UK, and less commonly from other countries; ST68 has been isolated from clinical and environmental sources, and ST27 has been isolated mostly from clinical samples in the UK where it caused an outbreak in the West Midlands in 1985. 4 to 36 isolates (mean, 14.9) were obtained from each room, yet more than one ST was detected in only 3/16 rooms. Of 25 samples from which we sequenced multiple (up to 15) picks, variation at the ST level was found in 5. Pairwise SNP differences ranged between 0-5 for the ST87s, 0-18 for the ST27s and 0-19 for the ST68s.

Conclusion.Three different clones (i.e. ST87, ST68 and ST27) were isolated demonstrating diversity within a single water system with cultivable legionella. Only 1 ST was detected in most rooms, suggesting spatial structuring.
Furthermore, detection of genomic diversity within some samples, up to the ST level, highlights the importance of analyzing multiple picks for a more complete understanding of Lp population structure.

The 9th International Conference on

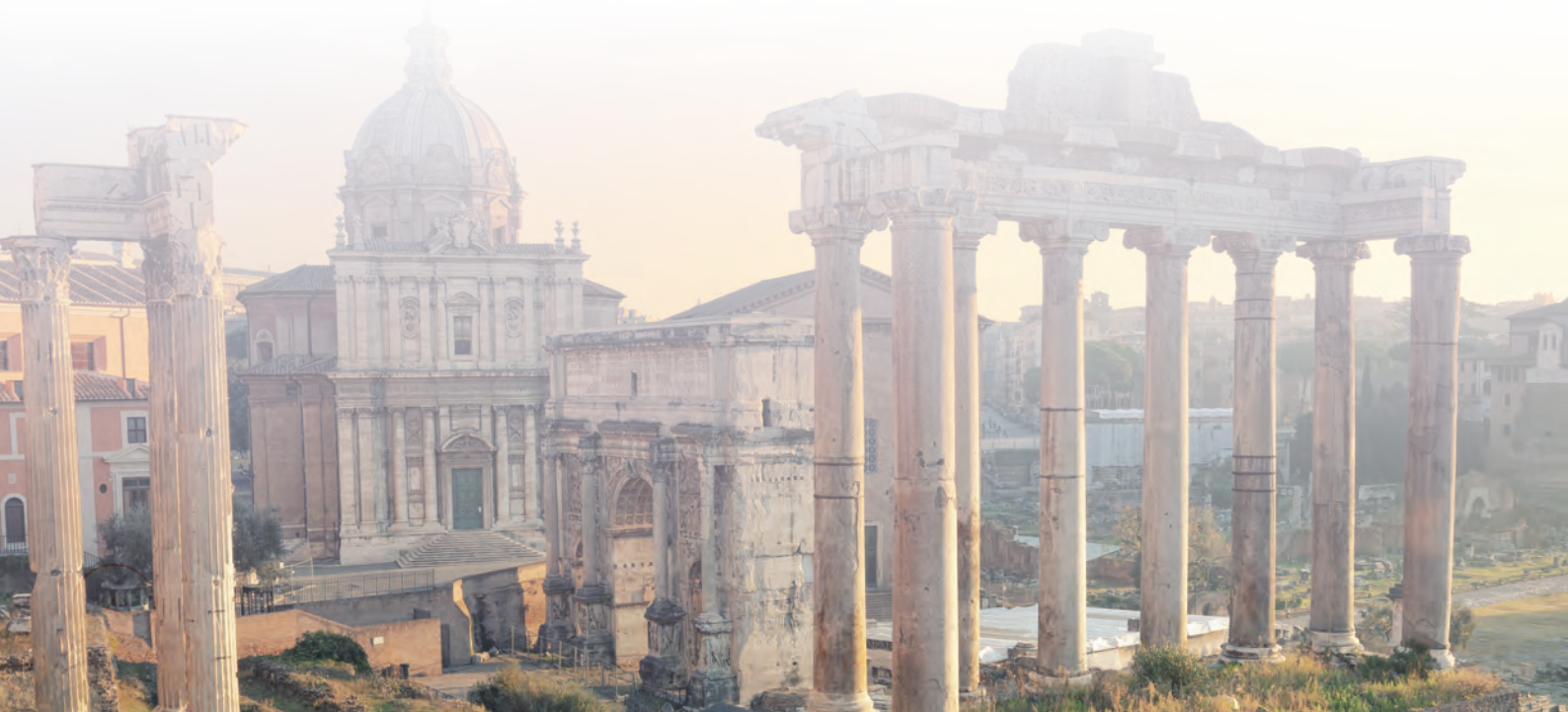
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

THURSDAY, SEPTEMBER 28TH, 2017

08:15-09:15 **SESSION 4 - CLINICAL ASPECTS, TREATMENT, ROUTE OF EXPOSURE AND HOST RELATIONSHIP**



Clinical Characteristics of *Legionella* Infection

Authors Paul H. Edelstein

Usual and unusual features of Legionnaires' disease and non-pneumonic Legionella infection will be presented

Route of Exposure to Aerosol and Legionnaires' Disease or Pontiac Fever

Authors Bentham R.

Legionellosis has two major clinical presentations, Legionnaires' disease and Pontiac fever. The former is a serious and sometimes fatal pneumonia the latter a self-limited 'flu-like' illness. The factors determining which clinical presentation will result from exposure are currently unclear. In the great majority of outbreak scenarios only one clinical presentation is reported. Copresentation of clinical symptoms from a common source is relatively rare. These observations point to the exposure route as a critical factor in determining disease presentation.

A large proportion of cases of Pontiac fever have been attributed to persons using spa pools. An interesting observation is that in three instances where cases of Legionnaire's disease were attributed to spa pools all three were demonstration models only and affected individuals did not use them. Pontiac fever was not reported in any of these three outbreaks. Proximity to the implicated spa pool was also a key determinant of disease.

This paper reviews documented reports of disease and theories of transmission and possible links between clinical presentation, source and exposure. The combination of the nature of exposure and the biophysics of aerosols may explain why using spa pools is more likely to result in Pontiac fever rather than Legionnaire's disease.

Legionella Aerosols from Shower to an Ex Vivo Human-Porcine Respiratory Model

Authors Allegra S., Girardot F., Riffard S., Pourchez J., Leclerc L., Forest V., Perinel S.

Background: How Legionella are aerosolized and enter the respiratory tract remains poorly documented. Data using animal experimentations led to the establishment of mathematical models whose extrapolation to humans is questionable. More physiological models of aerosols inhalation are needed.
The aim of our study, in a context close to the human anatomy and its physiological respiratory functions, was to (i) characterize the aerosols dispersed from sanitary hot water (SHW) through showers and (ii) determine the deposition pattern (sites, concentration and physiological state) of aerosolized Legionella in the lung region.

Materials/Methods: The physiological state and concentration of Legionella were assessed by qPCR for total cells, culture for viable and cultivable Legionella (VC), and flow cytometry for viable but non-cultivable Legionella (VBNC). Dispersed aerosols from showers were characterized using a 13-stage cascade low-pressure impactor. The human anatomy and the physiological respiratory functions were mimicked using a human replica of the upper respiratory airways connected to a pump mimicking the breathing parameters that correspond to an adult male physiology at rest. The deposition sites of aerosolized Legionella were determined using an ex vivo porcine lung and CellVizio technology.

Results: We determined that aerosols within the 0.26-2.5 µm size range may reach the pulmonary alveoli. Overall 0.7 % of viable cells (VC+VBNC) from dispersed suspensions were detectable. In the porcine lung, Legionella were found in the whole respiratory tract. 0.2 to 51% of the aerosolized Legionella populations reached the left upper lobe.

Conclusion: To our knowledge, it is the first time that experiments mimicking so closely a real human exposure were performed. New insights on aerosols dispersion and human doseresponse for LD are provided.

ABSTRACT BOOK

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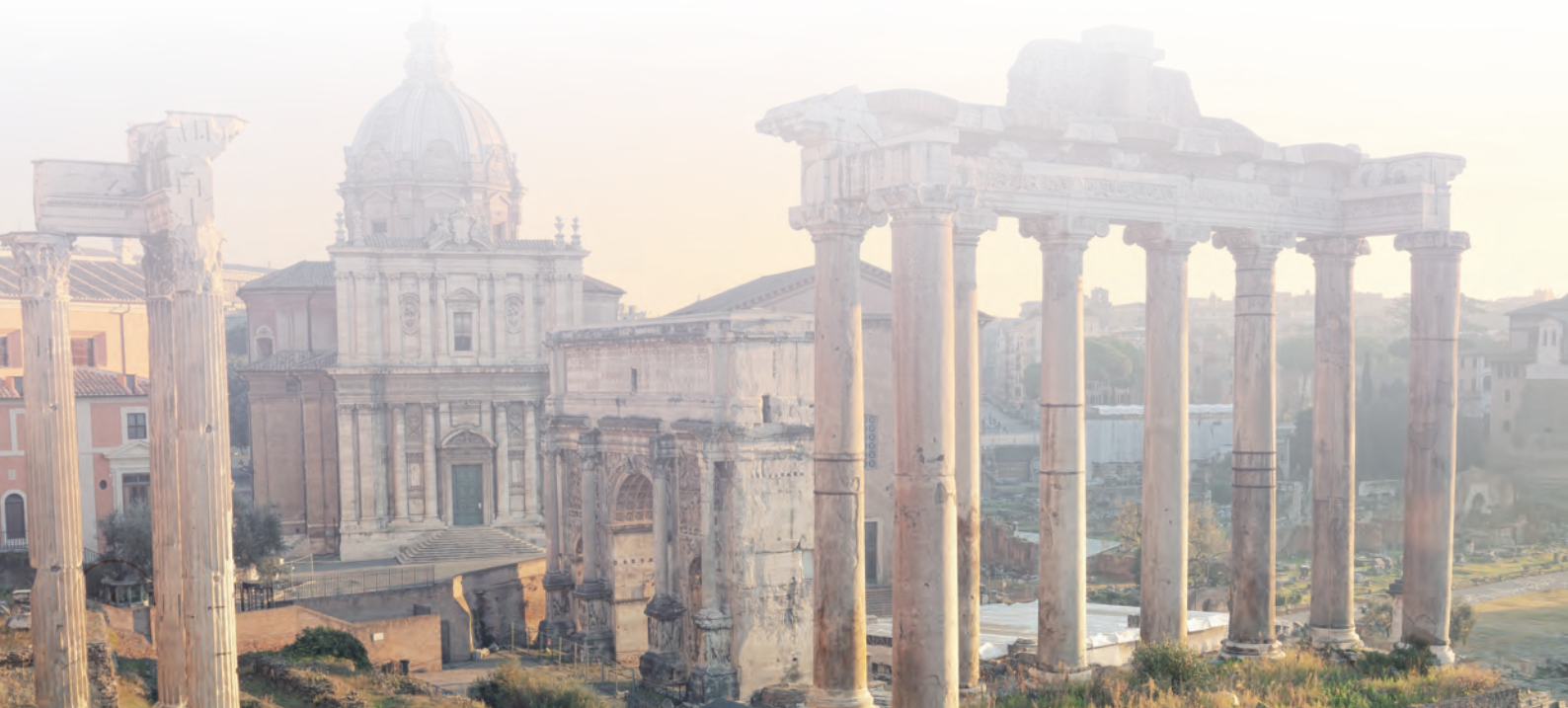
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

THURSDAY, SEPTEMBER 28TH, 2017

09:45-11:45 **SESSION 5 - DIAGNOSIS AND TYPING**



Diagnosis and *Legionella* typing of atypical forms of legionellosis

Author Sophie Jarraud

Through the description of 13 atypical cases of legionellosis characterized by the detection of Legionella in the lung over an abnormally long period of time, we will discuss the different hypotheses to explain the duration of these infections (recurrence, reinfection, resistance to treatment , presence of abscess). These Legionnaires' diseases are characterized by a persistent culture-positive over a period of 2 months to 1 year, with or without period of clinical improvement or cure during this period, despite treatment with appropriate antimicrobial agents.

Our aim is to better understand the microbiological, epidemiological, and clinical implications that may explain these persistent forms. During the investigations related to these cases, the available isolates were sequenced, the resistance to antibiotics evaluated by phenotypic and / or molecular methods such as targeted NGS for detecting minority subpopulations, the presence of abscesses sought after by imaging. The evolution of the bacterial and the fungal diversity in the lungs during infection and antibiotic treatment was evaluated by deep sequencing (Illumina) of the V3-V4 region of the 16S rRNA gene (bacteria) and the ITS1 region (fungi) on 7 respiratory samples from one patient.

These various investigations revealed a confirmed re-infection, a possible re-infection at the patient's home and 11 cases of Legionella persistence in the lungs over several weeks. No resistance to treatment could be demonstrated. The presence of an abscess rendering the treatment ineffective appears to be the cause of 5 persistent infections. The particular immune status of 7 patients appears to be the cause of the other persistent infections.

The Future of *Legionella* Typing in the Genomics Era

Author Jacob Moran-Gilad

In recent years, cluster investigation of Legionnaires' disease (LD) is increasingly carried out based on whole genome sequencing (WGS) of Legionella pneumophila (Lp). WGS gradually replaces the gold standard sequence-based typing (SBT) method with over a dozen LD outbreak investigations using WGS having been published over the last 2-3 years and a similar number of papers describe various applications of WGS for Lp control. Nevertheless, in order to implement WGS as a standardised and robust method for public health, many scientific, technical and operational aspects should be addressed.

The ESGLI Working Group (WG) on WGS of Legionella has been tasked with the development, validation and implementation of a WGS-based approach for Lp typing. Specifically, this WG is exploring alternatives for setting up a global WGS-based scheme that is standardised, robust, reproducible, scalable and fit-for-purpose. Moreover, the WG evaluates practical aspects of Lp WGS data sharing, storage, quality control and management, the nomenclature underpinning a WGS-based typing schemes and assurance of backwards compatibility with SBT.

The WG has agreed on the development and implementation of a core-genome MLST-based scheme for Lp typing, whereas other approaches (e.g. SNP calling) may be more suitable for ad hoc outbreak analysis. The proposed cgMLST scheme will involve ~50 loci out of >1,500 published targets, in order to balance discriminatory power with nomenclature, complexity and practicability. Selected core genome targets may also be useful for nested/direct typing on samples.
The WG has been exploring different working arrangements for genomic data management. The development of a computational solution for calling the mompS from WGS samples, ow ensures backwards compatibility. It is therefore envisaged that cgMLST will become the new ESGLI gold standard for Lp typing in the very near future.

LegioTyper: a Fully Automated and Rapid Detection Method for Serotyping of *Legionella pneumophila*

Authors Gründel A., Kober C., Petzold M., Herr C., Heese C., Lück C., Seidel M.

Background. Outbreaks of Legionnaires´disease (LD) occur worldwide. However, fast screening assays and sensitive detection methods are limited. Standard analysis of samples to identify and type L. pneumophila (L.p.) is time-consuming mainly due to a 10-day cultivation protocol.
The development of culture independent tools for serotyping in environmental and clinical samples is necessary for a fast and reliable comparison of different samples during e. g. outbreak investigations. The chemiluminescence sandwich microarray immunoassay (CL-SMIA) equipped with specific monoclonal antibodies (mAb) is a tool to differentiate serogroups and monoclonal subtypes of L. p. fully automated directly in different aqueous or urine samples.

Materials and Methods. CL-SMIA method was established on the microarray platform MCR-LT (Inst. Hydrology, TU Munich). Over 20 mAb's were immobilized on a microarray for subtyping L.p. in clinical and environmental samples. MAb's are based on the 'Dresden panel' with slight modifications (Inst. Med. Microbiology, TU Dresden). Biotinylated polyclonal antibodies detect bound antigen, followed by an incubation with poly-streptavidin-labeled horseradish peroxidase. A CL reaction is quantified by using the readout system MCR-LT. The procedure can be performed in less than 40 minutes.

Results. Detection and simultaneous typing of L.p. in different sample types was feasible and showed unambiguous and clear results. We were able to directly detect and type legionellae in water samples that were culture-negative e.g. due an induced viable but not culturable (VBNC) state. Furthermore, urine samples from culture-positive patients could be confirmed and subtyped.

Conclusion. This method proofed its capacity to directly detect and type L.p. in environmental and clinical samples independent of cultivation. The MCR-LT platform can be applied as monitoring tool for environmental samples and as diagnostic tool for clinical urine samples to detect and type L.p.

The Average Nucleotide Identity Index is a Taxonomic Tool That Can Also Be Useful for Determining the Epidemiological Relationship Between *Legionella pneumophila* Genomes

Authors Figueras M. J., Sanchis M., and Perez-Cataluña A.

Legionella is an important human pathogen causing nosocomial and community-acquired pneumonia mainly in immunosuppressed population. When an outbreak occurs the use of appropriate genotyping methods able to identify the focus of contamination and routes of transmission during epidemiological survey are essential.

A large number of subtyping techniques have been used such as AFLP or PFGE but The European Working Group for Legionella Infections establish the sequence based typing (SBT) as the current "gold standard" and propose an epidemiological typing scheme for clinical and environmental isolates of Legionella pneumophila (Lp).

To improve the epidemiological concordance in outbreaks of legionellosis, the new trend is the used of whole-genome sequences and it was proposed that a scheme with ± 50 genes provides optimal epidemiology concordance. However, this approach is still complex and time consuming.

The objective of this study is to evaluate if the average nucleotide identity (ANI) that is a taxonomical tool to compare genomes to determine if they belong to the same species (values >96%) could be used for the epidemiological characterization of outbreak strains. We selected 31 existing annotated Lp genomes from the NCBI associated with specific outbreaks and others unrelated to be used as controls.

The OrthoANI (Orthologous Average Nucleotide Identity) software tool was used to compare the genomes. The ANI obtained for all tested Lp strains was > 96% indicating that all were correctly assigned to these species. However, ANI values obtained when we compared strains associated with outbreaks (ex: the Philadelphia strains) ranged from 99.99% to 100% evidencing that the compared genomes came from the same strain.

In conclusion, ANI showed to be a fast and easily to handle tool that enabled to recognize if genomes coming from clinical Lp cases were identical to those recovered from the environment, demonstrating the specific strain responsible of the outbreak.

Utility of rapid techniques in the Legionnaires´ disease outbreak in Manzanares, Spain, December 2015-February 2016

Authors Cebrián F., Fernández P. J., Montero J. C.

Background: An explosive outbreak of Legionnaires' disease (LD) with 277 confirmed cases was identified on 11 December 2015 in Manzanares, Spain, and was declared closed by 03 February 2016. This is the outbreak with the highest attack rate (14.9/1000 inhabitants) in the entire history of the illness.
Rapid microbiological analysis identified several sources of risk, among others an industrial cooling tower and a public fountain, to be the probable source of the infection. Utility of rapid techniques is presented.

Materials/Methods: Environmental samples were tested by both a rapid technique based on the immunomagnetic separation (IMS) and the culture method (ISO11731). All testing was carried out by the Regional Public Health Laboratory of the Instituto de Ciencias de la Salud in Talavera de la Reina (Toledo, Spain). The strains isolated by culture were identified by sequence-based typing.
Results: 307 environmental samples were analyzed, 171 of them processed by culture method (positivity of 5.3 %) and 149 processed by IMS method (positivity of 10.7 %).

Results of the environmental investigation were consistent with epidemiological hypothesis eyeing the cooling tower (OR = 3.9; p: 0.003) and the decorative fountain (OR = 5.7; p: 0.03) initially positive by IMS method as most likely causes of this outbreak.

Conclusion: The identification and the shut-down of the suspicious sources in the early stage (hours) of the outbreak may have contributed to reduce the Legionnaires' disease impact on the public health, by significant reduction of the time for which the population was exposed to the bacteria. This is the first time that an immunomagnetic test method certified by AOAC-RI has been used in the early stage of an outbreak investigation. Preventive closure of the positive facilities has been able to contribute to the low mortality rate (1.4 %). Rapid laboratory environmental testing must be carried out to reduce the impact of similar LD outbreaks in the future.

ABSTRACT BOOK

The 9th International Conference on

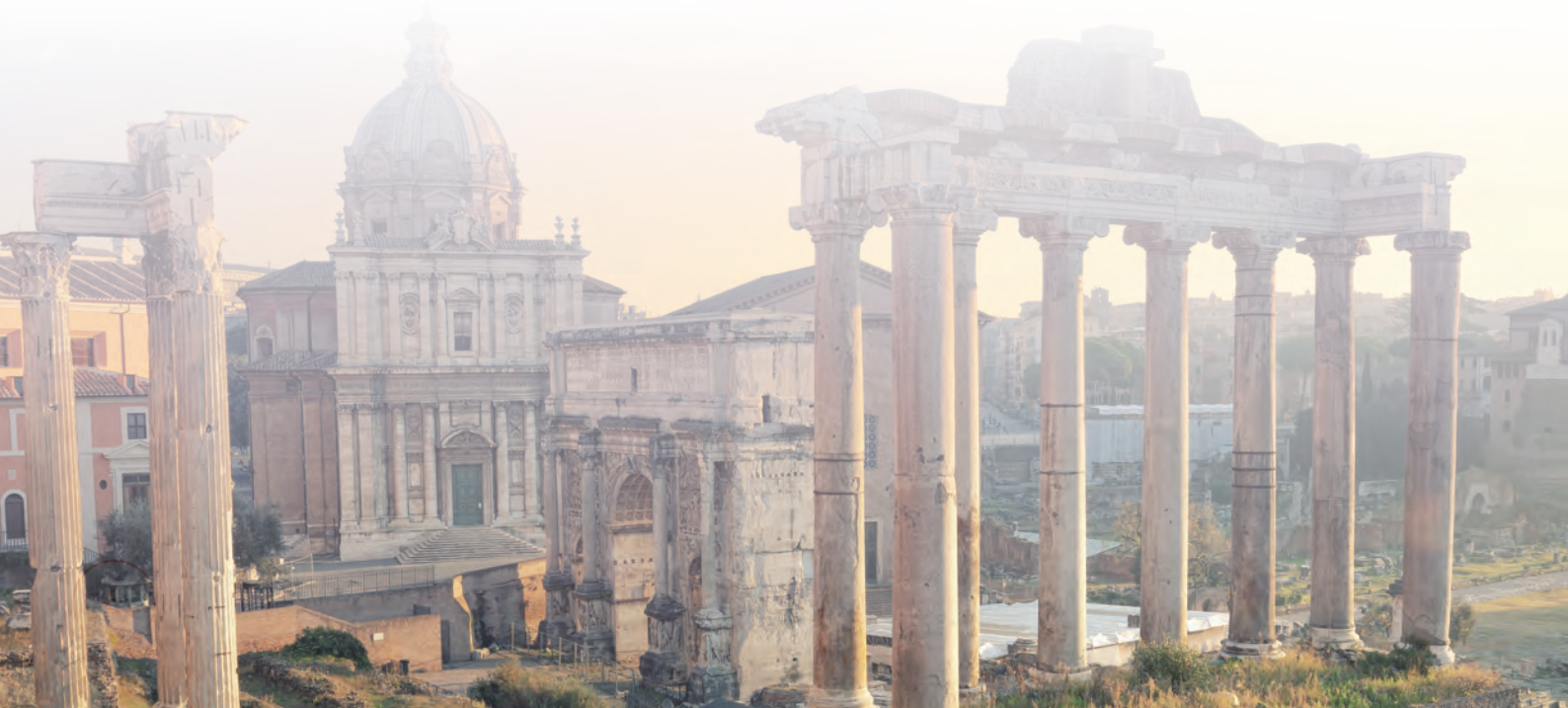
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

THURSDAY, SEPTEMBER 28TH, 2017

14:30-16:15 **SESSION 6 - SURVEILLANCE AND OUTBREAK INVESTIGATIONS**



Legionnaires' disease from the other side of the world: the New Zealand perspective

Authors Murdoch D. R., Priest P. C., Slow S., Chambers S. T.

Our current understanding of the epidemiology, diagnosis, treatment and prevention of Legionnaires' disease is based on dogma largely arising from the northern hemisphere. This view has changed little over recent decades, driven by an emphasis on Legionella pneumophila as the causative agent, a focus on outbreaks, reliance on traditional diagnostic tools, and the assumption that Legionella species are a rare cause of pneumonia.

New Zealand has among the highest reported incidence of Legionnaires' disease globally. Although New Zealand has a relatively high incidence of Legionnaires' disease caused by the species L. pneumophila, about two thirds of cases are caused by Legionella longbeachae. As a consequence, Legionnaires' disease in New Zealand has a mixed epidemiology, with a large proportion of cases occurring sporadically associated with gardening activities in spring and summer. The early and widespread adoption by diagnostic laboratories of nucleic acid detection methods in the country is partially responsible for New Zealand's relatively high reported incidence of Legionnaires' disease through improved case detection.

The innovative use of laboratory diagnostics in New Zealand has also likely led to improved detection of cases caused by non-L. pneumophila species, and a better understanding of the national epidemiology of Legionnaires' disease than any other country. This knowledge is being used to establish improved management guidelines for cases of suspected Legionnaires' disease and to better direct preventative strategies for this disease.

A National Cross-Sectional Study to Uncover the Hidden Burden of Legionnaires Disease: The LegiNZ Study

Authors Priest P. C., Slow S., Chambers S. T., Sheerin I.G., Hope V. T., Murdoch D. R., the LegiNZ Investigators

Background: Legionnaires' disease is regarded as being relatively uncommon, but the true burden has not been rigorously assessed. Many parts of the world have limited, if any, data on the incidence of Legionnaires' disease simply because diagnostic testing is infrequently or not performed. We hypothesised that many cases of Legionnaires' disease are undiagnosed and there would be a marked increase in cases following the introduction of routine PCR testing.

Materials/Methods: LegiNZ was a prospective cross-sectional study, in which active surveillance was used to maximise the identification of cases of Legionnaires' disease over a 1-year period from 20 hospitals covering a catchment area of 96% of New Zealand's total population. Lower respiratory samples from patients hospitalised with pneumonia were routinely tested for Legionella by PCR, whether specifically ordered by clinicians or not. Additional cases of Legionnaires' disease were identified through mandatory notification to Public Health Units.

Results: During the study period, 6044 lower respiratory samples were tested by PCR, from which 222 cases of Legionnaires' disease were diagnosed. An additional 26 hospitalised cases were detected through notifications. 60% of all cases were caused by Legionella longbeachae and 23% by Legionella pneumophila. The calculated incidence of hospitalised Legionnaires' disease was 5.4 per 100,000 population for the country, and as high as 9.8 per 100,000 in some regions. This compares to an average annual reported incidence for New Zealand of 2.6 per 100,000 over the 10 preceding years. Cases were identified across the country, including at high incidence in some regions that had identified few cases in the past.

Conclusion: Routine PCR testing doubled the detection of Legionnaires' disease across New Zealand. Many cases of Legionnaires' disease remain undetected if the ordering of diagnostic tests relies solely on the discretion of clinicians.

Community-Acquired Cases of Legionnaires' Disease: the Proportion Technically Preventable by Applying the German Drinking Water Ordinance?

Authors Buchholz U, Jahn HJ, Reber F, Lehfeld A-S, Brodhun B, Haas W, Lück C, Gagell C, Schaefer B, Stemmler F, Otto C, Bärwolff S, Beyer A, Geuß-Fosu U, Hänel M, Larscheid P, Mähl P, Morawski K, Peters U, Pitzing R, von Welczeck A, Widders G, Wischnewski N, Eichendorff C, Hinzmann A, Klosinski M, Nürnberger E, Schilling B, Schmidt S, Schumacher J, Sissolak D, Zuschneid I, Angermair S, Arastéh K, Behrens S, Borchardt J, Creutz P, Danckert J, Deja M, Elias J, Gastmeier P, Kahnert H, Laun R, Lehmke J, Leistner R, Naumann M-B, Pankow W, Pross M, Scherübl H, Stocker H, Sturm A, Wilbrandt B.

Background It is assumed that residential drinking water (RDW) is responsible for a large proportion of community-acquired cases of Legionnaires' disease (CALD). Therefore, Germany adopted in 2011 an amendment to the Drinking Water Ordinance requiring the mandatory monitoring of Legionella in publicly and commercially used complex drinking water systems. It established a so-called technical measures trigger value set at 100 colony-forming units per 100 ml water for the presence of Legionella spp. Exceeding the value requires a risk assessment of the DW system followed by remediation of structural deficiencies. We devised a study to calculate the proportion of CALD preventable by applying the ordinance.

Methods We set the study up in the 12 health departments of Berlin and started the pilot phase in December 2016. We interviewed CALD cases and elicited the type of RDW system (complex vs. non-complex system). We endeavored to take water samples from the households of all CALD cases, as well as from non-RDW sources cases were exposed to. We determined Legionella concentration in water samples and typed strains for serogroup, monoclonal antibody (mAb) and – if applicable – sequence type. We did two analyses: (1) using numerator data and applying them to the total number of CALD cases (direct/conservative approach); (2) using the percentages of CALD with known information and extrapolating them (multiplicative/estimative approach). Analysis was done with MS Excel, v2010.

Results Between December 2016 and April 2017 35 CALD cases were notified. One case was attributed to a non-DW source using mAb-typing. Of the remaining cases, the direct and conservative approach yielded between 19% and 66% of CALD cases that may be preventable by applying the drinking water ordinance.

Conclusion So far only a small proportion of CALD cases could be attributed to non-RDW sources. The potential preventative effect of the drinking water ordinance remains to be elucidated.

Legionella pneumophila in the Flint Water Supply: A Molecular Epidemiology and Virulence Assessment

Authors Byrne B., McElmurry S., Sadler R., Kilgore P., Love N., FACHEP research group, and Swanson M.

Background: Flint, Michigan had outbreaks of Legionnaires' disease in 2014 and 2015, when their water source and treatment changed. To inform risk management strategies, we are analyzing legionellae in southeast Michigan.

Materials & Methods: To evaluate legionellae diversity, Sequence Type (ST) was determined for 20 strains isolated from Flint and Detroit residences in Fall 2016 and 33 clinical isolates from hospitals in three counties in 2013 -16. Serogroup (SG) was verified by immunostaining.
Virulence of the environmental isolates was gauged by quantifying infection of primary mouse macrophages by microscopy and enumerating colony forming units.

Results: Only SG1 ST1 L. pneumophila was common to both environmental and clinical samples. Infection of macrophages was similar for the one residential and four clinical SG1 ST1 isolates. In residences, SG6 strains were the most common L. pneumophila isolated, and the majority were the closely related ST367 and 461. Macrophage infection was similar for the 16
SG6 ST367 and 461 isolates and the SG1 clinical isolates (>100-fold CFU increase in 72 h).
Intracellular replication was also similar for the 16 strains obtained from Flint homes and 2 strains from residences on a different water source.

Conclusion: Globally, ST1 L. pneumophila strains are frequently isolated from patients and the environment. Others isolated ST1 strains from a Flint hospital that treated Legionnaires' disease patients. Additional analysis may determine whether these ST1 isolates are clonal.
Previous studies from Spain, Greece, and Israel isolated SG6 ST367 and 461 from the environmental and patients. Because the Urinary Antigen Test does not readily detect non-SG1
L. pneumophila, disease caused by SG6 is likely under-diagnosed worldwide. By increasing knowledge of residential colonization and the risk of SG6 and ST1 L. pneumophila, we aim to extend the proud legacy of Michigan's public health and research community who discovered Pontiac Fever.

Spatial Distribution of Legionellosis at the Small Area Level in the City of Barcelona: 2000-2016

Authors Gallés P., Valero N., Marí-Dell'Olmo M., Gómez A.

Background In the city of Barcelona the majority of the reported cases of Legionnaire's Disease (LD) are sporadic cases not associated with an exposure source.
Although studies have shown the link between sporadic cases and sources as cooling towers or home water supplies the source of infection remains unknown for most cases.
The study aims to analyse the spatial distribution of sporadic LD cases at small area level in the city of Barcelona from 2000 to 2016 and to identify spatial clusters of risk.

Materials / methods We have conducted a cross sectional ecological study. Two spatial unit were used to perform the analysis: neighbourhood (73 areas) and statistical basic area (SBA) (233 areas). Raw and standardized incidence rates by age were performed. Moran's I global and local indexes were used to analyse spatial autocorrelation rates.
Smoothing of these rates was performed by the Full Bayes method for studying the spatial pattern. Results were represented in maps of the two spatial units used in the study. All the analyses were performed by sex.

Results In total, 851 sporadic LD cases with unknown source of exposure were recorded (80% of total reported cases) of which 64,7% were men and 35,3% were women, and 75,4% were over 45 years of age in the 6 years studied.
The incidence rates for the whole study period for the SBA were from 0 to 2,02 for the ten thousand inhabitants, from 0 to 4,01 for men and from 0 to 1,82 for women.
We have found three separate cluster areas with high values for the considered period.

Conclusions Spatial analysis techniques are a useful tool to identify geographical pattern of disease and they provide added value to the epidemiological surveillance. Identification of small areas with a high incidence of LD could help to identify unknown exposure sources of LD cases and prioritize prevention and control resources.

Factors Associated With the Occurrence of Further Cases in Accommodations Associated With a Cluster of Travel-Associated Legionnaires' Disease Cases After Implementation of Control Measures

Authors Beauté J., Sandin S., de Jong B., Payne Hallström L., Robesyn E., Giesecke J., Sparén P.

Background: The detection of a cluster of travel-associated Legionnaires' disease cases (TALD) in any EU Member State prompts action at the accommodation, follow-up by health authorities and reporting to ECDC of measures taken. Some accommodations are reported with further cases despite the presumed implementation of adequate control measures. The objective of this study is to identify factors associated with the occurrence of further cases after implementation of control measures to improve prevention.

Methods: Hotel and holiday rental accommodation sites located in the EU associated with two or more TALD cases with onset dates less than two years apart (a 'cluster') and notified during 1 June 2011?31 December 2016 were included. The occurrence of a further case was defined as any case with onset date after the report on measures taken. We fitted a Cox regression model to estimate the association between accommodation characteristics and the occurrence of a further case.

Results: Of the 372 accommodations notified with a cluster during the study period, 358 (96%) had available information on follow-up. These accommodations were notified with an average of 2.5 TALD cases. Of these, 91 (25%) were associated with at least one further case after the report on measures (12 per 100 accommodation*years) after a median time of 305 days (IQR 166-618). Accommodations previously notified with at least one TALD case before the cluster notification were more likely to be associated with further cases (hazard ratio 1.7; 95%CI 1.1-2.7). Accommodations with 40-79 and 80-159 rooms were more likely to be associated with further cases compared to those with less than 20 rooms. Neither the detection of Legionella in the water system nor the type of disinfection were found to be associated with the risk of reporting a new case.

Conclusion: TALD cluster sites of medium size or previously notified with a TALD case should receive special attention with possibly scaled-up control measures.

Epidemiology and Microbiology of Recurrent Accommodation Sites Implications for Surveillance and Prevention

Authors Crespi S., Ricci M. L., Nicolau A., Drasar V., Scaturro M., Caporali M. G., Bella A., Gumá M., Rota M. C.

BACKGROUND The existence of accommodation sites repeatedly associated with cases of travel-associated Legionnaires' disease (TALD) has long been recognized but little is known about their underlying causes. In addition, present ELDSNet cluster definition could be suboptimal for both identifying and managing these sites.

METHODS We have analysed retrospectively all TALD cases occurred in the period 2005-2015 in Italy and Balearic Islands (Spain), applying the current ELDSNet cluster and single case definitions and a new proposed definition of recurrent accommodation sites, defined as sites associated with multiple linked cases, regardless of the time elapsed between them.
In 54 sites across a higher number of countries, all associated with 3 or more cases and positive Legionella testing results (hereby recurrent sites series), we additionally conducted a microbiological study.

RESULTS Overall, 385 cluster associated sites and 1151 clustered cases were identified in the study period, of which 358 sites and 1070 cases were identified in the Italian database and 27 sites and 81 cases in the Balearics database. Using the recurrent site definition, 63 additional sites (16,4% increase) and 225 additional linked cases (19,5% increase) were identified (60 sites and 210 cases in Italy and 3 sites and 15 cases in Balearics).
In our microbiological study, Legionella pneumophila sg1 was isolated in 49 (90,7 %) sites. For the subgroup (30 sites) with available MAb typing, 24 (80 %) were colonized by MAb 3/1 positive strains.

CONCLUSIONS 1) The use of recurrent site definition would allow identifying more potentially preventable situations than the present ELDSNet definitions both in terms of new sites and cases. 2) The high prevalence of MAb 3/1 positive strains in recurrent accommodation sites suggests that this is a key causal factor and highlights the relevance of such finding in TALD investigations.

ABSTRACT BOOK

The 9th International Conference on

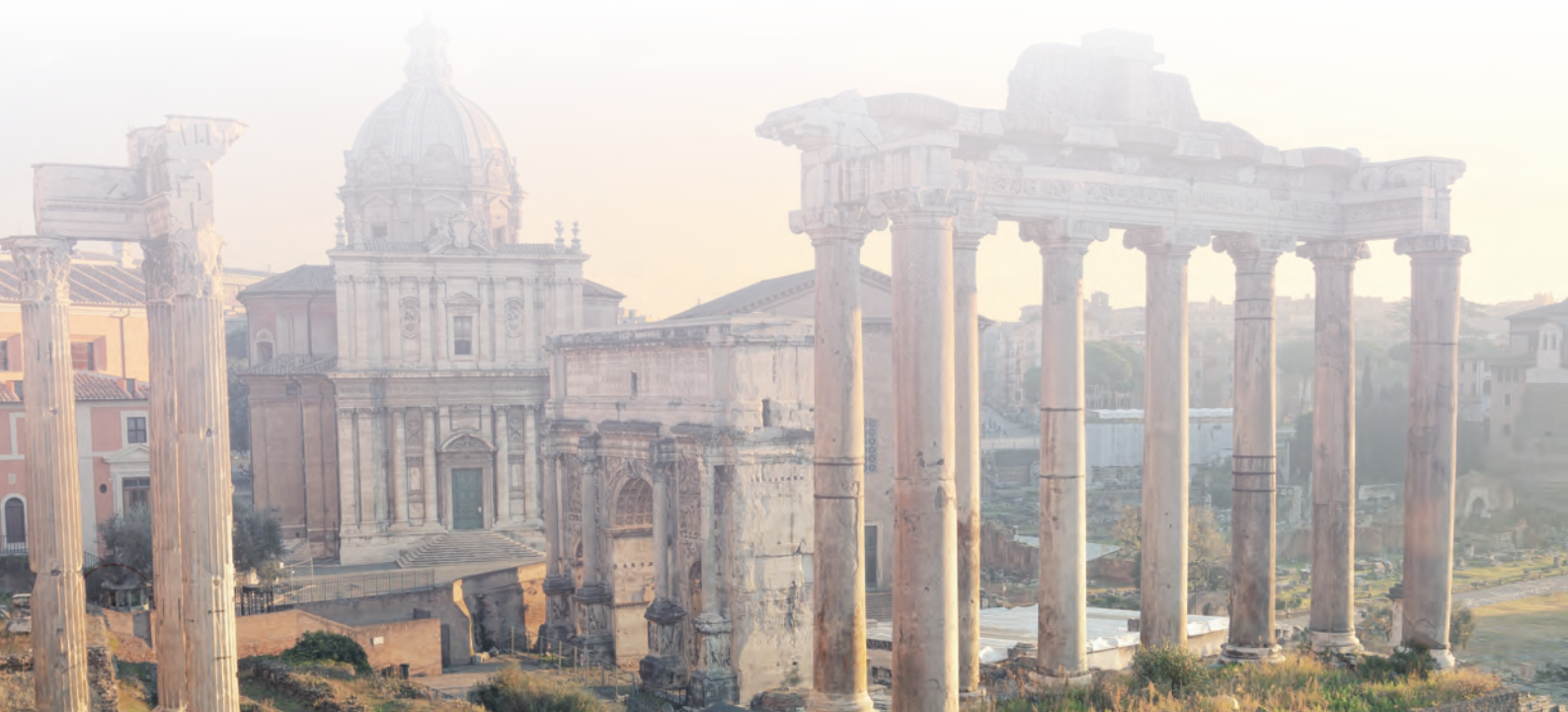
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

FRIDAY, SEPTEMBER 29TH, 2017

08:15-10:00 **SESSION 7 - ECOLOGY OF *LEGIONELLA* AND INTERACTION WITH ENVIRONMENTAL RESERVOIRS**



The original cat and mouse interplay: with picky amoebae and persistent *Legionella pneumophila*

Author Ashbolt N. J.

Background: The colonization of free-living amoeba (FLA) by bacteria is an ancient relationship, as seen through conserved genes such as in epibionts, where the fibronectin type III domain proteins bind bacteria via flagellin. Some of these functional genes have evolved into 'virulence' genes, likely from beneficial host-microbe interactions in various symbionts. Beyond that, feeding habits of amoebae and defensive responses in host and prey are not well described; Given that FLA (amongst other likely environmental host cells) are a major host for L. pneumophila (Lp) amplification in drinking waters, and human macrophages are an accidental host. The focus here is on developing a mathematical model to identify key features of this host-prey interaction that may inform exposure assessments and ultimately control strategies for legionellosis.

Approach: As the genomes of most amoebae are poorly described, we started by identifying what we do know about Lp-Acanthamoeba interactions, and from surveys that identified Acanthamoeba, Naegleria, and Vermamoeba spp. as the most common amoebae in drinking water systems (the main source for cases in North America). We replaced Naegleria, with Willaertia magna, and compared its features for Lp predation and growth to members of the other two genera. Next, a conceptual model was described for Lp growth within drinking water biofilms, largely to highlight what data needs maybe more important to estimate factors important to Lp exposures.

Results: Our mathematical model is hierarchical, starting with hydraulic condition (stagnant vs flowing), sessile cell detachment during flow, and Lp feeding/growth/release from susceptible trophozoites vs digestion by resistant amoebae. This helped highlight possible critical amoebal densities in water biofilms and hence identifying amoebae as possibly useful targets for early management of conditions conducive for Lp amplification. Additional elements now considered important for research include preferential grazing on none-Lp cells, which may explain why stagnant conditions are not only important for biofilm growth, but also reduced wash-out of released Lp from infected amoeba and a greater chance for amoebal grazing to provide a positive feedback growth loop. In using W. magna we also identified 1) over 1-year long persistence of active but non-growing Lp cells within trophozoites and 2) that excreted Lp-vesicles could be an important environmental form, previously not specifically modelled in Lp exposure assessments. Next partitioning factors (bulk water to aerosols) of free cells, Lp associated with trophs, cysts and vesicles will be added to evaluate their importance.

Conclusion: Exposure modeling is useful in the absence of quantitative data to aid the identification of important factors, here described for Lp growth in piped water systems. In addition to amoebal density, understanding the well-developed picky feeding habits of common amoebae has highlighted subtle points not previously model that seem critical for blooms of Lp in drinking water systems.

Natural antimicrobial substances to control proliferation of *L. pneumophila*

Authors Berjeaud J.M., Loiseau C., Portier E., Depayras S., Rouxel M., Corre M. H., Verdon J.

L. pneumophila is frequently encountered in hot-water distribution systems, cooling towers as well as in other manmade aquatic systems. In these environments, L. pneumophila is generally found associated to amoebae and / or biofilms. The interactions which may occur between the microbial flora and L. pneumophila in these aquatic communities are important in regard to the regulation of population dynamics in bacterial ecosystems particularly by the production of antimicrobial compounds.

The aim of the present study was to investigate the capability of aquatic bacteria, particularly Pseudomonas spp. to inhibit L. pneumophila by producing antagonistic compounds. The anti-Legionella molecules were purified and characterized.
Pseudomonas strains were tested, using spot on lawn assays, for their antagonistic activity towards L. pneumophila. The diffusible antimicrobial compounds were purified from culture supernatant, using ethyl acetate extraction and reverse phase HPLC then characterized using LC-MS.

Pseudomonas strains (17/25) were found to secrete anti-Legionella compounds. The anti-Legionella activity of active Pseudomonas strains was correlated to the secretion of biosurfactants. Pseudomonas is known to produce many biosurfactants classified in two families lipopeptides and rhamnolipids. Finally, the anti-Legionella activity was directly correlated with all the isolated compounds belonging to these two types of biosurfactant.
Interestingly, these compounds were found to display activity towards all the Legionella spp. tested. Moreover, the activity of these molecules was shown to be higher towards Legionella strains than all other bacteria, Gram positive or Gram negative, assayed.

In conclusion, this study confirms the particularly sensitivity of Legionella to detergent-like molecules. Moreover, these results reveal that natural biomolecules secreted by aquatic bacteria could represent potent tools for the biological control of L. pneumophila.

Survival patterns and virulence of viable but non-culturable Legionellae induced by starvation

Authors Schrammel B., Cervero-Aragó S., Dietersdorfer E., Sommer R., Repic A., Stockinger H., Walochnik J., Kirschner AKT.

Background: In response to environmental stress, Legionella cells are able to switch into a viable but non-culturable (VBNC) state in which they are unable to form colonies on standard media. This state is seen as survival strategy to escape unfavourable conditions, but it is still unclear to what extent VBNC Legionella cells are relevant for human health.

Methods: We induced the non-culturable state of six Legionella strains by starvation in ultrapure water at 45°C. The transition to the VBNC state was monitored for up to 13 months by a set of different parameters like culturability, membrane integrity, esterase activity, the presence of virulence markers and uptake of amino acids. Distinct VBNC cell subpopulations were identified by flow cytometry. In parallel, virulence against Acanthamoeba and three human macrophage cell types was assessed.

Results: Legionella strains became fully unculturable between 10 and 60 days of starvation. After initial 0.1 to 3 log reduction of viable cells, a constant part of the population showed stable signs of viability. Distinct VBNC sub-populations were identified: cells with high and low esterase activity and cells with intact and partly damaged membranes. 1 to 85% of all cells remained with intact cell membrane and 0.04 to 5% with high esterase activity for ~200 days of starvation. LPS and surface protein virulence markers were present at high levels throughout the experiment. Most interestingly, all L. pneumophila SG1 strains remained infective for all four hosts up to 360 days after loss of culturability. However, the VBNC cells exhibited significantly reduced virulence, with longer incubation periods, higher necessary multiplicities of infection and lower percentages of infected host cells.

Conclusion: This is the first report that starved VBNC Legionella cells can directly infect human macrophages. Thus, they may pose a risk for human health, however with significantly reduced virulence in comparison to culturable cells.

Legionella pneumophila Prevents Cell Division and DNA Replication of Acanthamoeba Castellanii

Authors Mengue L., Régnacq M., Aucher W., Portier E., Héchard Y and Samba-Louaka A.

Acanthamoeba castellanii is considered as a “trojan horse” of the microbial world as it allows multiplication and protects several bacteria. Legionella pneumophila, the causative agent for legionellosis, is one of the most studied A. castellanii resisting bacteria. Many host cellular pathways are modulated by L. pneumophila. Our objective was to test whether L. pneumophila would modulate the proliferation of A. castellanii.

In order to achieve our goal, A. castellanii were co-cultured 2 h at 30°C with L. pneumophila Paris. To study Acanthamoeba proliferation, cell division and DNA synthesis were determined respectively through time-lapse imaging experiments and incorporation of an analogue of thymidine (EdU). Determination of the protein essential for multiplication of A. castellanii was performed through genetic complementation experiments within the yeast Saccharomyces cerevisiae.

We found that L. pneumophila is able to impair proliferation of infected A. castellanii. Time lapse microscopy showed that, in addition to impair cell division, L. pneumophila induced modifications in shape, motility of A. castellanii. Use of Edu demonstrated that infection inhibited DNA replication within A. castellanii. This effect seemed controlled by an effector secreted by L. pneumophila since a mutant in its type IV secretion system (dotA) did not impair proliferation. Thus, we searched for cyclin dependent kinase (CDK) genes in the A. castellanii genome and found one gene, CDC2b, which is similar to the main cell cycle regulator gene in human (CDK1) and Baker’s yeast Saccharomyces cerevisiae (CDC28). By genetic complementation experiments, we establish that the amoebal protein CDC2b might be a CDK. To our knowledge, L. pneumophila could be the first bacterium regulating the ukaryotic cell cycle through downregulation of the host CDK expression. In conclusion, L. pneumophila impairs Acanthamoeba castellanii cell cycle by a mechanism which remains to be elucidated.

Temperature Induced Viable but Non-Culturable *Legionella pneumophila* Cells are Virulent Against Amoebae and Macrophage-Like Cells

Authors Cervero-Aragó S., Schrammel B., Dietersdorfer E., Sommer R., Repic A., Stockinger H., Walochnik J., Kirschner AKT.

Background: In response to environmental stress, Legionella cells are able to switch into a viable but non-culturable (VBNC) state in which the bacterial cells are unable to form colonies on standard medium. This state is seen as a survival strategy to escape unfavourable environmental conditions, but it is still unclear to what extent VBNC Legionella cells are relevant for human health.

Methods: We induced the non-culturable state of two L. pneumophila strains by incubation in ultrapure water at two temperatures relevant for the management of hot-water systems (55°C, 60°C). The transition of the bacteria to the VBNC state was monitored at different time points by following culturability and measuring viability parameters such as esterase activity and membrane integrity by flow cytometry. In parallel, the virulence of the two strains against an Acanthamoeba strain and a macrophage-like cell line (THP1) was assessed.

Results: Although differences were observed between the two L. pneumophila strains, complete loss of culturability (8 logs) at 55°C and 60°C occurred after 3 to 24 hours and 60 min, respectively. Depending on the temperature, after two weeks up to 2.5% (1.5 log reduction) of the population was still viable. At 55°C and 60°C, the two L. pneumophila strains remained infective for both hosts up to 75 days after complete loss of culturability. However, the VBNC cells exhibited significantly reduced virulence, with longer incubation periods, required higher multiplicities of infection and revealed a lower percentage of successfully infected host cells.

Conclusion: This is the first report that thermally induced VBNC Legionella cells can directly infect human macrophage-like cells. Thus, they may pose a risk for human health, however with significantly reduced virulence in comparison to culturable cells.

ABSTRACT BOOK

The 9th International Conference on

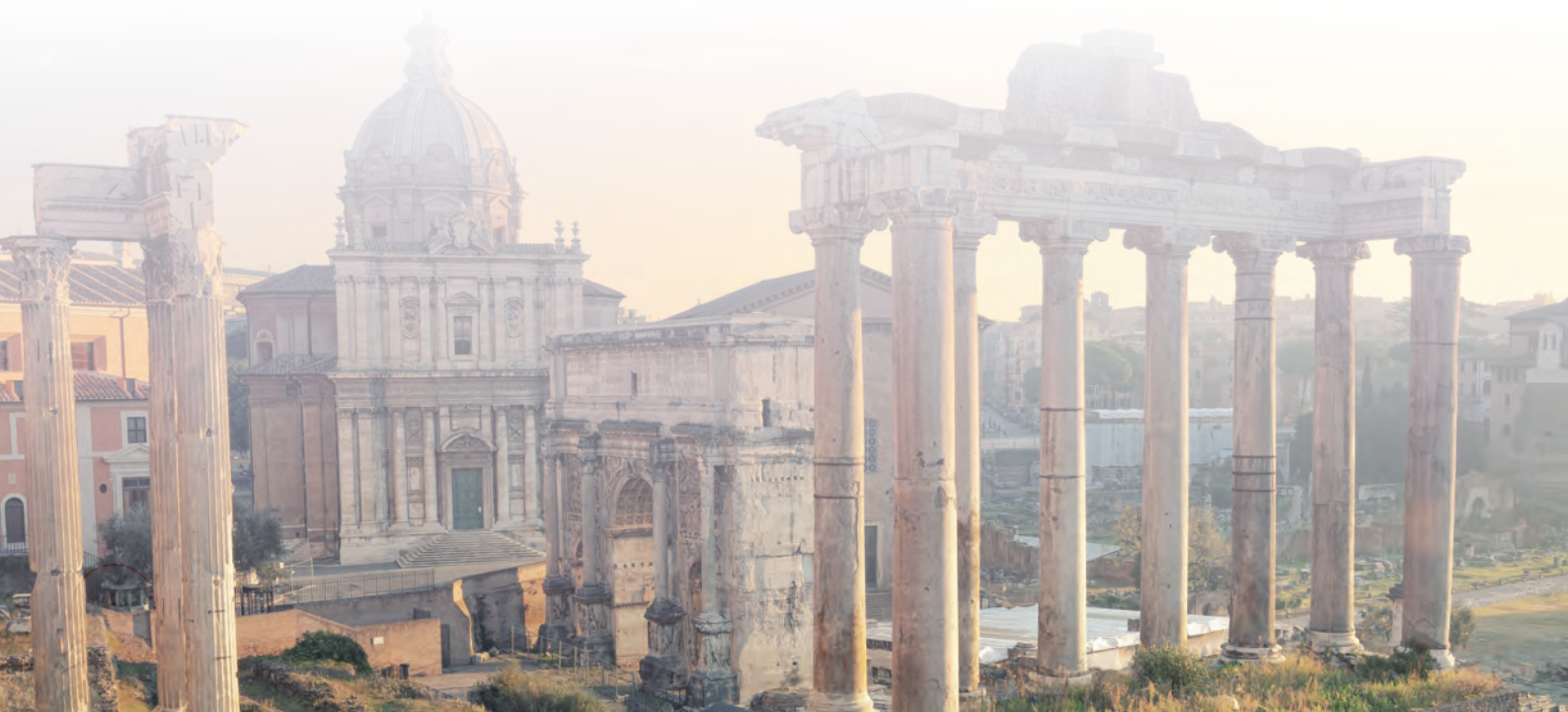
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

FRIDAY, SEPTEMBER 29TH, 2017

10:30-12:00 **SESSION 8 - *LEGIONELLA* DETECTION AND DIFFUSION IN THE ARTIFICIAL RESERVOIRS**



Legionella Colonization of Water Systems Aboard Passenger Ships and Differences with Accommodation Sites (Hotels Etc)

Authors Mouchtouri V., Kyritsi M., Kostara E., Nakoulas V., Hatzinikou M. and Hadjichristodoulou Ch.

Background: Hotel-associated Legionnaires' disease remains a significant cause of travelassociated respiratory tract infection among tourists in Greece. The aim of the study was to assess the colonization of hotel water supply systems in touristic places of Greece with Legionella spp., and to investigate possible association of physicochemical parameters with the presence of the bacterium.

Materials/Methods: 556 water samples from 51 hotels were analyzed. Water sampling and microbiological analysis were performed following the ISO methods. 158 cold water samples were further analyzed for chemical parameters (pH, free disinfectant concentration, hardness, conductivity and trace metals). Risk assessment and standardized hygiene inspection was conducted in each hotel, and the results were associated with those of microbiological and chemical analysis.

Results: The hygiene inspection classified 17.6% of the hotels as satisfactory, 15.7% as adequate and 66.7% as unsatisfactory. Legionella spp. was isolated in 160 out of 556 (28%) of the samples. 41,8% of hot water samples and 21,8% of cold water samples were positive for legionella detection. 38 out of 51 (75%) of the hotels were found to be colonized and 31,4% required intervention measures according to the EWGLI criteria. In cold water supply systems free disinfectant concentration

Legionella detections in environments and their impacts on the occurrence of legionellosis in Japan

Authors Kura F., Amemura-Maekawa J.

Legionellosis cases in Japan were about 1,600 in 2015 and 2016, resulting in the incidence rate of 1.25 per 100,000, similar to that of Europe. In about half of the cases, the sources of infection are suspected to be bath facilities, but in the remain those are unknown.
The major causative agent has been Legionella pneumophila SG1 (Lp1).
Legionella spp. harbour in various environments. To clarify the relation between the genotype and the environmental habitat of Lp1, isolates from various environments were sequence-based typed and mapped in a minimum spanning tree (MST).
The sequence types (STs) of cooling tower water (CT) isolates were found to be similar and 74% were ST1. STs of bathwater (BW) isolates and soil isolates indicated more diversity and formed 3 groups each. STs of puddle isolates were contained in soil
ST-groups.
The difference of Lp1 in ST groups may be derived from the difference in host amoebae in each environment. The extent of Legionella growth was examined using several types of amoebae. However, factors determining the relation between the
ST groups and the environmental habitats of Lp1 remain unclear. As National Reference
Center of Legionella, clinical isolates are voluntarily collected and STs of these isolates with a MST including hundreds of Japanese isolates have been informed back to local institutes of health, local departments of public health and hospitals for assistance to estimate the source of infection since 2014.
In environmental surveillance, 23%, 22%, 29%, 48%, and 6% of BW, CT, shower water, and puddle water and topsoil, respectively, were positive for Legionella spp. The gene of Lp1 likely associated with pathogenicity, lag-1, was detected in 26%, 14%, and
2% of BW, topsoil and CT Lp1 strains, respectively, corresponding to the finding that major infection sources have been bath facilities in Japan. Until now, annual legionellosis cases were not decreasing. Assured disinfection of water and daily sanitation should be coherent.

Prevalence and Diversity of *Legionella* in US Cooling Towers

Authors Llewellyn A. C., Lucas C., Roberts S., Brown E., Raphael B.H., Winchell J.

Background: Cooling towers (CT) have been implicated in several large communityassociated Legionnaires' disease (LD) outbreaks. The purpose of this study was to determine the prevalence of Legionella in CTs in different regions of the US and to characterize the microbiomes of samples obtained for routine testing.

Methods: Samples from 196 CT sites were shipped blinded to CDC by several water testing laboratories. The samples represented various US geographical regions and were tested for Legionella DNA using a multiplex real-time PCR assay. PCR positive samples were cultured and resulting isolates were further characterized to the species and/or serogroup level. Bacterial community abundance in PCR positive samples was surveyed using 16S rRNA amplicon sequencing.

Results: Legionella DNA was detected in 84% of CT samples and from every region studied. Legionella was isolated from 48% of the PCR-positive samples (40% overall) and nearly half of the culture-positive samples contained more than one type of Legionella. Overall, 144 unique Legionella isolates were recovered. The most commonly isolated Legionella spp. included: L. pneumophila (53%), L. anisa (22%), and L. rubrilucens (8%). The bacterial microbiomes of these samples were remarkably homogenous and dominated by Proteobacteria.

Conclusions: This study represents the largest survey of Legionella prevalence in US CTs conducted to date. Legionella spp. were detected and characterized from samples representing diverse geographical regions, indicating the ubiquity of this organism in US CTs. These results underscore the potential for LD outbreaks to occur throughout the US and the need for water management strategies to help prevent outbreaks from a CT source.

Identification of Cooling Tower Microbiota Supporting the Growth of *Legionella pneumophila*

Authors Paranjape K., Bédard E., Lévesque S., Fontaine Y., Raymond F., Corbeil J., Prevost M., Faucher S. P.

Background: Cooling towers are the most frequent source of outbreaks of Legionnaires' disease. In cooling towers, L. pneumophila (Lp) can be found in a planktonic state, associated with biofilms or inside unicellular hosts. Many abiotic factors are associated with the presence of Lp, but the role of biotic factors, beside the presence of host cells, is poorly understood. The hypothesis of this work is that the microbiota of the cooling tower is a key factor for the colonization by Lp.

Materials/Methods: To identify microbiotas supporting the growth of Lp, water was collected from 9 different towers located in Quebec, Canada. The samples were initially characterized by measuring standard water parameters and heterotrophic plate counts. The microbiotas were concentrated by filtration, adjusted to 108 cells/ml, and seeded in 24-well plates. The wells were then spiked in triplicate with a variety of GFP-labeled Lp strains. Spiked filtered water was used as a negative control. Fluorescence was measured over 7 days with a fluorescent plate reader. Metagenome sequencing was used to investigate the microbial population of interesting towers. Libraries were constructed with the Nextera preparation kit and sequencing was performed on a HiSeq.

Results: Strong increase in fluorescence was observed for 4 towers, indicating that their microbiota supports the growth of Lp. A drastic drop in fluorescence was observed for 1 tower, suggesting that its microbiota is refractory to Lp. The other 4 towers showed variable results or change in fluorescence in the negative control. Metagenomic analysis revealed huge diversity between the cooling towers. Interestingly, over 90% of the reads from an Lp-supporting tower was mapped to Limnobacter sp., a sulfur oxidizing bacterium.

Conclusion: Our analysis identified cooling towers susceptible to be colonized by Lp and a cooling tower that could resist colonization. Diverse microbial populations seem to be able to support the growth of Lp.

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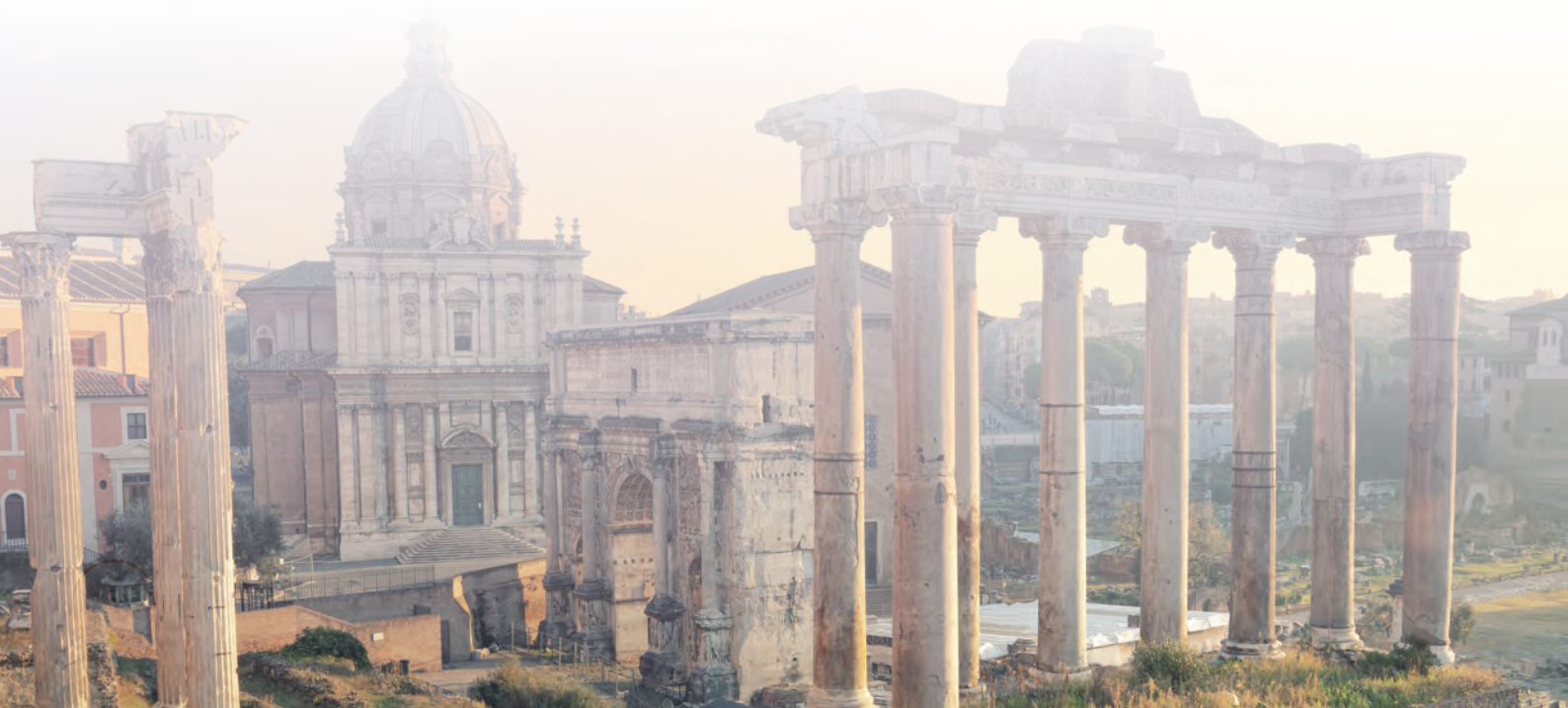
ORAL PRESENTATIONS

FRIDAY, SEPTEMBER 29TH, 2017

15:30-17:15 **SESSION 9 - PREVENTIVE AND CONTROL STRATEGIES**



The evaluation of mandatory *Legionella* environmental surveillance in healthcare facilities in Taiwan: clinical and environmental perspectives

Author Yusen E. Lin

Legionella is a common cause hospital-acquired pneumonia, especially for immunosuppressed patients. Cooling towers were originally thought to be the main reservoir for Legionella, but subsequent reports identified that the water distribution systems were also important as the source of Legionnaires' disease (LD) in hospitals. Culturing hospital water supply for Legionella is the first step to assess the risk of hospital-acquired Legionnaires' disease. This approach is widely adopted by national and international guidelines, and Taiwan CDC also published the recommendation for environmental surveillance of Legionella in healthcare facilities (>49 bed) in 2010. Both clinical and environmental surveillance data will be presented to demonstrate the cost effectiveness of such recommendation and possible preventive modalities in Taiwan.

Controlling Legionella in Building Water Systems: How Does Monochloramine Measure Up?

Author Janet E. Stout

Corrosive Effect on Various Pipe Materials Following Different Treatments for *Legionella Spp* Control in Hospital Water Systems

Author Isabella Marchesi

The 9th International Conference on

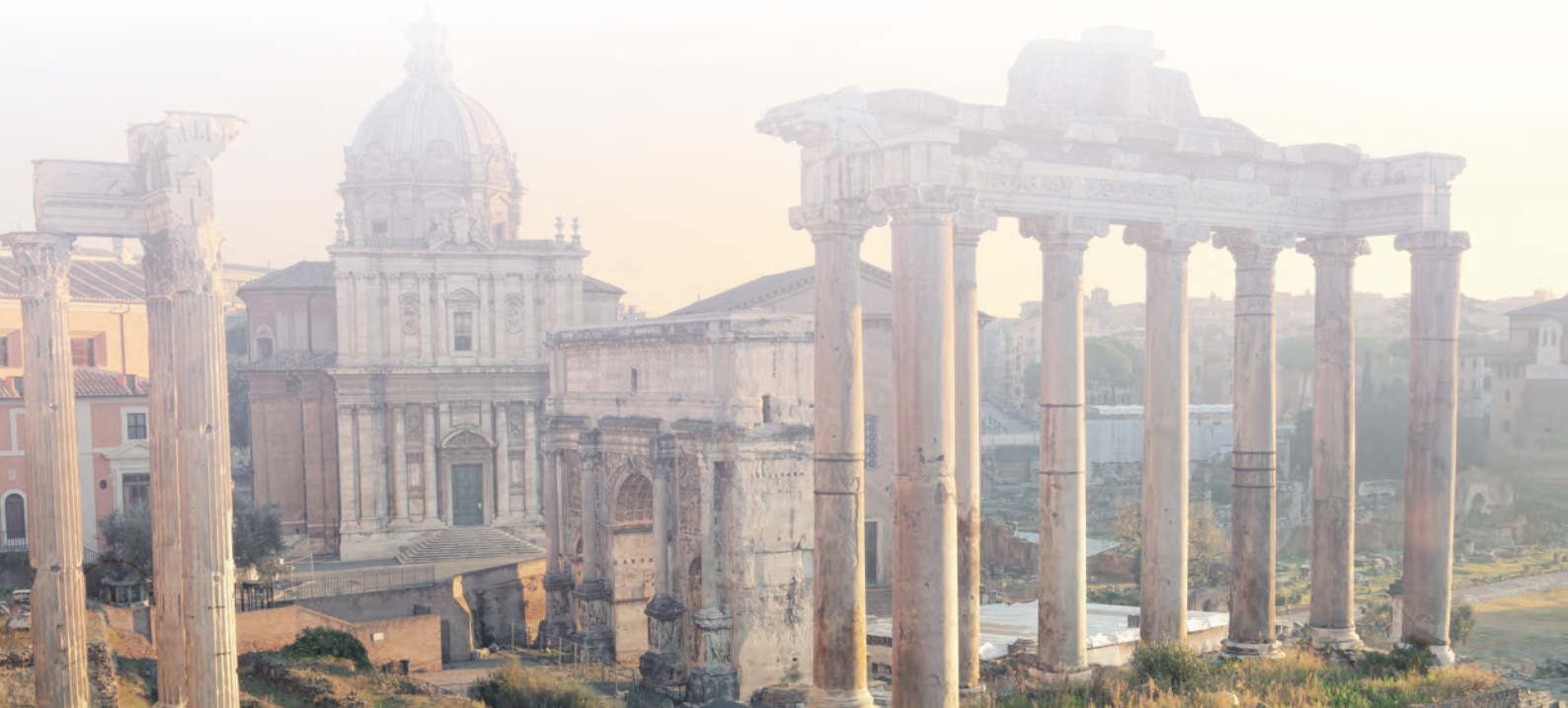
Legionella

Rome, 26th - 30th September 2017

ORAL PRESENTATIONS

FRIDAY, SEPTEMBER 29TH, 2017

17:45-19:00 **SESSION 9 - PREVENTIVE AND CONTROL STRATEGIES (CONTINUED)**



A Multicenter Study on *Legionella* Air Contamination in Italian Healthcare Facilities: Comparison of Different Sampling Methods

Authors De Giglio O., Montagna M. T., Diella G., Divenuto F., Pacifico C., Rutigliano S., Cristina M. L., Napoli C., Agodi A., Baldovin T., Casini B., Coniglio M. A., D'Errico M. M., Delia A. S., Deriu M. G., Guida M., Laganà P., Liguori G., Moro M., Mura I., Pennino F., Privitera G., Romano Spica V., Sembeni S., Spagnolo A. M., Tardivo S., Torre I., Valeriani F., Albertini R., Pasquarella C & GISIO-SItI, AIA and IMPIOS Working Groups.

Background: Healthcare facilities (HF) represent an at-risk environment for legionellosis transmission occuring after inhaling aerosols produced from contaminated water sources. The control of water is preferred to that of air because to date there are not standardized protocols sampling.

Methods: Legionella air contamination was investigated in the bathrooms of 11 HF contaminated with Legionella by active sampling (Surface Air System and Coriolis®?) and passive sampling. During the 8-hour sampling, hot tap water was sampled three times and analyzed for Legionella. Air samples were evaluated using culture based methods, whereas liquid samples collected using the Coriolis®? were also analyzed by real-time PCR. Legionella presence in air and water was compared by sequence-based typing (SBT) methods.

Results: Air contamination was found in four HF (36.4%) by at least one of the culturable methods. The culturable investigation by Coriolis®? did not yield Legionella. However, molecular investigation using Coriolis®? resulted in eight HF being positive for Legionella in air, which was greater than the number of positives obtained by culturable methods.

Maxi Impact of Temperature, Copper and Silver Exposure on the Viability and Recovery of Clinical and Environmental Strains of *Legionella pneumophila*

Authors Michèle Prévost, Margot Doberva, Sébastien Faucher, Severine Allegra, Émilie Bédard

Disinfection procedures in large building water systems can promote a viable but non culturable (VBNC) state of Legionella pneumophila (Lp) undetected by culture.
Environmental strains in premise plumbing are likely to develop resistance over time to stressors from treatment. The main objective was to evaluate the impact of copper, silver, chlorine and temperature on the viability and culturability of various Lp strains using viability flow cytometry, culture and qPCR.

A total of 13 strains, 3 reference, 2 clinical, and 8 environmental (sg.1&5) isolated from hot water systems (biofilm &water) and cooling towers were studied. After starving, strains were exposed to Cu (300 to 5000µg/L), Cu/Ag ions (300/30 to 800/80µg/L), chlorine (3 & 20ppm) and temperatures (45 to 70°C). Lp was monitored over 1 week of contact time by culture (AFNORNFT90-431), flow cytometry (Thiazol Orange™ & PI) and qPCR. Intracellular multiplication after treatment was evaluated in Acanthamoeba castellanii.

The exposure (dose/temperature X contact time) leading to V varied considerably depending on the strain. For example, an exposure of >3d at 5000 µgCu2+/L were required to inhibit culturability of the biofilmisolated strain vs. 6 hours for the water-isolated strain. For chlorine, a gradual shift to VBNC was observed and the ability to recover viability and growth after 1-week was inversely stringent stressor conditions prevented regrowth.

Findings show that monitoring Lp in building water systems by culture may underestimate their presence, especially when subjected to stressors such as copper, silver or high temperature.

These may induce a VBNC state, with a potential to regain infectivity following internalization or suppression of stress conditions. Treatment conditions should be set to ensure the elimination of VBNCs to lower the potential exposure when more favorable growth conditions are present.

Prevention and Control of *Legionella spp.* Colonization in Dental Units

Authors Casini B., Totaro M., Cristina M. L., Di Cave D., Valentini P., Spagnolo A. M., Poli A., Baggiani A., Privitera G.

Background: Dental Unit Waterlines (DUWs) have shown to be a sources of Legionella infection. We report the experience of different dental healthcare setting where a risk management plan was implemented.

Materials/Methods: In 2 Hospital Odontostomatology Clinics (HOC) and 3 Private Dental Clinics (PDC) housing 21 and 6 dental units (DU) respectively, an assessment checklist was applied to evaluate staff compliance with guideline recommendations. DUWs were investigated for microbial parameters. 3%-6% hydrogen peroxide (HP) for 1 hour was the prevalent shock disinfection practice followed by 0,2% peracetic acid or 2,5% quaternary ammonium compounds in continuous treatment. Simultaneously to shock disinfection, point-of-use filters were installed.

Results: In the HOCs a poor adherence to good practices was demonstrated, otherwise protocols were carefully applied in PDCs. L. pneumophila sg 1 was detected in hospital DUs from handpieces (2/21, 9%) whilst L. p. sg 2-14 in 19% (4/21) and 33% (2/6) in HOC and PDCs respectively, mainly from handpieces (22%, 6/27) with counts 102-105 CFU/L. P.aeruginosa was associated with high TVCs mainly from hospital spittoons (67%, 14/21). FLA were recovered only from hospital DUs (28%, 6/21) and all species belonged to Vermoamoeba vermiformis (99% identity). Legionella was eradicated after 6% HP shock disinfection and pointof-use filters installation, whilst P.aeruginosa after the third shock disinfection with a solution of 4% HP and surfactants. In one PDC, the integrated strategy “3% HP disinfection-filter installation” allowed no longer to detect Legionella. The shock disinfection showed a limited effect, with a recolonization period of about 4 weeks.

Conclusion: Our data suggest the presence of a large contamination and biofilm persistence that explain the inefficacy of low-level disinfectants. This occurrence requires a risk management and an effective choice of disinfectant to obtain an hazards control during dental practices.

Legionella Colonization Of Hotel Water Supply Systems In Touristic Places Of Greece: Associations With System Characteristics and Physicochemical Parameters

Authors Mouchtouri V., Kyritsi M., Kostara E., Nakoulas V., Hatzinikou M. and Hadjichristodoulou Ch.

Background: Hotel-associated Legionnaires' disease remains a significant cause of travelassociated respiratory tract infection among tourists in Greece. The aim of the study was to assess the colonization of hotel water supply systems in touristic places of Greece with Legionella spp., and to investigate possible association of physicochemical parameters with the presence of the bacterium.

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Risk Assessment of *Legionella* in Hospital Washbasins: Water and Aerosol

Authors Laganà Pasqualina, Campanella Giovavvi, Mazzù Francesco, Palermo Roberta, Delia Santi.

Background: Legionella diffuses into the environment from several indoor spots (showers, sinks, tubes, etc.), causing Pontiac Fever and Legionnaires' Disease. Until inter-human transmission was demonstrated, in the 2016, the only way to contract the disease was by inhaling contaminated droplets. In hospital, the best prevention strategy applicable to preventing or reducing the risk of contracting the disease is the constant monitoring of water supply. This study evaluates presence of Legionella in Messina (Italy) University Hospital wards both in water and aerosol samples.

Materials/Methods: To recover Legionella from water samples the standard procedures reported in the Italian Guidelines (2015) were used. Aerosols samples were collected from the aerosol formed around the faucet when the water flows. In this case, simulating handwashing, the plates were inoculated only for the fall of the droplets and this are an even greater risk for patients who use the washbasins.

Results: Totally 270 samples of water and aerosols were collected from 135 washbasins, distribuited in all wards. Legionella was isolated only from water in 68 samples (50,4%) and both from water and aerosols in 50 samples (37%). Completely negative resulted 17 sites.
Serogroups isolated were L. pneumophila 1, 3, 6 and Legionella gormanii.

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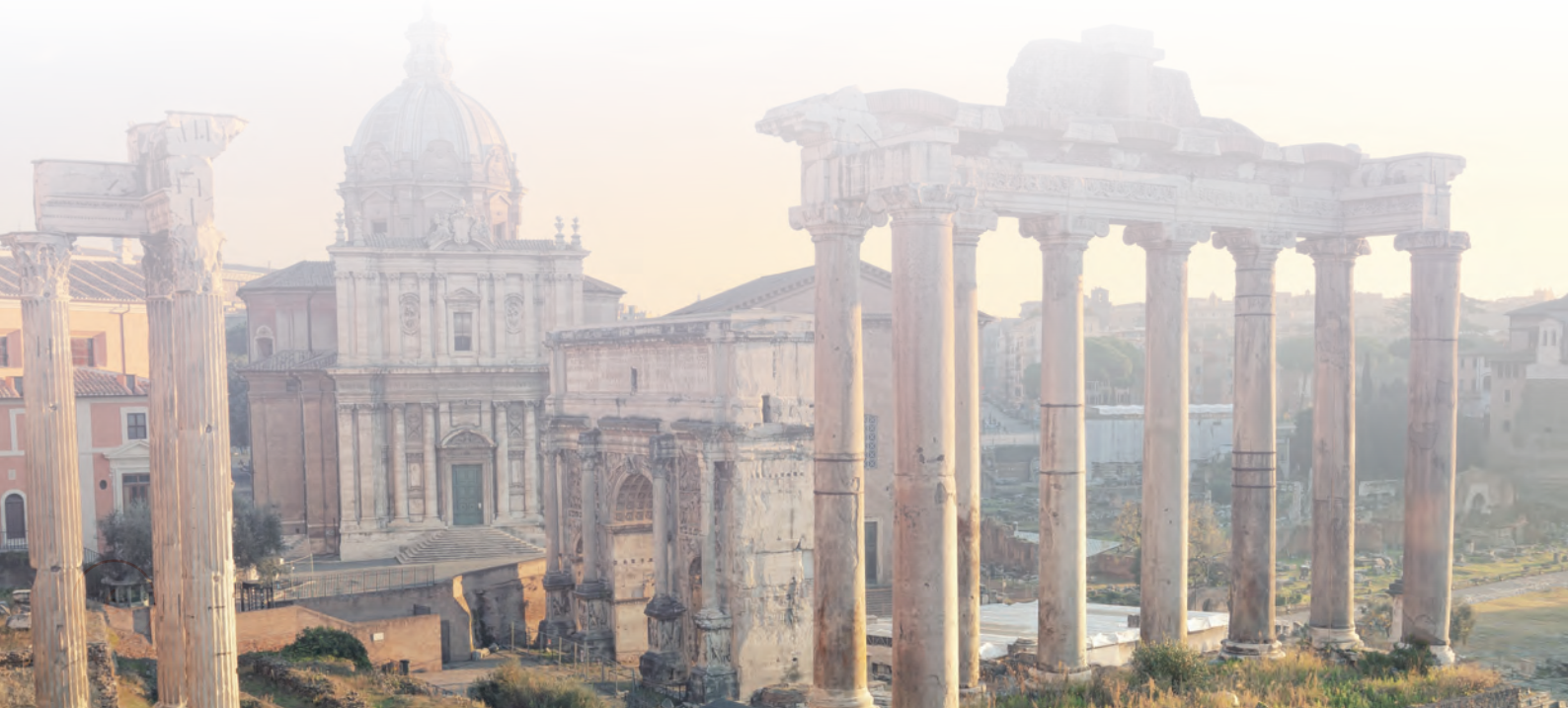
ORAL PRESENTATIONS

SATURDAY, SEPTEMBER 30TH, 2017

08:30-11:00 **SESSION 10 - *LEGIONELLA* GUIDELINES IN DIFFERENT COUNTRIES**



European *Legionella* Guidelines

Author Susanne Lee

U.S. Guidance and Standards

Author Janet E. Stout

The German experiences with *legionella* risk regulation

Authors Martin Exner, Stefan Pleischl

1. Epidemiology of Legionellosis in Germany

Germany has 82 million inhabitants living in 16 federal states of Germany. In 2016 992 legionellosis were notified in 2016. 96% of these notified patients with legionellosis were hospitalised. The mortality rate of legionellosis was 4,7% in 2016. 13 outbreaks had been registered including 1 outbreak with 24 patients in 2016. According to the competence network of community acquired pneumonia (CAPNETZ) it is estimated that between 15,000 to 30,000 cases of legionellosis are occurring each year which means that the numbers of notified cases are a significant underestimation of the true numbers of legionellosis in Germany. Therefore legionellosis is assessed as an important public health risk and is jugded - resulting exclusively from the environment - as a preventable disease. Therefore there is strong public health reason to regulate this risk.

Infection sources and transmission pathways for legionella which are regulated in Germany are as follows:

- water intended for Human consumption-
- cooling towers and evaporated condensers
- swimming pool's and
- sewage water treament

2. Specific conditions in Germany

Specific basic conditions are to be taken into account as follows:

- the precautionary principle codified in the "Protection against Infection Act"
- minimising principle for disinfectants (like chlorine) codfied in the ordinance for water intended for Human consumption
- obligation to inform responsible authorities.and consumers in case of deviation

The competent authorities for water intended for Human consumption and swimming pool Water are public health departments, competent authorities for cooling towers and evaporative condensers are environmental protection department's.

3. Ordinances and technical rules for prevention and control of legionella

3.1 Water intended for Human consumption

In 1987 a official proposal by the Federal German Public Health agency was published in which it was proposed that numbers of legionella in technical systems should be as low as reasonably achievable (ALARA principle) by technical measures. Epidemiogical studies in late 80ies and reports of outbreaks were published in Germany; in a study in Berlin it was documented that up to 10% of all pneumoniae were legionellosis. In one nosocomial outbreak with 11 cases and three deaths the associated hospital was closed temporarily due to a systemic contamination of the drinking water installation until an expertise could certify that by technical measures the risk of legionella would have brought under control. Until to this time no regulation existed in which accetabel concentrations for legionella not harming the health of patients in health care facilities were regulated. This outbreak was the trigger event to develop a proposal published in 1990 < 100 Legionella spp. / 100 mL could be an acceptable concentration for water in the drinking water installation and when exceeded, would give reason to fear an avoid-able health hazard related to the drinking water installation and lead to measures to check the sanitary and technical condition of the drinking water installation being taken in the form of a risk analysis. In 1993 and 2004 the technical rules for Legionella in Drinking water installations were published by the German technical and scientific Association of gas and water and values for Legionella for verification purposes were proposed . In 2001 the German ordinance on the quality of water intended for human consumption mentioned for the first time legionella spp.. In the same ordinance 2013 a technical action value of 100 Legionella spp. / 100ml , and obligation of notification in case of not fulfilling the technical action value and an obligation of expertise on the status of the drinking water installation were integrated. The details of these regulations and the experiences will be discussed in detail.

4.2 Cooling towers and evaporative condensers regulation

Until 2015 no technical rules and ordinance with an integration of an action value for Legionella concentrations exist. Even after a cooling tower associated outbreak in 2010 in Ulm no efforts were undertaken to develop such guidelines. This situation was heavily criticised by public health societies. Only after the biggest cooling tower associated legionella outbreak in Warstein in 2013 a technical rule was published in 2015 and a federal emission protection ordinance with clear criteria for registration, technical action values and criteria for maintaining was published in 2017.

The details will be discussed in detail.

4.3 Swimming pool and spa pool regulation

An ordinanance for swimming pools regulating legionella risks in the swimming pools does not exist until today, instead of this a very strong proposal and technical rules regulating the risk of legionella and swimming pool in Germany have been published in 2014.

4.4 Sewage plants

The Legionella Warstein Outbreak in 2013 was associated with legionella in areation ponds of sewage treatment plants in which up to 100,000,000 legionella/100ml including the epidemic strain could be isolated by which a river was contaminated from which the cooling water for a cooling tower was abstracted identified as one of the infections reservoirs. Therefore an ordinance was published by one German state (North Rhine Westfalia) was published in 2016. This ordinance will be discussed in detail.

Legionella Guidelines in the Russian Federation: Harmonization in Accordance to International Standards

Authors Tartakovskiy Igor, Demina Yu., Grusdeva O., Portenko S.,Karpova T., Dronina Yu

In the last ten years 7 different national guidelines,recommendations and 1 national sanitary rules "Prevention of Legionellosis" introduced in Russian Federation on the issue of legionellosis.

The documents include modern aspects of Legionellosis surveillance at the national and international level: governmental sanitary-epidemiological surveillance, standardized case definitions, approaches to risk management included control of potential dangerous water systems (cooling towers,potable water, hot tubes,swimming pools and etc.), management of outbreaks, prevention of nosocomial legionellosis.

Modern approaches for prevention of nosocomial legionellosos included in the new national sanitary rules "Prevention of infections in hospitals'. Methodical aspects of laboratory diagnostic of Legionellosis are reflected in the new national guidelines "Laboratory diagnostic of pneumonia" with special emphasis for different approach to diagnostic of community-aquired legionellosis and nosocomial legionellosis in immunocompromised patients.

The introduction of the new guidelines - important step of harmonization the surveillance and prevention of Legionnaires' disease in Russian Federation in accordance to international standards.

Legionella Regulation in Australia

Authors Bentham R., Jones R.

The system of Federation in Australia adds considerable complexity to Legionella regulation. There is no national regulation directly dealing with Legionella control. Legionellosis is a nationally notifiable disease. There is a national plumbing code which is enforceable.
There are national standards and newly released guidelines. Public health regulation is the responsibility of the individual States.

All States have regulations that deal with cooling water systems. Some States have regulations dealing with health care institutions (hot and warm water systems). Some States require sampling for Legionella, others do not. Frequency of required sampling is dictated in South Australia, but based on the stipulations of the risk management plan in other jurisdictions (Queensland, Victoria).

All States require Legionella testing to Australian Standard 3896 which sets a limit of detection of 10 cfu/mL. No States specify a sample volume to be collected or a sampling method (first flush etc.). Positive test results may or may not be notifiable to local or State government.
In some States legionella legislation is administered centrally, in others it is devolved to local government. Local government in some instances may pass responsibility for auditing and routine sampling to third party contractors.
This disparity in regulation means that there is no national consistency in approach. Further, the nature of the State regulations and their specifications means that a unified national approach is highly unlikely to occur.

Although the regulatory system is rather disparate there have been notable successes in Legionella control in different States. The move towards risk management based control strategies is one of these successes. Risk based management of high risk systems has been introduced in different forms in two States and is being developed in a third. The move from prescriptive to risk based management approaches will probably improve consistency across jurisdictions.

Italian *Legionella* Guidelines: a special focus on dental unit waterlines

Author Ricci Maria Luisa

The “Guidelines for Legionella Prevention and Control” published in 2000, were the first Italian document addressed to provide updated information on Legionella and legionellosis.
In 2005 two additional guidelines were released: the first one was elaborated in order to offer to the owners of accommodation sites elements of judgment for the Legionella risk assessment and control measures to reduce Legionella contamination in water system; the second document described the requirements for laboratories performing Legionella detection and enumeration.

In 2015 a review of the documents mentioned above was released. The new guidelines are divided in two parts: a general one, where an updated description on the most important topics of Legionella is described and a technical part consisting of 13 annexes. Among them are described procedures on diagnosis, both of clinical and environmental samples, sampling, questionnaire for outbreak investigation, checklist for Legionella risk assessment, methods for prevention and control of contamination, etc. In the new document are fully reviewed the following 3 topics: 1. Alert and action levels, with the introduction of epidemiological, percentage of positive samples and concentration (CFU/L) criteria. 2. Use of qPCR for Legionella detection in water samples, in order to quickly identify the positive samples that will be further analysed by culture for CFU/L quantification. On the contrary, negative qPCR results can be used for official reports and for risk assessment analysis without culture confirmation. 3. A chapter devoted to the risk of acquiring LD in working places, focusing on dental office. Prescriptions for dental unit waterlines (DUWL) as avoiding stagnation of use of sterile water for circuit feeding, disinfection with continuous and discontinuous methods, etc are provided. Results of a survey of 24 European countries concerning how Legionella contamination of dental DUWL is dealt with will be also presented.

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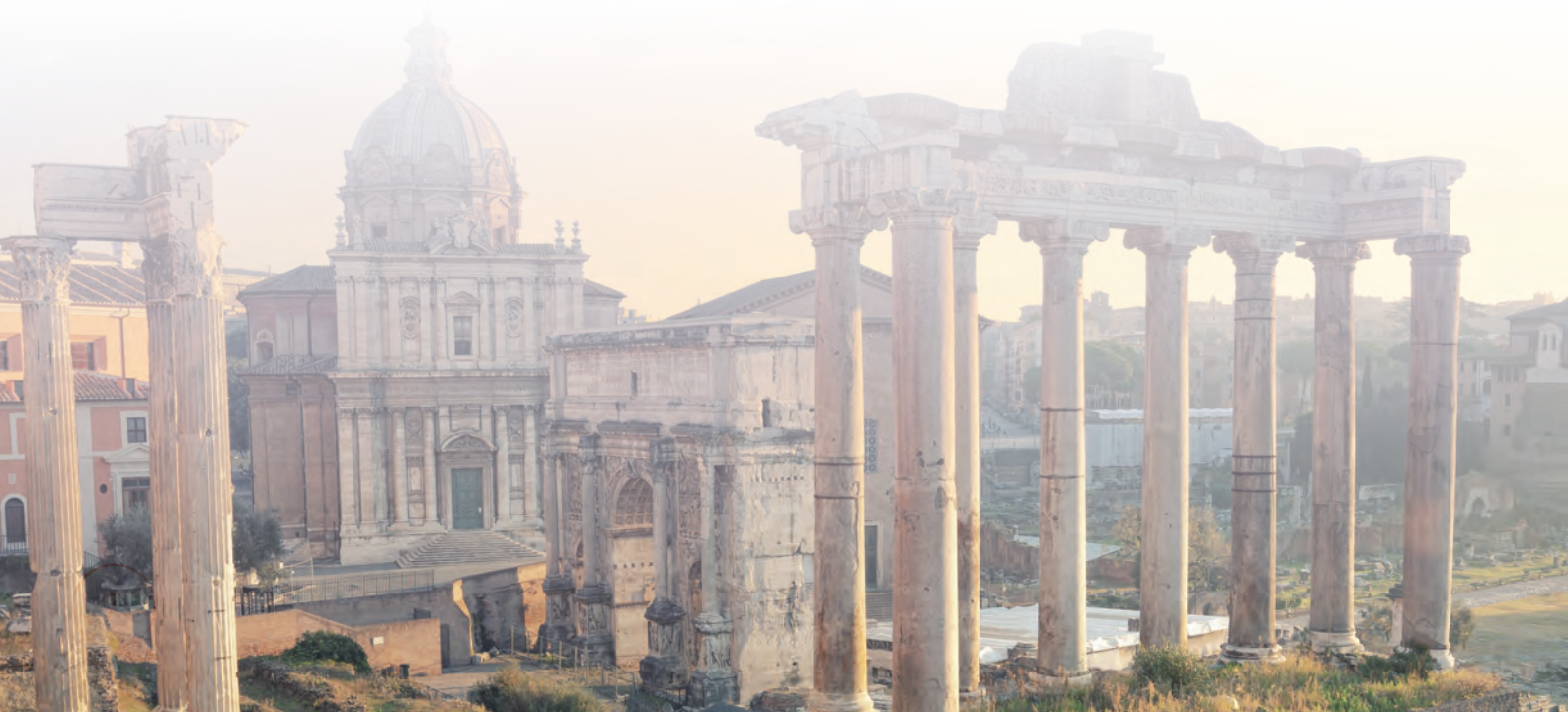
SATURDAY, SEPTEMBER 30TH, 2017

11:30-13:30 **SESSION 11 - CHALLENGES OF *LEGIONELLA* AND FUTURE PERSPECTIVES**



Advancing a Framework for Prebiotic/Probiotic Control of *Legionella* in Distribution Systems and Building Plumbing

Authors Pruden A., Ji P., Rhoads W. J., Garner E. D., Strom O. R., Schwake D. O., Falkinham J. O., Edwards M. A.

Controlling Legionella in water distribution systems and building plumbing presents a grand challenge. While incidence of Legionnaires' Disease has steadily risen among developed countries, existing regulatory frameworks are generally focused only on fecal sources of pathogens and are poorly equipped to control Legionella. The various Legionella spp. associated with Legionnaires' Disease differ from fecal bacteria in that they are integral members of the microbial ecosystems that are characteristic of potable water systems. In particular, they reside in complex biofilms, thrive under clean, oligotrophic conditions, are relatively tolerant of disinfectants, and are dependent on amoebae hosts to reproduce and propagate their virulence. Thus, traditional disinfection regimes formulated for fecal pathogens, which are non-native and thus more susceptible to dieoff in potable water systems, are not sufficient for Legionella. Here we present a synthesis of recent research and discuss an alternative framework for Legionella control, a "prebiotic/probiotic" approach, which derives from an understanding of its microbial ecology.

Specifically, it must be recognized that disinfectants such as chlorine, do not kill everything in the water, rather, they select and shape the native microbial community. Likewise, trace nutrients, such as assimilable organic carbon and iron, can have a profound effect on the ability of Legionella to thrive, as can antimicrobials, such as copper. Further, synergistic and antagonistic interactions with various other members of the microbial community, especially amoebae hosts, should be considered. Ultimately, these factors must be boiled down within the context of real-world water systems and choices made with respect to water treatments, materials, flow regimes, temperature settings, plumbing configurations, disinfectant types, and doses in order to inform more effective regulations and guidelines for protecting public health.

Technology Advancement Supporting *Legionella* Control in Dubai

Authors Eng. Redha Hassan Salman

Dubai's Buildings Health and Safety Self-Compliance Reporting System BHSCRS, is the latest tool that have been adopted to track compliance of water systems in order to prevent and control legionella outbreak incidents.

The smart interactive platform between Dubai Municipality and the building owner's or operators ensures the timely compliance with health & safety regulations and guidelines related to maintaining, operating and monitoring building systems, The remotely operated online system features include following up corrective actions as well as generating historic trends and reports.

Ensuring safe environment and protecting all emirate's residents and visitors through improving the level of health and safety in buildings has been a top priority and reflected clearly in Dubai Strategic Plan 2021. BHSCRS which came into implementation almost three years ago, was one of the most important initiatives that has been implemented under the above-mentioned strategic plan.

The surveillance and monitoring programs aimed at controlling legionella in buildings have started long back since 2003, in accordance with the Local Order 11 of 2003 regarding Public Health & Community Safety. These programs and efforts have been evolving utilizing advancement in technology and corresponding to increased challenges in the city.

Controlling and sustaining high level control of Legionella in water systems is a complex matter that includes many aspects such as policies, regulations, guidelines, standards, testing facilities, and most importantly enforcement. Dubai Municipality Legionella control program have mapped out responsibilities, requirements and resources to achieve a high level control and maintain buildings health and safety.

The European Perspectives of *Legionella* Surveillance and Control

Author Valeria Gaia

Since the first outbreak of Legionnaires' disease (LD) in 1976 in Philadelphia, many progresses have been made in understanding Legionella and the transmission of the disease. More accurate diagnostic tools were developed and natural habitats have been studied. Despite all these progresses, the incidence of Legionnaires' disease in Europe has been constantly increasing and many outbreaks have been described worldwide.

There is clear evidence that several challenges concerning distinct aspects of LD such as diagnosis, typing of the isolates, identification of community sources , influence of climate changes, etc. still need to be solved.

PCR has brought more sensitivity and rapidity to the diagnosis of LD, but the EU case definition still does not consider PCR for the definition of a confirmed case.
Molecular methods, flow cytometry and many other rapid detection tests may help improving the detection of Legionella spp. in water, but in most European guidelines only culture results are used for the action level definitions.
New typing techniques based on Next Generation Sequencing have been very useful to investigate outbreaks and to understand the phylogeny and the taxonomy of these fascinating bacteria but they have also evidenced new challenges.
These and many other evidences show that often the laboratory techniques are more advanced than the guidelines or legislation and that a lot of work still need to be done.