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WELCOME

Dear Delegates,

A warm welcome to the 4th meeting of the ESCMID Study Group for Legionella Infections (ESGLI) held in Amsterdam, The Netherlands, September 22-23, 2016. This conference is organized by the Royal Netherlands Water network (KNW), The National Institute for Public Health and the Environment (RIVM) and the Regional Public Health Laboratory Kennemerland. The aims of ESGLI involve the improvement of diagnostics, treatment, control and prevention of legionellosis.

Since the first described outbreak of Legionnaires' disease in 1976 in Philadelphia forty years ago, much has been learned about Legionella and the disease. Diagnostic methods were developed and knowledge has been gained about the natural habitats and other environmental sources of Legionella. Legislation for Legionella prevention in water systems was implemented in many countries. Nevertheless, annually more than 6000 patients with Legionnaires' disease are diagnosed in Europe, and every year new outbreaks of various sizes occur worldwide. This emphasizes that many challenges for public health and scientific research still remain. The conference offers a unique opportunity to discuss these and other challenges with fellow researchers and other parties. The organizing committee has drafted, with the help of the scientific committee, an interesting program based on the many abstracts that were submitted by researchers from all over the world who wish to share and discuss their scientific knowledge.

The ESGLI 2016 conference will present the latest scientific developments and knowledge about the ecology of Legionella, diagnostics, typing methods, outbreak investigations, travel associated Legionnaires' disease, epidemiology and surveillance, environmental sources of Legionella, climate associations, and Legionella prevention.

We are proud and honored to host this conference in The Netherlands, and hope that you will find it interesting and of use for your own research and education. We wish you a pleasant stay in Amsterdam.

The Organizing Committee

Outline program

Wednesday

21-09-2016

16.30 – 18.00

Welcome reception at the Hortus Botanicus Amsterdam

Thursday

22-09-2016

08.30 – 09.00

09.00 – 09.10

09.10 – 10.40

10.40 – 11.20

11.20 – 12.20

12.30 – 14.00

14.00 – 15.50

15.50 – 16.20

16.20 – 18.00

18.00 – 18.15

18.15 – 19.15

19.15 – 22.00

Registration

Opening of the conference by Dr. J. K van Wijngaarden

session 1: *Epidemiology and Surveillance*

Coffee break and posters

session 2: *Prevention and Control Strategies*

7th ESGLI business meeting - Lunch and posters

session 3: *Genomics*

Tea break and posters

session 4: *Outbreaks and Case Reports*

Drinks

Transport by boat to the conference dinner

Conference dinner at the Olafs Chapel Barbizon Palace

Friday

23-09-2016

08.00 – 09.00

09.00 – 10.50

10.50 – 11.20

11.20 – 12.40

12.40 – 14.00

14.00 – 15.40

Registration for one day attendants

session 5: *Microbial Ecology*

Coffee break and posters

session 6: *Diagnosis and Typing*

Lunch and posters

Session 7: *Environmental and Surveillance*

Conference program

Thursday

22-09-2016

08.00 – 09.00

09.00 – 09.10

Registration

Opening of the conference by Dr. J. K van Wijngaarden
Ministry of Health, Welfare and Sport, The Netherlands

Session 1 Chair

Epidemiology and Surveillance E. IJzerman

09.10 – 09.40

O1. Epidemiology of Legionnaires' disease in Europe, 2015
Keynote presentation by Birgitta de Jong, Sweden

09.40 – 10.00

O2. Legionnaires' disease among patients hospitalised with pneumonia in South Africa
N. Wolter, M. Carrim, C. Cohen, S. Tempia, S. Walaza, L. de Gouveia, F. Treurnicht, O. Hellferscee, A.L. Cohen, H. Dawood, E. Variava, A. von Gottberg, South Africa

10.00 – 10.20

O3. Connectedness of two outbreaks of legionellosis; Bremen, 2015 and 2016
U. Buchholz, J. Dullin, C. Lück, R. Lachmann, H.J. Jahn, B. Brodhun, A. Gründel, M. Lelgemann, Germany

10.20 – 10.40

O4. Investigating the spatial component of Legionnaires' disease outbreaks: a geographic analysis tool for field epidemiologists
E. Robesyn, S.L. Ionescu, B. de Jong, Sweden

10.40 – 11.20

Coffee break and posters

Session 2 Chair

Prevention and Control Strategies S. Lee

11.20 – 11.40

O5. DSS: Monitoring biofouling tendency on the way to Legionella prevention
A. Pereira, J. Martins, Portugal

11.40 – 12.00

O6. Development of new UK guidance for the delivery and management of safe water in healthcare premises for the control of Legionella
J. Walker, P. Ashcroft, United Kingdom

11.20 – 11.40

O7. Energy efficiency and hygiene in drinking water installations
C. Lück, M. Petzold, T. Koshkolda, C. Schreiber, G. Schaule,
R. Rothmann, K. Rühling, Germany

11.40 – 12.00

7th ESGLI business meeting & Lunch and posters

Session 3 Chair

Genomics S. Jarraud and S. David

14.00 – 14.30

O8. The Legionella genus genome: diversity and plasticity, the key to adaptation
Keynote presentation by Prof. Dr. Carmen Buchrieser, France

14.30 – 14.50

O9. An evolutionary genomic history of Legionnaires' disease in Scotland
B.A. Wee, D. Lindsay, B.L. Jones, A. Smith, N. Loman, R. Fitzgerald,
United Kingdom

14.50 – 15.10

O10. Whole Genome Sequencing supports the role of waste-basin aeration as a direct source of Legionnaires' disease transmission during Lens outbreak
S. Jarraud, G. Kapatai, S. David, C. Ginevra, M. Mentasti,
J. Sweetman, R. Tewole, C. Campese, A. Underwood, N. Fry,
V. Chalker, T.G. Harrison, France

15.10 – 15.30

O11. Comparison of sputum microbiome of legionellosis-associated patients and other pneumonia patients: indications for polybacterial infections
M. Halpern, H. Mizrahi, A. Peretz, R. Lesnik, Y. Aizenberg-Gershtein,
S. Rodriguez-Martinez, Y. Sharaby, N. Pastukh, I. Brettar,
M.G. Höfle, Israel

15.30 – 15.50

O12. Molecular detection of a clinically important clone of Legionella pneumophila serogroup 1 – the 'ST47 clone' by the ESCMID Study Group for Legionella Infections (ESGLI)
M. Mentasti, P. Cassier, S. David, C. Ginevra, L. Gomez-Valero,
A. Underwood, B. Afshar, C. Buchrieser, J. Etienne, V. Chalker,
T. G. Harrison, S. Jarraud, United Kingdom

15.50 – 16.20

Tea break and posters

Session 4 Chair

Outbreaks and Case Reports G. Wewalka

16.20 – 16.40

O13. Microbiology investigation of two-phase outbreak in Bremen, Germany
A. Gründel, M. Seidel, M. Leigemann, M. Berges, K. Lück, C. Lück,
Germany

16.40 – 17.00

O14. Case report: Legionella bozemanii arthritis caused by wound contamination
L. Beraud, A. Ranc, H. Lemaire, G. Descours, C. Ginevra, G. Lina,
L. Baret, S. Jarraud, France

17.00 – 17.20

O15. Legionella pneumonia associated with Continuous Positive Airway Pressure (CPAP) therapy
J.M. Stolk, A. Russcher, E. van Elzakker, E.F. Schippers,
The Netherlands

17.20 – 17.40

O16. Construction deficiencies in swim-spa pools: a new major contributing factor in outbreaks of legionnaires' disease
S. Crespi, J. del Barrio, B. Santandreu, M. Gumá, J. Riu, Spain

17.40 – 18.00

O17. A large community outbreak of Legionnaires' disease in Manzanares, Ciudad Real, Spain
C. Pelaz, R. Cano, M. Chico, G.I. Asensio, B. Bellido, A. Iascu,
I. Perez, G. Gutiérrez, Spain

18.00 – 18.15

Drinks

18.15 – 19.15

Transport by boat to the conference dinner

19.15 – 22.00

Conference dinner at the Olafs Chapel Barbizon Palace

Friday

23-09-2016

08.00 – 09.00

Registration for one day attendants

Session 5 Chair

Microbial Ecology M.L. Ricci

09.00 – 09.30

O18. Legionellae in the viable but non culturable state: how can we detect them and what could they mean for human health?
Keynote presentation by Prof. Dr. Alexander Kirschner, Austria

09.30 – 09.50

O19. Legionella growth potential of drinking water produced by reverse osmosis and materials in contact with reverse osmosis produced drinking water
P. van der Wielen, K. Learbuch, M. Lut, W. Knibbe, The Netherlands

09.50 – 10.10

O20. Legionella pneumophila ecophysiology in drinking water distribution systems
Y. Sharaby, S. Rodríguez-Martínez, M. Pecellin, I. Brettar, M. Höfle, M. Halpern, Israel

10.10 – 10.30

O21. Legionella spp. in treated wastewater in Bavaria: a public health problem?
D. Koeck, S. Huber, S. Walser, B. Brenner, S. Kolb, D.E. Koeck, C. Herr, C. Höller, Germany

10.30 – 10.50

O22. Detection of viable Legionella bacteria at the different process stages of wastewater treatment plants treating communal wastewater
P. Räsänen, J. Kusnetsov, Finland

10.50 – 11.20

Coffee break and posters

Session 6 Chair

Diagnosis and Typing V. Gaia

11.20 – 11.40

O23. Typing of Legionella pneumophila serogroup 1: the MALDI-TOF mass spectrometry could be an efficient screening method
A.G. Ranc, S. Bidah, S. Mailler, I. Maffre, A. Miclot, R. Ottoviani, A. Bricout, C. Chavent, M. Arsac, C. Vidal, Priscillia Courault, C. Ginevra, L. Beraud, G. Descours, M. Welker, F. Vandenesch, G. Durand, O. Dauwalder, V. Girard, S. Jarraud, France

11.40 – 12.00

O24. Antibiotic susceptibility of Legionella pneumophila clinical isolates in Germany
T. Koshkolda, C. Lück, Germany

12.00 – 12.20

O25. Genomic diversity and evolution of Legionella pneumophila "ST1" within hospitals and implications for future nosocomial investigations
S. David, B. Afshar, M. Mentasti, C. Ginevra, I. Podglajen, S.R. Harris, S. Jarraud, T.G. Harrison, J. Parkhill, United Kingdom

12.20 – 12.40

O26. Contribution of the Amoebae Plate Test (APT) to the isolation of Legionella spp. from clinical samples: prospective analysis over a period of 9 months
A.G. Ranc, H. Hanneltel, J.V. Reynaud, C. Kolenda, A.G. Ranc, L. Beraud, C. Campese, C. Ginevra, S. Jarraud, G. Descours, France

12.40 – 14.00

Lunch and posters

Session 7 Chair

Environmental and Surveillance C. Lück

14.00 – 14.20

O27. A real study case comparing rtPCR and culture methods in a water distribution system
G. Saucedo Pagès, I. Manero, M.J. Arnedo, B. Galofré, Spain

14.20 – 14.40

O28. Occurrence of Legionella spp. in workplaces in Italy: a nationwide survey
G. Celenza, P. Bellio, L. Di Pietro, R. Iorio, C. Luzi, A. Mancini, M. Perilli, Italy

14.40 – 15.00

O29. Development of a public map of wet cooling towers in the Netherlands
W. Reinhold, The Netherlands

15.00 – 15.20

O30. Alternative sources of clinically relevant Legionella
E. van Heijnsbergen, F.M. Schets, J.A.C. Schalk, A.M. de Roda Husman, The Netherlands

15.20 – 15.40

O31. Presence and prevalence of Legionella spp in collected rain water
L. Steege, G. Moore, United Kingdom

15.40 – 15.45

Closing of the conference



ESGLI

ESCMID STUDY GROUP
FOR LEGIONELLA INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

01. Epidemiology of Legionnaires' disease in Europe, 2015

Keynote presentation by Birgitta de Jong, Sweden

Objectives

Epidemiological trends in Legionnaires' disease (LD) in the EU area are monitored by ECDC through annual enhanced surveillance of the disease together with the Member States. In addition, through the European Legionnaires' Disease Surveillance Network (ELDSNet), real-time 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

Nominated ELDSNet members from each EU/EEA country collect and report to The European Surveillance System (TESSy) database at ECDC. Data on TALD cases are entered on a real-time daily basis to TESSy and an annual retrospective data call undertaken for all LD cases in April 2016. The two data sets were analysed separately.

Results

For all LD cases, 6 969 confirmed and probable cases were reported by 28 countries. The overall notification rate was 1.4 per 100 000 inhabitants in 2015. Male-to-female ratio was 2.5:1.

People aged over 64 years accounted for 47% of 6 962 cases with age reported. Of 5 594 cases with a known outcome, 455 were reported to have died, giving a case fatality ratio of 8%. Eighty-nine percent of the 6 969 cases reported, were diagnosed by urinary antigen test. On the basis of the EU case definition for TALD, 1 141 cases with onset of disease in 2015 were reported by 25 countries. Of these, 96% were confirmed cases. Two thirds of all reported TALD cases, were reported by the United Kingdom (213), Italy (202), France (200), and the Netherlands (142). Over two thirds of the cases (69%) were male. The median age of the reported cases was 62 years (range 10 to 94). In 2015, ELDSNet detected 167 new TALD clusters, which were associated with accommodation sites in 33 countries (or ships) worldwide.

Conclusions

The epidemiological picture of LD in 2015 continued to show an increase in the number of reported cases compared to previous years. The number of reported cases in 2015, in both systems, are the highest since surveillance began.

The added value of a European surveillance is demonstrated by the TALD cluster detection where a large proportion of these clusters would not have been detected by any single country. International collaboration through ELDSNet has been an important factor in detecting TALD clusters in 2015 as in previous years.

02. Legionnaires' disease among patients hospitalised with pneumonia in South Africa

N. Wolter, M. Carrim, C. Cohen, S. Tempia, S. Walaza, L. de Gouveia, F. Treurnicht, O. Hellferscee, A.L. Cohen, H. Dawood, E. Variava, A. von Gottberg, South Africa

Objectives

Despite being a notifiable condition, cases of legionellosis are rarely diagnosed and reported in South Africa. We aimed to determine the prevalence and characteristics of patients with Legionella infection amongst individuals hospitalised with severe respiratory illness.

Methods

Syndromic pneumonia surveillance was performed at two sentinel sites: Klerksdorp-Tshepong Hospital Complex (KTHC) in North West Province and Edendale Hospital (EDH) in KwaZulu-Natal Province. Individuals hospitalised with severe pneumonia were enrolled from June 2012 through March 2016. Demographic and clinical information was collected using standardised questionnaires. Nasopharyngeal specimens and induced/expectorated sputum were collected and tested for Legionella spp. by real-time PCR. Positive specimens were further tested by real-time PCR to identify *L. pneumophila* serogroup 1 and *L. longbeachae*.

Results

During the study period, 6322 patients were enrolled and 5662 (89.6%) were tested for Legionella: 2854 (50.4%) on nasopharyngeal specimens only, 223 (3.9%) on sputum only, and 2585 (45.6%) on both specimen types. Of patients tested, 50.8% (2869/5644) were male, 35.7% (2016/5645) were aged <5 years, and 54.3% (2129/3922) were HIV infected. Legionella spp. were detected in 28/5662 (0.5%, 95% confidence interval 0.3-0.7%) patients, with 16/28 (57.1%) cases detected in 2012. Case patients were aged 19 months to 59 years, with a median of 38 years. The highest detection rate was in the 25-44 year age group (0.9%, 16/1780). HIV infection amongst Legionella cases was documented in 68.2% (15/22). Males accounted for 15 (53.6%) cases, and the majority of cases (20/28, 71.4%) were hospitalised at KTHC. The detection rate was higher in sputum (24/28, 85.7%) compared to nasopharyngeal specimens (5/28, 17.9%) ($p < 0.01$). Species were only identified for 3/28 (10.7%) cases, due to low specimen bacterial loads. Two case patients were infected with *L. longbeachae* and one with *L. pneumophila* serogroup 1.

Conclusions

Legionella infection was detected in 0.5% of patients hospitalised with severe respiratory illness, with cases predominantly occurring in the 25-44 year age group. Sputum was more sensitive than nasopharyngeal specimens for diagnosis. Species identification was limited, but demonstrates infection due to both *L. pneumophila* serogroup 1 and *L. longbeachae* occurring in South Africa

O3. Connectedness of two outbreaks of legionellosis; Bremen, 2015 and 2016

U. Buchholz, J. Dullin, C. Lück, R. Lachmann, H.J. Jahn, B. Brodhun, A. Gründel, M. Leigemann, Germany

Objective

In the German city of Bremen, two outbreaks of legionellosis had occurred in November/December 2015 and February/March 2016, respectively. We analyzed properties of both outbreaks to understand if they were linked to each other.

Methods

We defined a suspect outbreak case as the occurrence of a laboratory confirmed illness with respiratory symptoms compatible with Legionella infection with residence, workplace or stay in the city of Bremen within 10 days prior to illness onset. A "2015-case" was defined as a case with onset of illness in November or December 2015, a "2016-case" was defined as a case with onset of illness in February or March 2016. We compared the distribution of gender, age, and the place of residence by area (North, South, West, East, Middle) and district (N=23). We conducted sequence-based as well as monoclonal antibody typing in cultures of patient samples. Potential outbreak sources were sampled and tested for contamination with Legionella bacteria.

Results

There were 19 suspect cases in 2015 and 26 in 2016. While all typable (N=2 in 2015 and N=9 in 2016) strains belonged to monoclonal subtype Benidorm, one suspect case in 2016 was infected with the monoclonal subtype Knoxville and was excluded from further analysis. In 2015, 79 % (15/19) of cases were male, in 2016 64 % (16/25) were male ($p = 0.28$). Both 2015-cases as well as 2016-cases were on average 58 years old. The age distribution did not differ significantly between outbreak years ($p = 0.09$). Both in 2015 as well as in 2016 Bremen West was the area where the most number of cases resided. Moreover, the frequency distribution of residence by area or district did not differ significantly in 2015 and 2016, $p = 0.92$ and $p = 0.40$ respectively. Among suspect cases whose culture could be sequence typed, we identified a new sequence type (ST 2151) in both outbreak years. No Legionella strain from an environmental source could be identified so far that matched the epidemic strain.

Conclusions

2015-cases and 2016-cases were similar in relation to person characteristics and place, and were likely caused by an identical source. The most probable area of infection was Bremen West.

O4. Investigating the spatial component of Legionnaires' disease outbreaks: a geographic analysis tool for field epidemiologists

E. Robesyn, S.L. Ionescu, B. de Jong, Sweden

Objectives

A literature review in 2012 revealed only few documented Legionnaires' disease outbreak investigations where geographic information systems (GIS) had been used. However, depending on the availability of data on case locations, potential source locations, demography, and meteorological conditions, a variety of analytical techniques can support the identification of the outbreak source. ECDC built an easy-to-use GIS tool that makes the most important spatial analytical techniques available to field epidemiologists. We describe the production and functionality of the tool.

Methods

Following an expert meeting, held in 2011 with ECDC Legionnaires' disease surveillance experts, GIS experts, and national epidemiologists, we identified a specific, published investigation as a model for the tool (Sarpsborg, Norway, Clin Infect Dis. (2008) 46 (1): 61-69). The construction of the tool was outsourced and took place between September 2015 and April 2016. ECDC experts guided closely the production work to ensure the expectations from the future user would be met in terms of utility and user-experience. After completion of the tool, we shared the tool for user-feedback with investigators of three real outbreaks. We further requested data from the Norwegian model-outbreak for comparison of the published results with the outputs from our tool.

Results

The tool, integrated in the Legionnaires' disease outbreak toolkit, allows the outbreak control team to quickly visualise and inspect the outbreak area, plot cases and potential sources (e.g. cooling towers), and describe the event from a spatial perspective.

The tool further allows investigators to create a density map of cases, or a spatial risk map based on a population grid. There are four models, two for the initial situation where no potential sources are suspected, and two for the situation where the outbreak team wants to compare the likelihood of potential sources to have caused the outbreak. In the latter situation, densities or risks are mapped in concentric doughnut-shaped rings around the potential source locations.

The outputs can be adjusted for the time each case had spent at different locations in the days before falling ill. The output maps can supplement the evidence from environmental and microbiological investigations. No data are stored to preserve case confidentiality. The output maps can also be displayed without the sensitive exact case locations and can therefore also facilitate outbreak communication. Before making the tool publicly available, we reproduced the output of the published investigation to learn about the comparability of our tool.

Conclusions

We built a publicly available web-based software application* allowing field epidemiologists to quickly exploit the spatial hints contained in their outbreak data and support source identification. We recommend outbreak teams to use the tool as an important extension of ECDC's toolkit for Legionnaires' disease outbreak investigation.

* <https://legionnaires.ecdc.europa.eu/gistool/>

05. DSS: monitoring biofouling tendency on the way to Legionella prevention

A. Pereira, J. Martins, Portugal

Objectives & Methods

The increase of Legionella in water system is undoubtedly potentiated by the existence of (bio)fouling layers in water systems. A good water treatment program should include the online assessment of fouling layers to correctly address the preventive and counter-measures. The DSS fulfills the need for online, non-intrusive, real-time monitoring of the (bio)fouling formation and removal tendency. This works describes how the DSS can be implemented in Cooling Water System to change the traditional paradigm of 'Looking for the water chemistry' to 'looking for the deposit'. This new approach showed to be very effective on the optimization of the preventive measures (chemical usage, overdosages, side-stream filtration, cleaning strategies and its efficacy, etc) to decrease the deposit formation and it was observed that this strategy had positive impact on the Legionella found in such systems. The DSS provides online, real-time and integrated information about the deposit layers attached to the inner surface of the monitored piping. Its functioning principles is based on the effect that the adhesion/ removal of the deposits has on the vibration of the monitoring tubing. It uses an actuator (excites the system) and a sensor (captures the vibrating response). The acoustic wave captured by the sensor is then mathematically processed and its output is correlated with the amount of the deposit attached to the monitored surface. The DSS operates in a bypass of the main system (e.g heat exchanger) and assesses non-intrusively (no contact between the sensing elements and the fluid and fouling layers) the information about the fouling trend.

Results

This new monitoring tool was implemented in a Cooling Water Tower (CWT) from a Food Industry. The water taken from the system recirculation was fed into the DSS at the hydrodynamic conditions that favors biofilm formation (0.5 m/s, Re: 5500). This system historically had some recurrent Legionella positivities. During the system auditing, the DSS output response was followed and evaluated and after establishing the fouling tendency baseline (which pointed out for HIGH FOULING TENDENCY), the preventive measures was adjusted and fine-tuned. It was seen that the DSS output response decreased to a LOW FOULING TENDENCY threshold and since then the Legionella positivities have been significantly reduced.

Conclusions

The DSS measures the direct impact of the water treatment regarding (bio)fouling phenomena. Being so, and since Legionella proliferation is directly conditioned by the existence of biofouling layers, a pro-active prevention, minimization and monitoring of such deposits strongly contribute for the mitigation of Legionella occurrence.

06. Development of new UK guidance for the delivery and management of safe water in healthcare premises for the control of Legionella

J. Walker, P. Ashcroft, United Kingdom

The Department of Health in England (DH) has recently produced new guidance for the delivery and management of safe water in healthcare premises. This new guidance focuses on a holistic approach via the integration of water safety groups (WSG) and water safety plans to manage and minimise the risks to health including clinical, microbiological and chemical aspects.

The DH worked with over 20 individual UK steering group members who were engaged in multiple face to face meetings and teleconferences in 2015 and 2016 to write and agree the content. The documents have been produced in three parts; A (Design, installation and commissioning), B (Operational Management) and C (*Pseudomonas aeruginosa* – advice for augmented care units). The most comprehensive updates from previous DH guidance includes; measures to control waterborne pathogens such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, mycobacteria as well as Legionella; alignment with Health and Safety Executive's (HSE's) revised Approved Code of Practice for Legionella (L8) and its associated HSG274 guidance documents (2013/14); the WSG being informed of decisions that affect the safety and integrity of the water systems and associated equipment before they go ahead and include decisions on the procurement, design, installation and commissioning of water services, equipment and associated treatment processes; that there is hygienic storage and installation of fittings and components and that installers/plumbers working on healthcare water systems as well as cleaners and clinical staff should complete a water hygiene awareness training course. In addition, those engaged to carry out any risk assessments associated with water safety should demonstrate to the WSG their experience and competence in assessing specific risks from microbiological, chemical and physical hazards on the specific healthcare population.

Following completion of draft documents the DH consulted with relevant stakeholders whose individual comments were assessed and commented on by the steering group prior to finalisation of the guidance document.

This new guidance which takes the format of a Health Technical Memorandum gives comprehensive advice and guidance to healthcare management, design engineers, estate managers, operations managers, contractors and procurement on the legal requirements, design applications, maintenance and operation of hot and cold water supply, storage and distribution systems in all types of healthcare premises and was published on 20th May 2016.

07. Energy efficiency and hygiene in drinking water installations

C. Lück, M. Petzold, T. Koshkolda, C. Schreiber, G. Schaule, R. Rothmann, K. Rühling, Germany

Objectives

Since the amendment of the German Drinking Water Ordinance in 2011, routine testing for *Legionella* spp. is obligatory in buildings with large volumes of hot drinking water (PWH). A common practice to prevent contamination of drinking water plumbing systems (DWPS) by *Legionella* spp. is heating-up of drinking water tanks to temperatures that are adverse for legionellae growth. However, temperature induced *Legionella* prevention needs energy. In larger buildings, PWH temperatures at 60 °C or even more consume more than 50 % of the energy needed for heat supply. The balance between energy costs and health is not yet fully evaluated.

In the transdisciplinary research project Energy Efficiency and Hygiene in Drinking Water Installations (EE+Hyg@TWI) in context to the Domestic Hot Water (DHC) in Low Temperature Systems focuses on the intersection of possible energy reduction potentials in PWH without increasing the health risk by *Legionella* spp.

Methods

Up to now, 72 buildings were included in the study. Water samples were taken from central hot water tanks (2nd liter of hot water flow, cold water supply and circulation return flow) and peripheral taps (1st, 2nd and 5th liter hot water; 5th liter cold water). Samples were analyzed for the presence of *Legionella* spp. and *Pseudomonas aeruginosa* according to ISO 11731-2 and ISO 16266, respectively. Furthermore, qPCR-based quantification *L. pneumophila* and *Legionella* spp. was performed using the PALL system (PALL Extractor and GeneDisc) or the BioRad system (Aquadien/iQ-Check). Next to microbiological analysis, we monitored water temperature and tapping profiles of each building over two weeks to reveal stagnation periods of the system and temperature shifts.

Results

Culture based analysis revealed that 32 % of the buildings were tested positive for *Legionella* spp. from which 13 % even exceeded the German technical threshold level (TTL) for *Legionella* spp. in drinking water of > 100 cfu/100 ml. In 18 % of the buildings *Legionella* spp. were isolated from cold water samples, with 3 % exceeding the TTL. By using qPCR, almost all buildings were tested positive for the presence of *Legionella* spp. DNA (98 %) with 45 % buildings tested positive for *L. pneumophila*.

Conclusions/Outlook

Extended sampling of DWPS showed a not negligible amount of *Legionella* spp. in cold water samples. Especially the 5th liter of cold water samples revealed a systemic contamination of legionellae. The influence of hot water temperatures on cold water systems needs to be evaluated.

08. The *Legionella* genus genome: diversity and plasticity, the key to

Keynote presentation by Prof. Dr. Carmen Buchrieser, France

Currently there are 61 species/subspecies described within this genus *Legionella*. We have sequenced 47 *Legionella* species and have characterized their genetic composition through comparative genomics. Together with the already published genomes, we have analyzed and compared 80 genomes representing 58 species-subspecies, a knowledge that is providing us with a quasi-complete overview of the genus *Legionella*. Furthermore all strains had been tested for their ability to infect and replicate in *Acanthamoeba castellanii* and in the human macrophage cell line THP-1.

Our results show that the *Legionella* genomes differ considerably in size (2.2-4.2Mb), gene content and effector repertoire. Results of this global comparative study will be presented. This new knowledge will not only be essential for *Legionella* research, but also a step towards the study of genomic diversity at the bacterial genus level.

O9. An evolutionary genomic history of Legionnaires' disease in Scotland

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Objectives

Approximately 6-9 cases of Legionnaires' disease per 100,000 inhabitants are reported each year in Scotland, and in 2012, a relatively large outbreak occurred resulting in 92 laboratory confirmed or probable cases and four deaths. The primary aim of this study is to apply whole genome sequencing to investigate the genomic diversity and evolutionary history of *Legionella pneumophila* isolated in Scotland over the last 30 years to identify the relationships between clinical isolates and environmental isolates in the context of the global diversity. Our second aim was to identify the repertoire of the *Legionella*-specific MPFG type IV secretion system (T4SS), that we have previously shown to contribute to the heterogeneity of virulence within a single outbreak.

Methods

We performed 250bp paired end high-throughput whole genome sequencing of 400 *Legionella pneumophila* strains isolated and cultured from clinical and environmental samples in Scotland from 1984 to 2015.

Results

Phylogenetic analyses indicate that these 400 strains of *L. pneumophila* represent a heterogeneous population of endemic and globally distributed lineages, and horizontal gene transfer and recombination have played a major role in shaping the genome of this species. Additionally, we analysed the distribution and diversity of the *Legionella* Genomic Island-associated T4SS (LGI-T4SS) that we previously correlated with increased virulence in a *Galleria mellonella* infection model. The LGI T4SS cluster can be divided into two main genetic subtypes that were present in 89% (Type I) and 62% (Type II) of the Scottish *L. pneumophila* isolates, respectively. Of note, Type 2 LGI-T4SS exhibited considerable genetic diversity and was distributed among *L. pneumophila* and *Legionella longbeachae* isolates, consistent with inter-species horizontal transfer. These data suggest an important role for the LGI T4SS cluster in *Legionella* spp. genome evolution and pathogenicity.

Conclusions

We have generated and analysed the largest collection of *L. pneumophila* whole genome sequences from a single country spanning 30 years, providing new insights into genome evolution, global distribution, and pathogenic capacity.

O10. Whole Genome Sequencing supports the role of waste-basin aeration as a direct source of Legionnaires' disease transmission during Lens outbreak

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Objectives

In 2004, a large legionellosis outbreak in Pas-de-Calais provided evidence of long-distance airborne transmission of *Legionella pneumophila* via cooling towers (CTs) at Plant A. Bacteriological data supported the hypothesis that contaminated waste-basin sludge was the source of CT contamination whilst epidemiological data suggested that waste-basin aeration was a direct source of LD transmission. However, no bacteriological typing data (PFGE and SBT) was able to confirm these hypotheses. The epidemic strain was also isolated from a plant B CT and car wash station (CWS) both located < 1km from Plant A. Our objective was to determine whether whole genome sequencing (WGS) could be used to support or refute the role of the waste-basin as the direct source of contamination and also determine the potential role of plant B and CWS.

Methods

WGS data from 78 *L. pneumophila* serogroup 1 (Lp1) ST15 isolates including 67 strains isolated during the outbreak (21 clinical isolates and 46 isolates from biological seeding, sludge, air above waste-basin, waste-basin, plant A and plant B CTs, CWS), 8 ST15 strains isolated in Plant B in 2007 and 3 ST15 strains from UK, were analysed. Mapping/SNP-based analysis using the Lens strain (ST15) as a reference genome and a core genome MLST (cgMLST) approach using 50, 100, 500 and 1455 genes were performed.

Results

A maximum likelihood phylogeny of SNPs identified 3 distinct clusters separating strains isolated during the 2004 outbreak, in 2007 and in the UK. SNP differences between the 67 isolates from the outbreak ranged from 0 to 16. The tree also revealed 2 subgroups amongst these strains, one of which includes 1 clinical strain and 9 strains isolated only in the waste-water treatment system (WWTS) and CWS. 50-gene cgMLST and 100-gene cgMLST were unable to distinguish ST15 strains isolated in 2004, in 2007 and in the UK in contrast to 500-gene and 1455-gene cgMLST. 1455-gene cgMLST showed that outbreak strains differ by 0 to 10 alleles and identified 4 allelic profiles that are shared by 15, 5, 2 and 2 strains respectively. Two of these allelic profiles contained one clinical isolate and strains isolated only in the WWST.

Conclusions

SNP-based typing and 500-gene and 1455-gene cgMLST performed well in distinguishing between epidemiologically related and unrelated ST15 isolates. SNP-based typing and 1455-gene cgMLST were successfully used to support the suspected direct link between the waste-basin and some of LD cases and between the waste-basin and the CWS.

O11. Comparison of sputum microbiome of legionellosis-associated patients and other pneumonia patients: indications for polybacterial infections

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Objectives

Bacteria of the genus *Legionella* cause water-based infections resulting in severe pneumonia. Here we analyze and compare the bacterial microbiome of sputum samples from pneumonia patients in relation to the presence and abundance of the genus *Legionella*.

Methods

The prevalence of *Legionella* species was determined by culture, PCR (using *Legionella* genus-specific primers), and Next Generation Sequencing (NGS). We compared bacterial communities of sputum of pneumonia patients with respect to richness, diversity, and relative abundances of bacterial genera in correspondence with presence and abundance of the genus *Legionella* and *L. pneumophila*.

Results

Nine sputum samples out of the 133 analyzed were PCR-positive. In contrast, *Legionella* was isolated by culture only from one sample. Illumina MiSeq 16S rRNA gene sequencing analyses of 21 sputum samples (8 *Legionella*-positive by PCR and 13 *Legionella*-negative) confirmed that *Legionella* was present in the PCR-positive sputum samples. The NGS approach allowed the identification of the bacterial community of the sputum at the genus level, and for the genus *Legionella* at the species and sub-species level. At the genus level, although a major fraction (42%) of the sputum samples was dominated by *Streptococcus*, a broad variety of seven different dominant genera (*Acinetobacter*, *Stenotrophomonas*, *Escherichia-Shigella*-complex, *Haemophilus*, *Proteus*, *Corynebacterium*, *Prevotella*) was observed. Interestingly, *Legionella* was never dominant but reached a maximum of 2.9% relative abundance with *L. pneumophila* as the dominant species. Comparison between NGS and the culture results that were obtained by the hospital laboratory were highly divergent in many cases species at a low abundance in NGS were cultured. Aquatic bacteria and especially bacteria that have been observed in an amoeba microbiome were identified in sputum samples that were positive for *Legionella*.

Conclusions

We identified for the first time the sputum major bacterial commensals and pathogens profiles with regard to *Legionella* presence. Our results demonstrate that there is a need to reevaluate the regulation of medical laboratory routine procedures and to consider using molecular methods for more accurate diagnosis. The presence of aquatic bacteria in *Legionella* positive samples may indicate that amoeba or amoebal vesicles might have transferred *Legionella* and its accompanying microbiota to the patients. Further studies at larger scales are essential to study the diversity and co-occurrence of *Legionella* and other pathogenic and non-pathogenic bacteria in lung and sputum samples, in order to elucidate *L. pneumophila*'s interactions and competitiveness.

O12. Molecular detection of a clinically important clone of *Legionella pneumophila* serogroup 1 – the ‘ST47 clone’ by the ESCMID Study Group for *Legionella* Infections (ESGLI)

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Objectives

While *L. pneumophila* serogroup 1 (Lp1) ST47 is the leading cause of legionellosis in Northern Europe, it is very rarely isolated from environmental samples. A comparative genomic approach was applied to develop a rapid PCR assay and better understand the evolution of Lp1 ST47.

Methods

Comparative analysis of 36 genomes representative of Lp species and preliminary in vitro results on an initial set of Lp strains, identified LPO_1073 as ST47-specific. A total of 604 Lp genomes (86 different STs), of which 129 ST47, were then examined for LPO_1073 presence. A real-time PCR was designed to detect a 142bp fragment of LPO_1073. A total of 387 *Legionella* strains isolated worldwide were analysed in vitro to validate this method. The LPO_1073 PCR was tested on 106 Lp1 PCR positive respiratory samples and 3 water samples from 2 proven ST47 sources.

Results

LPO_1073 was 100% conserved in all 129 ST47 sequenced genomes and the PCR was positive for all ST47 strains. When non-ST47 strains were tested, nine ST109 strains isolated in France were also positive, but interestingly eight UK ST109s were negative. Phylogenetic analysis based on SNPs revealed that ST47 evolved from “France” ST109 via recombination events. “France” and “UK” ST109s fall into two sub-groups, with the former more closely related to ST47, thus explaining the different PCR result. Our analysis suggests that “France” ST109 acquired LPO_1073 then recombined with ST62 to generate ST47. We analysed additional 27 ST109s present in the Lp SBT database by LPO_1073 PCR: of a total of 44 ST109s, 18 were negative [UK (11), USA (5) and Germany (2)] and 26 were positive [France (11), Netherland (7) Germany (5) DK (2), Belgium (1)]. These results suggest that the recombination events leading to the emergence of ST47 may have occurred somewhere in mainland-Northern-Europe. The LoD of the LPO_1073 PCR was determined as 34.3 copies/reaction (95% confidence). In accordance with previous culture results, 71/106 clinical samples resulted LPO_1073 PCR negative while 22/106 were positive. Four out of 13 (30.8%) culture negative samples were also positive by LPO_1073 PCR. The 3 environmental samples from 2 ST47 culture positive sources also gave a positive LPO_1073 PCR result.

Conclusions

We developed a sensitive PCR assay to detect the leading cause of LD in Northern Europe, also useful to investigate the distribution of Lp1 ST47 in the environment. The Genomic approach helped designing the PCR assay and understanding the evolution of Lp1 ST47.

O13. Microbiology investigation of two-phase outbreak in Bremen, Germany

A. Gründel, M. Seidel, M. Lelgemann, M. Berges, K. Lück, C. Lück, Germany

Objectives

In November 2015, 19 cases of Legionnaires' disease (LD), including one fatal, were reported in the city of Bremen. From three patients *Legionella* spp. isolates were obtained and analyzed using monoclonal antibodies (mAb) and sequence based typing (SBT). The outbreak strain was characterized as *L. pneumophila* serogroup (Sg1), mAb-subgroup Benidorm, and the novel sequence type (ST) 2151 (allele formula 2, 6, 17, 6, 13, 11, 59), whereas the neuA allele represent a new allele number. The source of infection was not identified and no additional cases were counted 25 days after the last reported case. In February 2016, a new cluster of LD cases in Bremen was reported.

Methods

LD cases were laboratory confirmed by *L. pneumophila* PCR (n=20), *L. pneumophila* sg 1 PCR (n=5), urine antigen test (n=42) or culture (n=13). Since the majority of Legionella outbreaks were caused by cooling towers (CT), the focus has been put on companies with CT's near by the living and working places of the patients. Seven companies were sampled. In total, 549 *L. pneumophila* Sg1 isolates were further typed by a panel of six mAb's (mAb 81-2, mAb 48-3, mAb 8-4, mAb 3, mAb 20-1, mAb 30-1) using an automated ELISA. The results were validated by the established "Dresden Panel" and analyzed by SBT.

Results

During the second outbreak 26 LD notifications were reported with another fatal. The outbreak strain was identical to the first outbreak. Several cooling towers as putative source were sampled. Several mAb-subgroups were found including mAb-subgroup Benidorm, however, the Benidorm mAb subgroup outbreak strain had different ST. The new mAb's tested were chosen due to its isotype (IgG1 or IgG2a/b) which is optimal for purification. The new panel showed excellent results and was in full accordance with the established panel for all tested isolates.

Conclusions

The outbreaks in Bremen proved to be in fact one repeated outbreak with biphasic character having the same putative source of infection. Despite the high number of *L. pneumophila* isolates obtained from different environmental samples, we were not able to detect the outbreak strain so far. In total, 43 LD cases and two deaths were reported. The case-fatality rate was 4.65 %. The lack of a central registry for CT's in Germany made the search for potential sources difficult. However, the automated ELISA helped to subgroup rapidly and reliably the high amount of samples.

O14. Case report: *Legionella bozemanii* arthritis caused by wound contamination

L. Beraud, A. Ranc, H. Lemaire, G. Descours, C. Ginevra, G. Lina, L. Baretts, S. Jarraud, France

We describe a case of a 56-year-old woman who received glucocorticoids, methotrexate and tocilizumab (a humanised monoclonal antibody that blocks the Interleukin-6 (IL-6) receptors) for an anti-synthetase syndrome. She fell down on the floor in a street market on the 5th of February and had a superficial wound on the middle left finger. Three days later, she received an intra articular corticosteroid injection in the left wrist. On the 11th of February, she came back to hospital for arthritis. A joint infection due to corticosteroid injection was initially suspected and anti-staphylococcal antibiotics (oxacillin and gentamicin) were started. Joint fluid puncture was realised and sent to microbiology laboratory on the 15th of February after starting anti-staphylococcal antibiotics. Because of the poor clinical evolution, antibiotics spectrum was enlarged on the 20th of February (piperacillin-tazobactam and vancomycin).

Ultimately, surgery was performed on the 25th of February and four samples of wrist abscess were sent to microbiology laboratory. Standard culture remained negative for all samples in spite of a 14 day-incubation and the use of various culture media. Universal 16S rRNA PCR was positive for *Legionella* spp. in 3 of the 5 samples analysed.

PCR amplification and sequencing of 23S-5S ribosomal intergenic region performed on 4 samples was positive for *Legionella bozemanii*. The culture of all samples was then performed on *Legionella* media: BCYE, BMPA and MWY. Only one sample was positive on BCYE after 9 days of culturing. Various colours and sizes of colonies and blue fluorescence under UV light were observed. All of the strain morphotypes were identified as *L. bozemanii* by matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry. The species identification was confirmed by sequencing of the mip gene. Pulsed Field Gel Electrophoresis (PFGE) was also performed on the 5 strain morphotypes and the same sporadic profile was observed. A chest radiography performed on the 15th of February showed no difference from the last one performed on December 2015.

Conclusions

We report a case of *Legionella bozemanii* arthritis diagnosed by PCR. The culture was positive for one of the five samples and confirmed the PCR diagnosis. The wound infection, caused by a fall of an immunocompromised patient on wet ground seems to be the cause of the arthritis. The unchanged chest radiography rules out a pulmonary origin of the wrist joint infection.

O15. Legionella pneumonia associated with Continuous Positive Airway Pressure (CPAP) therapy

J.M. Stolk, A. Russcher, E. van Elzaker, E.F. Schippers, The Netherlands

Introduction

Legionella bacteria is a relatively uncommon cause of community acquired pneumonia. Aquatic systems such as water distribution systems and air conditioning systems can act as amplifiers for the growth and proliferation of Legionellae. Since 2013 only three cases had been described of a Legionella community acquired pneumonia with a potential association with CPAP equipment. However, there is no reported case of Legionella pneumonia related to CPAP equipment in which an identical Legionella was found in the patient and the CPAP equipment. We describe a case where we found identical Legionella bacteria in the patient and the CPAP equipment.

Case description

A 51-year-old man came to the emergency department with fever, confusion and dyspnea since 3 days. Six weeks earlier he was returned from a holiday in Italy where he had stayed in an apartment. He had not visited a sauna and did not stay in hotels. There was no contact with birds during the last weeks. No other persons in his environment showed the same clinical signs and he was a non-smoker. His medical history included obstructive sleep apnea, for which he used CPAP therapy at home since 12 weeks. Chest X-ray revealed left lower pulmonary lobe consolidation. He was diagnosed with community-acquired pneumonia with a Pneumonia Severity Index (PSI) class V. Based on the calculated PSI score there was a high probability of morbidity and mortality. We started empirical therapy with intravenous amoxicillin and ciprofloxacin. Laboratory investigation demonstrated a positive urine Legionella antigen test. His respiratory status declined and he was intubated and admitted to the intensive care unit. Further investigation showed that there was no exposure to high risk installation for Legionella during the incubation period with exception of the CPAP equipment. The CPAP equipment showed signs of poor maintenance. Water from the CPAP equipment and sputum from the patient revealed Legionella pneumophila. Serotyping and sequence-based typing showed an identical Legionella pneumophila serotype 1 ST37.

Conclusions

Because of an increase in incidence of OSAS in the last decade it is most likely that the use of CPAP therapy will increase. It is important to know that CPAP equipment can be colonized with Legionellae and might cause legionella pneumonia. Therefore it is necessary to ask for CPAP therapy in a patient with community-acquired pneumonia. Based on this case the Dutch centre for disease control and prevention implemented this question in the 'Legionellosis Hypothesis-Generating Questionnaire'. Furthermore, it is essential for providers of CPAP therapy to provide clear information on the correct maintenance of CPAP equipment.

O16. Construction deficiencies in swim-spa pools: a new major contributing factor in outbreaks of legionnaires' disease

S. Crespi, J. del Barrio, B. Santandreu, M. Gumá, J. Riu, Spain

Objectives

In 2012, the origin of a big outbreak of Legionnaires' disease in a hotel in Calpe, Spain, was traced to construction deficiencies in its swim-spa pool shell structure. Although this particular case has been regarded as exceptional, deficiencies in both the design and structure of these at-risk systems are rarely assessed during outbreak investigations and their impact is largely unknown.

Methods

We have investigated two outbreaks of travel-associated Legionnaires' disease (TALD) in hotels in Catalonia and Balearic Islands (Spain) that were eventually associated with their respective swim-spas. Additionally, we inspected three hotels, all in Spain, which had been associated with clusters and/or recurrent cases of TALD. During our investigations, we assessed the design and architectural characteristics of the swim-spa pools in the five hotels studied. In all the cases, we drilled holes in the pool shell structure in order to confirm or rule out the supposed deficiencies.

Results

We have detected important construction deficiencies in the five risk assessed pools. The drillings confirmed the presence of hidden cavities holding stagnant, non-treated water in all of them. Legionella pneumophila was detected either by culture or by qPCR in the cavities of three of the pools inspected. As for the two investigated TALD outbreaks, the environmental and microbiological findings strongly suggest that the hidden cavities found in the shell structure of the pools were the reservoir of the infecting strain.

Conclusions

Construction deficiencies in swim-spa pools can be a major contributing factor in Legionella infections associated with these water systems. Our findings suggest that this problem may be more widespread than previously considered.

O17. A large community outbreak of Legionnaires' disease in Manzanares, Ciudad Real, Spain

C. Pelaz, R. Cano, M. Chico, G.I. Asensio, B. Bellido, A. Iascu, I. Perez, G. Gutiérrez, Spain

Outbreak description

In December 2015, a large community outbreak was identified by the Public Health Services of Manzanares, a town with 18,642 inhabitants in the province of Ciudad Real, Spain. 558 cases were notified, 278 were classified as confirmed and 26 as probable cases. The onset of symptoms was from 19-11-2015 to 5-01-2016. 88% of the confirmed/probable cases occurred in two weeks. 36% of the confirmed cases were hospitalized and four patients died (CFR=1.4%).

Epidemiological investigation

Different epidemiological studies were carried out. All of them pointed out that the most probable source of the outbreak was in the district 3 in the North-East part of the town. The crude and adjusted incidence rate in district 3 was 2 times higher compared to the lower rate (district 1). ORs calculated in the case control studies were 6.7, and 4.07 for cases living near the two suspected sources of infection placed in district 3 (open-air decorative fountain and cooling tower). Relative risk of 2.10 was calculated by Kernell for cases living in district 3.

Environmental investigation

67 water installations were inspected, including cooling towers, decorative fountains, irrigation systems, etc. 170 water sample from 31 installations were analyzed. 7 water samples were positive for Legionella by culture and 9 were positive by immune-magnetic system.

Microbiological investigation

Legionella was isolated from respiratory samples of 30 patients and all isolates were identified as *L. pneumophila* SG 1, Philadelphia, ST899. There were other 17 patients in which Nested-PCR-ST899 was detected in absence of culture. Nine new STs were also detected among patients. The patient strain was recovered from two installations: a cooling tower and a water sprinkler placed out of the town. *L. anisa* was identified in a decorative fountain.

Control measures

On 12-12-2015, the protocol for the investigation of the outbreak was activated: two hospitals were informed and the environmental investigation initiated. Activity of all risk water installations was immediately suspended until inspection, sampling, and laboratory results were available. Inspections of installations were carried out, mainly in the industrial area.

Conclusions

Control measures were effective as LD cases ceased. The wide exposure of the population who lived in a 474 km² area hampered the epidemiological investigation. Epidemiological investigation focused in two installations as the most probable sources of infection in district 3, and microbiological results supported the cooling tower that seemed to be out of service during the most probable exposure period.

O18. Legionellae in the viable but non-culturable state: how can we detect them and what could they mean for human health?

Keynote presentation by Prof. Dr. Alexander Kirschner, Austria

For routine surveillance of legionellae in engineered water systems and outbreak investigations, cultivation-based standard techniques are usually applied. However, in many cases culture-negative results are obtained despite the presence of viable legionellae, and clinical cases of legionellosis cannot be traced back to their respective contaminated water source. Among the various explanations for these discrepancies, the presence of viable but non-culturable (VBNC) Legionella cells has received increased attention in recent discussions and scientific literature.

Alternative culture-independent methods to detect and quantify legionellae have been proposed in order to complement or even substitute the culture method in the future. Such methods should detect VBNC Legionella cells and provide a more comprehensive picture of the presence of legionellae in engineered water systems. However, it is still unclear whether and to what extent these VBNC legionellae are hazardous to human health. This presentation critically evaluates current methods to determine legionellae in the VBNC state, their potential to complement the standard culture-based method in the near future, and summarizes current knowledge on the threat that VBNC legionellae may pose to human health.

O19. Legionella growth potential of drinking water produced by reverse osmosis and materials in contact with reverse osmosis produced drinking water

P. van der Wielen, K. Learbuch, M. Lut, W. Knibbe, The Netherlands

Microbial growth in the drinking water distribution system in the Netherlands is prevented by limiting biodegradable compounds to such an extent that the water is biologically stable. Drinking water company Oasen is implementing membrane filtration with reverse osmosis (RO) to produce drinking water with a higher degree of biostability. Since RO removes microbial biodegradable compounds, it is hypothesized that remineralised RO-water limits biofilm formation and multiplication of *Legionella pneumophila*.

The objectives of our study were to (i) determine the *Legionella* growth potential (LGP) of remineralised RO-water and compare this to conventional treated water, and (ii) determine whether different materials, in contact with remineralised RO-water, can enhance growth of *L. pneumophila*. The LGP of water was determined with boiler biofilm monitors (BBM) that mimics the flow of water in a premise plumbing system, and that were fed with RO-water produced from groundwater, remineralised RO-water and drinking water from the conventional treatment. The LGP of materials in contact with remineralised RO-water was determined using the biomass production potential test for materials, with the modification that each flask with material was inoculated with *L. pneumophila* 16 weeks after the test started. The results from the BBMs demonstrated that the *L. pneumophila* numbers in de BBMs fed with RO-water or remineralised RO-water were relatively low, whereas *L. pneumophila* numbers in de BBMs fed with conventional treated water were 100 to 1000 times higher. Consequently, the LGP of RO-water or remineralised RO-water were approximately 500 to 700 times lower than the LGP of conventional treated water (23.1 to 34.7 CFU cm² and 1.7×10^4 CFU cm², respectively). The results from the material test demonstrated that enhanced growth of *L. pneumophila* was not prevented when the remineralised RO-water was in contact with PE-100, PE-XC and PVC-P. The *L. pneumophila* numbers were 10 to 100 times higher with these materials than with glass, which is an inert material. This enhanced growth of *L. pneumophila* is likely caused by growth promoting substances that leak from the materials.

Overall, our study demonstrates that distribution of remineralised RO-water does not enhance growth of *L. pneumophila* in the BBM system that mimicked the premise plumbing system. However, when materials as PE-X or PVC-P are used in the premise plumbing system, growth of *L. pneumophila* can still occur despite the immaculate quality of the supplied remineralised RO water.

O20. Legionella pneumophila ecophysiology in drinking water distribution systems

Y. Sharaby, S. Rodríguez-Martínez, M. Pecellin, I. Brettar, M. Höfle, M. Halpern, Israel

Objectives

Legionella pneumophila (L.p) cause water-based infections, resulting in severe pneumonia. Our aim was to improve our knowledge regarding L.p ecology in Drinking Water Distribution Systems (DWDS) in Israel with emphasis on the eco-physiological traits of different L.p genotypes colonizing DWDSs.

Methods

Three years survey was conducted and seasonal samples were taken from water and biofilm at different sampling points of a small drinking water distribution system. L.p was isolated and identified to its genotype level by Multiple-Locus Variable number of tandem repeat Analysis (MLVA). Different physiological traits related with the bacterial life cycle and virulence were studied in laboratory in-vitro experiments; growth traits kinetics modeling (λ , μ_m , A), pore forming activity (hemolysis) and temperature-dependent amoebae infectivity rates were used to determine and compare the physiological characteristics of each of the strains.

Results

Within the studied DWDS, *Legionella* exhibited a seasonal pattern with significantly higher counts in summer and a decrease during the colder months of autumn and winter. *Legionella* was isolated from six out of the seven selected sampling points, with counts up to 5.8×10^3 cfu/l. L.p counts were negatively correlated with chlorine. Five L.p MLVA-genotypes (Gt4, Gt6, Gt15, Gt17 and Gt18) were identified at different buildings along the DWDS route. The presence of a specific genotype, Gt4, consistently co-occurred with high *Legionella* counts and seemed to “trigger” high *Legionella* counts in cold water. Mathematical modeling of the bacterial growth at different temperatures revealed significant differences between the growth traits of the three L.p genotypes. Gt4 strains exhibited superior growth and adaptation to lower temperatures (25-30 °C) while Gt15 appeared to be best adapted to relatively higher temperatures (42-45 °C). Only Gt15 strains were able to proliferate at 45 °C. In addition, significant differences in the virulence traits were observed between the genotypes. Gt15 strains showed a significantly higher pore forming activity. In contrast, Gt4 and Gt6 strains were more infective for *Acanthamoeba castellanii*.

Conclusions

We showed, for the first time, that different L.p genotypes possess different physiological traits. These traits may influence their colonization at specific ecological niches within the DWDS. Moreover, the differences in their virulence traits suggest that different genotypes have different pathogenicity potential towards humans. Our findings highlight the importance of understanding the eco-physiology of different L.p genotypes and may be used in the future as a tool to improve the methods for L.p monitoring and for genotype-specific public health risk assessment in DWDSs.

O21. Legionella spp. in treated wastewater in Bavaria: a public health problem?

D. Koeck, S. Huber, S. Walser, B. Brenner, S. Kolb, D.E. Koeck, C. Herr, C. Höller, Germany

Introduction

In summer 2013 a large outbreak of Legionnaires' disease occurred in Warstein, Germany. Approximately 165 people become ill, 3 died. The outbreak was most probably caused by contaminated aerosols generated by a cooling tower which was fed by river water. High concentrations of Legionella spp. were detected in river water and effluents of the local waste water treatment plant (WWTP) discharging into the river. The local WWTP was contaminated by high concentrations of Legionella spp. from the WWTP of a brewery. This outbreak led to major concerns about the role of treated wastewater in causing Legionnaires' disease in Germany.

Objectives

There exist very little data about concentrations of Legionella spp. in Bavarian WWTPs and possible health risks. Therefore detection methods were established and WWTPs were sampled.

Methods

Biologically treated wastewater and WWTP final effluents were tested for Legionella spp. Moreover aerosol samples taken near aeration tanks were investigated. WWTPs from dairy and paper industries and breweries were selected, as the water temperatures are high and therefore favourable for Legionella growth. There are no standardized methods for cultivation of Legionella spp. in wastewater. A detection method including dilution and heat treatment of the samples was established. Bioaerosol samples were taken by Coriolis μ cyclone air sampler and also tested for Legionella spp. by cultural methods. Additionally Legionella spp. was detected by quantitative real-time PCR and antibody microarrays.

Results

Twelve WWTPs were investigated and Legionella spp. was detected in the water samples from ten WWTPs, in five plants by PCR and cultivation, in three plants only by PCR and in two plants only by cultivation. The maximum concentrations were 3.3×10^6 CFU / 100 ml from culture methods, 5.44×10^7 copies / 100 ml from PCR and 8.30×10^7 CFU / 100 ml from antibody detection, respectively. The lower concentration of Legionella spp. in the final sedimentation compared to the biological treatment step indicates an overall reduction of Legionella sp. during sewage treatment. Bioaerosol samples from four WWTPs were tested positive for Legionella spp. by PCR (above detection limit). But no growth of Legionella spp. was obtained from any aerosol sample. In the aerosol samples from one plant Legionella spp. could only be detected by microarray.

Conclusions

Despite the reduction of Legionella spp. during waste water treatment, high concentrations of Legionella spp. are still present in the WWTP effluents and consequently in surface waters. Concentrations in wastewater treatment plants can even grow under specific conditions (see, for example outbreak in Warstein). If searching for the cause of an outbreak of Legionnaires' disease, WWTPs should therefore always be considered.

O22. Detection of viable Legionella bacteria at the different process stages of wastewater treatment plants treating communal wastewater

P. Räsänen, J. Kusnetsov, Finland

Legionellae can cause severe pneumonia when aerosols containing Legionella bacteria are inhaled. In recent studies, very big concentrations of Legionella have been found from wastewater treatment plants (WWTP) treating industrial wastewater, also in association with legionellosis. High amount of other microbes, organic material and inhibitors are challenging culture and quantitative polymerase chain reaction (qPCR) based methods for detection of Legionella from wastewater. False negative culture results can be very misleading. Propidium monoazide qPCR makes inspection of viability stage bacteria cell possible without culture method. The aims were to detect Legionella bacteria and observe their viability from samples of different wastewater treatment stages of WWTPs treating communal wastewater. The detection methods were culture and the combination of propidium monoazide treatment with qPCR assays: Legionella spp. (16S rRNA), Legionella pneumophila (mip gene) and Legionella pneumophila serogroup 1 (wzm gene). Samples were collected and analysed in years 2012 and 2014. In wastewater samples of the plant A, Legionella concentrations were up to 2300000 CFU/l with culture and 31000000 cells/l with qPCR. From the sludge samples, Legionellae were detected up to 160000 cells/g with qPCR. From the plant B, concentrations up to 100000 CFU/l with culture and 32000000 cells/l with qPCR were detected from wastewater. The sludge contained up to 9900 cells/g by qPCR. All the three sludge samples studied from plants A and B were culture negative. The portion of viable Legionella cells varied from 29 to 100 % depending on the WWTP process stages. Serogroup 1 of Legionella pneumophila species was detected in one sample from year 2012 by culture, and from three samples with qPCR. Legionella spp. and serogroups from 2 to 15 of Legionella pneumophila species were more often detected from the samples.

Legionella concentrations were generally possible to estimate using these qPCR assays while culture method had more difficulties to detect Legionellae from these wastewater matrices. As the covalent binding between PMA and dsDNA is based on photoreaction, the higher amount of organic deposit may restrict its use to determine the viability of Legionella in some samples. The detected Legionella concentrations were throughout big even though this study was focusing on the WWTPs of communal wastewater, not industrial WWTPs. Basing on these results, the use of safety respirators for employees and visitors should be recommended while working and visiting also the WWTPs of communal wastewater.

O23. Typing of *Legionella pneumophila* serogroup 1: the MALDI-TOF mass spectrometry could be an efficient screening method

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Objectives

Sequence-Based Typing (SBT) is still the gold standard method recommended by ESGLI for *Legionella pneumophila* subtyping. Due to high discriminatory power, Whole Genome Sequencing (WGS) becomes the method of choice for epidemiological investigations. However, both SBT and WGS are expensive methods which do not allow their application to a large number of isolates during cases investigation. The usefulness of MALDI-TOF in typing methods have been described for some bacteria but only few studies concerned *L. pneumophila*. The aim of our study was the development of an easy-to-use typing method of *L. pneumophila* serogroup 1 (Lp1) through MALDI-TOF process.

Methods

A total of 247 strains (229 clinical and 18 environmental isolates) comprising 27 frequently isolated Sequence Type (STs) in Europe were tested. All these isolates were gathered into 11 groups: 4 clonal complexes (CC) CC1, CC23, CC701 and CC9 (including the corresponding ST and its single and double locus variants) and 7 other groups including only one represented ST as singleton. A specific protocol was developed to acquire spectra using sinapinic acid as a matrix. The performance of two sample preparations, standardized deposits (inoculum) and non-standardized deposits (colony) was compared. In order to determine the discriminatory power of this tool, a predictive identification model was built after processing of the spectra and its performance was evaluated by cross-validation.

Results

A total of 1515 spectra were analysed (751 and 764 for inoculum and colony protocols respectively). The two preparation methods gave equivalent results. Between 90% to 100% of correct identification was obtained for ST47 (99%), ST59 (91%), ST94 (93%) and for strains belonging to CC1 (100%), CC23 (96%), CC9 (100%) and CC701 (100%). Less successful correct identification was obtained for some singleton such as ST704 (63%), ST224 (70%), ST62 (75%) and ST75 (50%). These STs are the ones with the fewer spectrums available, explaining in part these low performances.

Conclusions

This method applied on 255 isolates from 27 STs showed promising performance for the major Lp1 STs isolated in Europe. A large collection of strains analysis is ongoing to validate our model. This method based on MALDI-TOF process allows the differentiation of *L. pneumophila* at the clonal complexes level which made it, together with its low cost, an interesting tool that could be used as a first-step screening in epidemiological studies.

O24. Antibiotic susceptibility of *Legionella pneumophila* clinical isolates in Germany

T. Koshkolda, C. Lück, Germany

Objectives

Recently the possibility of the selection for fluoroquinolone resistance by the antibiotic treatment for *L. pneumophila* has been reported (Shadoud et al. 2015). Further, the first ciprofloxacin-resistant strain has been isolated from a clinical sample (Bruin et al. 2014). The goal of this study was to verify a set of clinical isolates against 8 antimicrobial agents which are especially relevant for a therapy of legionellosis.

Methods

A total of 100 strains of *L. pneumophila* serogroup 1, which were isolated from the clinical specimens during the period from 2001 to the beginning of 2016, were chosen for the testing of their susceptibility. The minimum inhibitory concentrations (MICs) were determined after the incubation of the isolates on BCYE- α agar for 48-hour by using the E-test. Obtained results were interpreted using epidemiological cut-off values (ECOFFs) for *L. pneumophila* serogroup 1.

Results

Until now, all examined isolates showing a growth inhibition by the low concentrations of the antimicrobial agents and their MIC values are lower or equal to the ECOFFs. It was found that rifampicin is the most active against isolates under in vitro conditions. This study is not yet completed. However, the similar MIC distribution within the rest of the strains is expected. Conclusion The preliminary results have shown that, the in vivo resistant variants of *L. pneumophila* are still difficult to find by checking occasionally. The introduction of the routine susceptibility testing is necessary for better understanding of the in vivo antibiotic resistance development within the *L. pneumophila* population.

References

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O25. Genomic diversity and evolution of *Legionella pneumophila* “ST1” within hospitals and implications for future nosocomial investigations

S. David, B. Afshar, M. Mentasti, C. Ginevra, I. Podglajen, S.R. Harris, S. Jarraud, T.G. Harrison, J. Parkhill, United Kingdom

Objectives

Nosocomial-associated cases of legionellosis have been reported from many hospitals worldwide, and commonly involve *Legionella pneumophila* sequence type (ST) 1. The aim of this study was to determine whether whole genome sequencing (WGS) could be used to support or refute suspected links between hospital water systems and clinical infections.

Methods

WGS data from 229 ST1 isolates, including 92 that were newly sequenced for this study, were analysed. This collection comprises isolates from 16 countries and associated with over 20 hospitals.

Results

Phylogenetic analysis suggests that all hospitals from which multiple environmental isolates were obtained have been colonised by a single ST1 strain, with the exception of a French hospital, which was colonised by two very distinct ST1 strains over several years. All suspected links between hospital water systems and clinical infections are supported by WGS data.

Despite the discovery of distinct hospital lineages, the deep sampling of one UK hospital also revealed the existence of substantial diversity within the hospital population. Some evidence of microevolution in different parts of the hospital was observed, whereby isolates sampled from the same ward clustered together in a phylogenetic tree. Clinical isolates were also most closely related to environmental isolates sampled from the same ward in which the patient stayed.

Within the phylogenetic tree, epidemiologically unrelated isolates from the same geographical region often cluster closely, and we show evidence of hospital seeding via the local spread of ST1. However, some isolates from distant geographical regions also cluster closely, and we demonstrate that hospitals can also be seeded via the (possibly recent) international spread of ST1.

Conclusions

We demonstrate that WGS can be successfully used to support or refute suspected links between hospital water systems and clinical infections. However, deep sampling of hospital water systems may be required due to the substantial diversity within hospital populations, the co-existence of multiple strains within the hospital water system, and the similarity of hospital isolates to those from the local area.

O26. Contribution of the Amoebae Plate Test (APT) to the isolation of *Legionella* spp. from clinical samples: prospective analysis over a period of 9 months

A.G. Ranc, H. Hanneltel, J.V. Reynaud, C. Kolenda, A.G. Ranc, L. Beraud, C. Campese, C. Ginevra, S. Jarraud, G. Descours, France

Objectives

The isolation of *Legionella* strains from clinical samples is critical to conduct epidemiological investigations and the sensitivity of current cultural techniques needs is poor. We described and evaluated the performance of an amoebic coculture technique, the Amoebae Plate Test (APT), to the recovery of *Legionella* strains from respiratory samples.

Methods

From February to September 2015, we received 133 respiratory samples from 123 patients with confirmed (n=118) or suspected (n=15) Legionnaires' disease (LD) according to ECDC case definition. For each sample, we prospectively implemented 3 cultural techniques: - axenic culture by inoculating 400 µL of sample onto BCYE, MWY and BMPA agar plates upon sample arrival at the laboratory, 5 days a week; - “liquid” amoebic coculture (LAC) of 500 µL of sample using *Acanthamoeba castellanii* into six-well tissue culture plates, followed by subcultures on days 3 and 7 onto MWY and BMPA plates, as previously described, performed weekly after a pre-treatment of the sample (lysis of epithelial cells and coculture of the bacterial pellet); - amoebic coculture of 500 µL of sample by APT, also using *A. castellanii* and performed weekly after a similar pre-treatment; amoebae were spread onto a BMPA plate and the bacterial pellet was spotted; plates were incubated for 10 days.

Results

63 *Legionella* strains (62 Lp1, 1 *L. anisa*) (47.4%) were isolated from the 133 samples. Axenic culture was more sensitive than APT and LAC (sensitivities: 42.9%, 36.8% and 30.1%, respectively). Seven samples were positive by axenic culture only and grew less than 10 colonies (6 Lp1, 1 *L. anisa*). Four samples were positive by APT only: axenic culture and LAC were contaminated (>50 contaminating colonies). No samples grew by LAC only. The times to result (means) were 4.4 days for APT, 5.3 days for culture and 7.8 days for LAC. No difference between the 3 techniques was observed regarding the typing of the strains.

Conclusions

The APT showed better performance than LAC regarding its sensitivity and time to result, with the advantage of a one-step procedure. While APT was less sensitive than axenic culture, it provided additional strains, which are mandatory for the identification of the source of infection and for a better understanding of LD. Moreover, we recently succeeded in identifying a source of infection (water from aerosol therapy with negative axenic culture and LAC) by APT for a patient.

O27. A real study case comparing rtPCR and culture methods in a water distribution system

G. Saucedo Pagès, I. Manero, M.J. Arnedo, B. Galofré, Spain

Objectives

The main objective of this work was to obtain a better knowledge of Legionella presence in a water distribution treatment plant (WDTP) and network (DN). Real time PCR (rtPCR) is usually used to monitor outbreaks and to give a rapid response to urgent situations while Legionella culture by membrane filtration is the official method in the Spanish legislation frame. Although Legionella control is not mandatory for drinking water systems (DWS), it is very important for a water company as a management tool allowing to understand and correlate data obtained. Scientific bibliography systematically shows no correlations between both analytical methods.

Methods

During the first 6 months of 2015, monthly samples from DWTP and DN (including dead-ends) have been analyzed by rtPCR and culture. Both methods are validated and ISO 17.025 accredited by international protocols: NF T90-471 for rtPCR and ISO 11731 for culture.

Results

A total of 167 samples from different origins were analyzed. All result possibilities (positive and/or negative) for both methods were studied. 47,9% of the samples were negative in all cases and 12,5% positive for rtPCR and culture. A 34,1% of the samples were positive for molecular method and negative for membrane filtration (possibly caused by dead or non-culturable Legionella). Finally, a small percentage of the samples (5,4% , n=9) were negative for rtPCR and positive for the culture method: all these samples were quantified with low counts (<50 cfu/L) and 6 of them belong to dead-end samples.

In the specific case of the DN, 70,5% of the samples were negative for both methods, and this percentage increases to 93,2% when samples negative for culture but positive for rtPCR are added. Further studies are being completed, including the control of free chlorine, temperature, total organic carbon and other microbiological parameters such as amoebas.

Conclusions

The percentage of positive samples for Legionella spp. in the DN has been similar in 2015 to the last years (from 2011 to 2014: 4,5 to 7,5%). More than 94% of the results obtained are easily interpretable. Nevertheless, a small percentage of the samples that are positive for culture but negative for rtPCR always appears (around 5%), independently of the sample origin (DWTP, DN or dead end). This could be caused by the different detection limits of both methods (1 CFU/L and 100 GU/L) or by the PCR method inclusivity. Here relays the importance of quality control, validation and method knowledge.

O28. Occurrence of Legionella spp. in workplaces in Italy: a nationwide survey

G. Celenza, P. Bellio, L. Di Pietro, R. Iorio, C. Luzi, A. Mancini, M. Perilli, Italy

Objectives

The aim of this study is to determine the prevalence of Legionella spp. in water supply systems, in workplaces in Italy.

Methods

The study was carried out from February 2014 to March 2015. In this period 240 workplace sites were sampled in 17 out of 20 Italian Regions and 2,286 samples were collected and analysed for the presence of Legionella spp. The number of Legionella bacteria in water samples was determined by membrane filtration method. Presumptive colonies were confirmed by subculture in BCYE medium with and without cysteine. Species identification has been performed by latex agglutination and then by DNA sequencing of mip gene. To date, clonal relation of 22 Legionella pneumophila 1 coming from Southern regions of Italy was ascertained by Sequenced Based Typing. ATCC 33152 has been used as control.

Results

The analysis for the presence of Legionella spp. showed positivity (>100 CFU/L) in 702 water samples (30.71%) collected from 158 sites (65.83%). Positive samples were: 102-103 CFU/L, 16.71%; 103-104 CFU/L, 9.32%; 104-105 CFU/L, 4.16%; 105-106 CFU/L, 0.48%; >106 CFU/L, 0.044% (1 sample). 181 strains of Legionella were isolated. 30% were identified by latex agglutination as L. pneumophila 1, 36% as L. pneumophila 2-15, 15% as other species than L. pneumophila and 19% were classified as other Legionella species. The identification performed by DNA sequencing of mip genes exhibited a slight discrepancy between the two methods: 36% L. pneumophila 1, 27% L. pneumophila 2-15 and 37% other species than L. pneumophila (17% L. anisa, 1% L. birminghamensis, 1% L. bozemanii, 4% L. quateirensis, 1% L. santacrucis, 13% L. taurinensis). The Sequence Based Typing performed on 22 out of 65 L. pneumophila 1 isolates showed 12 different allelic profiles and 4 new allelic variants, 1 for flaA locus and 3 for proA locus. Allelic profile ST1 seems to be the most common in Southern Italy (11 out of 22). SBT analysis is still undergoing. Further Whole Genome Sequencing will be carried on.

Conclusions

This study is the first detailed nationwide investigation on Legionella spp. in workplaces in Italy. 74% of positive samples exceeded 104 CFU/L indicating a growing risk of infection. In this study we also report for the first time the allelic distribution of L. pneumophila 1 in water supply systems, showing, at the moment, high prevalence of ST1 allelic profile.

O29. Development of a public map of wet cooling towers in the Netherlands

W. Reinhold, The Netherlands

The Atlas Living environment provides information on the quality of the Dutch living environment, especially with the use of maps. It includes environmental issues that affect the physical and mental health, such as air quality, water quality and safety. Wet cooling towers are systems that are used for the removal of excess heat from production processes and buildings by means of the atomisation of water in an open construction. Worldwide, they are known as one of the main sources of Legionella pneumonia. If the system is poorly maintained and managed the Legionella bacteria in the water can reach large numbers, which can be spread over considerable distances and can lead to infection through inhalation of the bacteria. In case of an outbreak of Legionnaires' disease, it is therefore important to know where the wet cooling towers are located, in order to prevent further infections. Because they often stand on roofs, they are difficult to find from street level. Therefore, in recent years a digital map has been developed showing the wet cooling towers. The map is not only important for source detection, but also to supervise and enforce the safety regulations for wet cooling towers.

The supervisors (municipalities and environment services) enter the data on the wet cooling towers and keep the information up to date. The map is public, so that everyone knows where the wet cooling towers are in his area. Citizens, companies and institutions can also easily inform the supervisor about (possible) wet cooling towers. After verification, the wet cooling towers are entered into the map by the supervisor. In this way, a map is created that approximates the actual situation as much as possible, measures can be taken quickly and effectively in case of an outbreak, the owners of wet cooling towers are encouraged to manage them well, and the public is made more alert to the health risks of wet cooling towers.

The presentation focuses on both the development and the operation of the wet cooling tower map within the Atlas Living environment, and the experiences that have been acquired so far.

O30. Alternative sources of clinically relevant Legionella

E. van Heijnsbergen, F.M. Schets, J.A.C. Schalk, A.M. de Roda Husman, The Netherlands

Objectives

Legionella infections occur sporadically and in outbreaks, but most cases are sporadic. For the majority of sporadic cases, the source of the infection is never found. Legionella strains that are most often found in patients are rarely isolated from (patient-related) environmental samples. This may have methodological causes, but may also result from sources not being considered in source investigations.

Drinking water sources, such as showers and taps, are often targeted in outbreak investigations. Whirlpools, cooling towers, air conditioning systems, fountains, thermal springs and potting soil are well-known and confirmed sources of Legionella infections. Since a positive association between LD incidence and precipitation intensity, relative humidity and temperature was established, the natural environment and its potential role in human exposure to Legionella was studied.

Methods & Results

An amoebal coculture method using *Acanthamoeba castellanii* cells was used for the examination of various environmental samples. Viable Legionella pneumophila were isolated from pluvial floods after intense rainfall and from influent and aeration ponds at sewage water treatment plants. Viable *L. pneumophila* were also isolated from rainwater puddles on roads, and from soil that was sampled next to the roads where the puddles were sampled. To some, but not all, *L. pneumophila* isolates a sequence type (ST) could be assigned. In some cases, these STs had also previously been isolated from patients with LD.

The most frequently isolated *L. pneumophila* ST from patients in The Netherlands, Belgium and the UK is ST 47. In the Netherlands, this ST was isolated from environmental sources only three times, all concerning outdoor garden whirlpools. Examination of garden soil from two of the three gardens involved, demonstrated the presence of ST 47 in soil from the garden related to the most recent cases, suggesting possible contamination of the whirlpool with soil on bathers feet. A more extensive study of garden soil from gardens showed the presence of viable, clinically relevant Legionella species, including *L. pneumophila*, *L. saintelensi* and *L. longbeachae*, in 12% of the investigated garden soil samples. These findings indicate that garden soil is a potential source of Legionella bacteria, but the transmission pathway to humans is as yet unclear.

Conclusion

Potential alternative environmental sources of Legionella have been identified by using amoebal coculture, which allows detection of Legionella in samples with high numbers of other bacteria. However, the public health impact of these alternative sources needs further study. It is nevertheless recommended to consider these alternative sources in source investigations.

Conclusions

This study is the first detailed nationwide investigation on Legionella spp. in workplaces in Italy. 74% of positive samples exceeded 104 CFU/L indicating a growing risk of infection. In this study we also report for the first time the allelic distribution of *L. pneumophila* 1 in water supply systems, showing, at the moment, high prevalence of ST1 allelic profile.

O31. Presence and prevalence of Legionella spp. in collected rain water

L. Steege, G. Moore, United Kingdom

Objectives

The UK Government's National Adaptation Programme aims to make the country resilient to a changing climate. A key objective is to help people take action to prepare for the impacts of climate change in their homes and workplaces, including using water more efficiently. Rainwater collection has the potential to reduce household demand for water – particularly when used for outdoor purposes (e.g. the watering of gardens). Adults (>60 years) spend more time gardening than any other age group and are also more likely to collect and use rainwater. The presence of opportunistic pathogens including Legionella spp could represent a health risk to users. The aim of this study was to investigate the presence and prevalence of legionellae in collected rainwater.

Methods

Volunteers who collected and used rain water were asked to provide a water sample. Each sample was cultured using selective and non-selective media and assayed for the presence of legionellae using qPCR. In addition, new rainwater storage butts were installed at PHE Porton and positioned in direct sunlight or in the shade. Temperature (ambient and water), Legionella numbers (genomic units) and total culturable load were monitored on a monthly basis. Nitrate, oxygen and microbial gradients within the water butts were also investigated.

Results

63 volunteers provided water samples taken from 110 water butts. The mean number of bacteria recovered was 1.65×10^8 cfu/L (range: 3.00×10^5 - 1.61×10^9 cfu/L). Legionella spp were detected in 104 (95%) samples at a mean level equivalent to 4.71×10^4 gu/L (range: 4.6×10^1 - 5.01×10^5 gu/L). No *L. pneumophila* was detected. The temperature of rainwater stored in direct sunlight was significantly higher than that stored in the shade ($p < 0.05$). In both cases, the temperature of water taken from the base of the storage butt (i.e. via the tap) was also significantly lower than that of the surface water. Legionella spp (gu/L) in water taken via the tap was significantly greater than that taken from the surface. However, to date, no correlation between temperature, nitrate or dissolved oxygen and Legionella numbers has been observed. Monthly sampling is on-going and a full dataset will be presented.

Conclusions

The perceived risk of using rainwater for outdoor purposes is low. However, results of this study suggest that Legionella spp. are common contaminants of collected rainwater. Their aerosolisation through common gardening activities may have public health consequence.

POSTERS

P1. Public Health England's proficiency testing scheme for Legionella PCR – findings from a pilot study

N. Patel, United Kingdom

P2. Legionella pneumophila serotype 1, mAb 3/1+ve Philadelphia subtype isolation, from a patient with confirmed community acquired Legionnaires' disease (CA-LD) despite antimicrobial treatment

A. Flountzi, E. N. Velonakis, S. Melcher, C. Chronopoulou, T. G. Harrison, M. Mentasti, A. Vatopoulos, Greece

P3. Utility of real-time PCR for the identification and speciation of Legionella in South Africa

M. Carrim, N. Wolter, C. Cohen, S. Walaza, L. de Gouveia, F. Treurnicht, H. Dawood, E. Variava, A. von Gottberg, South Africa

P4. Legionella prevalence and risk of legionellosis in Japanese households

T. Kuroki, Y. Watanabe, H. Teranishi, J. Amemura-Maekawa, F. Kura, Japan

P5. Prevalence of Legionella pneumophila in patients hospitalized: twelve years of experience

M. Pecorari, G. Fregni Serpini, F. Frascaro, N. Nanni, G. Forbicini, R. Magnani, W. Gennari, S. Tagliazucchi, M. Meacci, B. Meccugni, A. Fabio, F. Rumpianesi, M Pecorari, Italy

P6. Evaluation of bactericidal activity of nine biocides against Legionella pneumophila serogroup 1

M.A. Casares Medrano, G. Reina, M. Fernández-Alonso, A. Ramos, J. Leiva, Spain

P7. Legionella bacteria in water systems of hospitals - criteria of a case study research design for risk management in the built environment

T.W. Leiblein, United Kingdom

P8. Characteristics of travel associated Legionnaires' disease in travellers from Europe to South Africa (1996 – 2015)

R. Stewart, T. Thomas, N. Fry, A. Duse, South Africa

P9. LegioType AS-1: Rapid microarray-based genotyping of Legionella pneumophila Sg1 isolates
M. Petzold, S. Jarraud, R. Ehricht, N. Jacotin, T. Meyer, P. Slickers, A. Ziegler, S. Monecke, C. Lück, Germany

P10. How long have Legionella culture media to be incubated?

G. Wewalka, S. Rehak, R. Paunovic, G. Jell-Wiesinger, L. Richter, Austria

P11. Free-living amoebae and Legionella in different microbiological complexity water systems

C. Avanzini, L. Franzin, V. Demarie, Italy

P12. Characterization for clinical Legionella species by Legionella Reference Center in Japan

J. Amemura-Maekawa, K. Chida, H. Ohya, J. Isobe, J. Kanatani, S. Tanaka, H. Nakajima, S. Yoshino, M. Ohnishi, F. Kura, Japan

P13. Detection of Legionella pneumophila and Streptococcus pneumoniae urinary antigens during the largest German outbreak of Legionnaires' disease in Warstein

C. Gagell, L. Humpert, I. Menke-Möllers, C. Lück, Germany

P14. Emergence of a Legionella pneumophila subsp. non-pneumophila ST701 clone

A. Campan-Fournier, C. Ginevra, C. Oger, F. Jauffrit, V. Navratil, A.G. Ranc, G. Perriere, S. Jarraud, France

P15. Why a positive Legionella urinary antigen test result may not be the final answer: the role of the National Legionella Reference Laboratory

F. Naik, M. Mentasti, G. Dabrera, V. Chalker, N.K. Fry, United Kingdom

P16. Molecular typing and monoclonal subgrouping of Legionella pneumophila serogroup 1 clinical strains in Piemonte, Italy

L. Franzin, V. Demarie, C. Avanzini, Italy

P17. Development of a new system for the detection of *Legionella pneumophila* in water samples

N. Párraga-Niño, S. Quero, N. Uria, O. Castillo-Fernández, M. García-Núñez, F.X. Muñoz-Pascual, M. Sabrià, Spain

P18. First survey and identification of *Legionella pneumophila* in tropical countries: environmental isolates from Cameroon, Cambodia and Senegal

C. Ginevra, J. Chastang, C. Massip, G. Descours, C. Gilbert, S. Jarraud, France

P19. Impact of urine samples concentration on Legionnaires' disease diagnosis with BinaxNOW® *Legionella* urinary test: a prospective study

L. Beraud, A. Ranc, R. Ruimy, C. Segonds, C. Ginevra, G. Lina, G. Descours, S. Jarraud, France

P20. Evaluation of *Legionella* K-set®, a lateral flow test for the detection of *Legionella pneumophila* serogroup 1 antigens in urine samples

A. Souche, A. Ranc, G. Descours, C. Ginevra, G. Lina, S. Jarraud, L. Beraud, France

P21. McFarland methodology for quantification of *Legionella* spp.

T. Graells, E. Jiménez, E. Padilla, P. Pérez, Spain

P22. Antibiotic susceptibility of environmental strains and clinical strain of *Legionella* spp. from a hospital nearby Barcelona (Spain)

T. Graells, E. Jiménez, E. Padilla, P. Pérez, Spain

P23. Detection of *Legionella pneumophila* urinary antigen by the rapid lateral flow ImmuView® kit

D. Lindsay, K.J. Scott, A. McNeil, S. Rankin, B. Jones, A. Smith, F. Alcock, P. Landsbo Elverdal, United Kingdom

P24. Genotypic diversity of *Legionella pneumophila* in a hospital water supply: comparison between 2 areas

M. García-Núñez, S. Quero, A. Hernandez, J.M. Modol, N. Parraga, A. Sagristà, M.L. Pedro-Botet, M. Sabria, Spain

P25. Impact of disinfection strategies in environmental legionellosis in a pediatric consulting room

J.M. Modol, M.L. Pedro-Botet, M. García-Núñez, S. Quero, N. Robert, A. Hernández, N. Parraga, A. Sagristà, M. Sabrià, Spain

P26. Presence of *Legionella* and high-throughput profiling of bacterial communities in the water distribution system of a university building before and after disinfection

E. Federici, S. Meniconi, E. Ceci, C. Casagrande, E. Mazzetti, E. Montalbani, G. Cenci, B. Brunone, Italy

P27. Clonal distribution of *Legionella pneumophila* macrolides specific efflux pump,

C. Ginevra, J. Chastang, C. Massip, G. Descours, C. Gilbert, S. Jarraud, France

P28. Fast and reliable screening of *Legionella* spp. and identification of *Legionella pneumophila* in water by real-time PCR including live/dead discrimination

F. Priller, S. Helbig, B. Junge, C. Grönewald, K. Berghof-Jäger, Germany

P29. NF validation of a fast real-time PCR method for the quantification of *Legionella* spp. and *Legionella pneumophila* in "clean" water samples

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

P30. CYTO-WATER: An integrated and portable platform for rapid detection and quantification of *Legionella* in environmental waters

B. Martinez, E. Soria, A. Yáñez, M.A. Fernández, E. Hill, D. Trouchet, J.M. Pérez, R. Götzen, S. González, Spain

P31. *Legionella* as a lower respiratory pathogen in India

S. Ponnoussamy, D.K. Kandy, India

P32. High prevalence of *Legionella* in water systems of merchant vessels

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

P33. Legionnaires' Disease in Ireland 2005-2013: How Complete is Reporting?

T. Mitchell, J. O'Donnell, D. O'Flanagan, Ireland

P1. Public Health England's proficiency testing scheme for *Legionella* PCR – findings from a pilot study

N. Patel, United Kingdom

The Food and Environmental Proficiency Testing Unit based at Public Health England, Colindale provides international external quality assessment (EQA) to microbiology laboratories that analyse food/water samples. Microbiology laboratories that examine water samples from the environment play an important role in the management of public health threats, supporting the appropriate management of facilities and controlling infection. Participating in EQA helps laboratories to demonstrate that their test procedures and the interpretation and understanding of test results are reliable, accurate and unequivocal.

The *Legionella* isolation scheme has been operating for over 10 years and focuses on challenging the laboratory in the detection and enumeration of legionellae. Advances in molecular testing have meant that laboratories are now using this method to analyse samples. To determine the performance of laboratory testing a pilot distribution was sent out to 35 laboratories that allowed samples to be analysed using molecular methods.

Sample A contained *Legionella pneumophila* serogroup 1 Sample B contained *Legionella jamestowniensis* A summary of the findings are:

- 97% (34/35) of the participants detected a *Legionella* in sample A and 77% (27/35) in sample B.
- Majority of the laboratories used ISO 12869:2012
- There was a large variation of methods used of which 89% were commercial assays.
- The limit of detection of the assay varied, this had an impact on the results reported

EQA supports the quality assurance of water testing in the laboratory by highlighting issues with test and method performance. Results from EQA distributions assists in the validation and improvement of methods used by testing laboratories so that surveillance data can be compared nationally and internationally. This presentation will cover a more detailed findings of the pilot distribution.

P2. Legionella pneumophila serotype 1, mAb 3/1+ve Philadelphia subtype isolation, from a patient with confirmed community acquired Legionnaires' disease (CA-LD) despite antimicrobial treatment

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Objectives

To investigate a confirmed CA-LD case, with isolation and molecular typing of a Legionella pneumophila serotype 1 isolate, from the patient, despite the antimicrobial treatment.

Methods

Bronchial secretions and sputum samples were brought to our lab, received from a Greek male patient, hospitalized, 60 years old, with a confirmed CA-LD. Water samples were also collected from his residence and possible sources from the area nearby, where he used to frequent (fountain). The samples' analysis was performed according to standard international guidelines methodology. Antibiotic susceptibility testing, Dresden panel mAb subgrouping, and Sequence Based-Typing (SBT-EWGLI protocol), were also performed.

Results

During the patients' hospital admission, he presented dyspnea, acute renal failure, mild -not productive cough, diarrhea, fever, low level of consciousness, anemia, medical history of diabetes mellitus, hypertension, coronary heart disease and looked really neglected. Subsequently, the cough became productive, the SaO₂ was decreased, and the lung X-ray showed severe diffuse pneumonia. The urinary antigen for Legionella pneumophila serotype 1, was positive. He immediately received antimicrobial therapy with ceftriaxone, doxycycline, and last added levofloxacin. He was released on his own volition, but he was re-admitted to another hospital, intubated and died in the ICU. Despite antibiotic treatment the clinical isolate recovered was a Legionella pneumophila serotype 1, mAb 3/1 positive, subtype Philadelphia; isolate, presenting a rare sequence type, the ST 436 (6th isolation in Europe, at the time). Environmental samples resulted negative, making the source of infection, impossible to identify.

Conclusions

In atypical pneumonias, the clinical diagnosis of CA-LD, the Legionella urinary antigen test, and the culture of clinical specimens, should not be omitted, even after antimicrobial treatment initiation. Clinical strains isolation: a) confirms the diagnosis in an absolute way, even for undetectable strains by the urinary antigen and b) enables the epidemiological investigation, the study, and the identification of infection's source.

P3. Utility of real-time PCR for the identification and speciation of Legionella in South Africa

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Objectives

Legionella is a fastidious organism and as a result, the prevalence of Legionella is largely underestimated. We implemented real-time PCR in our syndromic pneumonia surveillance programme in June 2012. We aimed to evaluate its utility in determining the species of Legionella positive specimens.

Methods

Individuals hospitalised with severe respiratory illness were enrolled from June 2012 through March 2016 at two sentinel sites in South Africa. Nasopharyngeal (NP) specimens included combined oropharyngeal and nasopharyngeal flocked swabs from ≥5-year-old patients and NP aspirates from <5-year-old patients, and induced/expectorated sputum were collected and transported to the testing laboratory on ice packs. From July 2013, sputum specimens were stored at -20°C after collection and transported on dry ice. Specimens were tested for Legionella spp. identifying the *ssrA* gene by real-time PCR, if positive, a multiplex real-time PCR was used to detect the *mip* gene for identification of Legionella pneumophila and the *wzm* gene for Legionella pneumophila serogroup 1 or a singleplex PCR was used to detect the LLO 1129 gene identifying *L. longbeachae*. Cycle threshold values (Ct-values) generated by real-time PCR were compared between sample types and transport conditions.

Results

Of the 5662 patients tested for Legionella, Legionella spp. was detected in 28 (0.5%, 95% confidence interval 0.3-0.7%) patients with 22 (79%) detected in sputum only, 5 (18%) in NP specimens only and 1 (4%) in both sputum and NP specimens. The overall mean (±standard deviation) Ct-value was 38.4±3.9, with a mean Ct-value of 38.1±4.3 and 39.5±1.9 in sputum and NP specimens, respectively (P=0.47). There was a significant difference in the mean Ct-value of Legionella spp. (*ssrA* gene) detected in sputum that were transported on ice packs (39.3±2.0, N=19) vs. specimens transported on dry ice (34.4±7.2, N=9) (P=0.01). Only after the change in transport methodology were species identified: *L. longbeachae* (n=2) and *L. pneumophila* serogroup 1 (n=1). A significant difference was observed between the mean Legionella spp. (*ssrA* gene) Ct-values of specimens for which a species was identified (30.7±8.7) vs. specimens for which a species could not be identified (39.1±1.9) (P<0.001).

Conclusions

PCR testing for Legionella overcomes many disadvantages associated with culture. Speciation was limited due to low bacterial loads in the samples which could be improved by transport conditions; however we were still only able to identify a species in the minority of cases.

P4. Legionella prevalence and risk of legionellosis in Japanese households

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Objectives

While the majority of sporadic cases of Legionella infection are associated with spas in public facilities and accommodations in Japan, the remaining cases may involve residential infections. We aimed to determine the occurrence of legionellosis in private houses.

Methods

We collected 149 water and 90 swab samples from aquatic environments in 19 houses from June 2013 to November 2014. Legionella DNA in these samples was detected via a loop-mediated isothermal amplification assay, and Legionella spp. were detected using culture techniques.

Results

DNA from Legionella spp. were detected in 77 water samples (51.7%) and 17 swab samples (18.9%). High Legionella DNA detection rates were observed in water samples from washing machines (92.9%, 13/14), aquariums (86.7%, 26/30), bathtubs (58.3%, 7/12), bathtub spouts (52.9%, 9/17), and wash hand basin taps (50.0%, 6/12). Legionella spp. were isolated from nine water samples (6.0%) and three swab samples (3.3%). Legionella pneumophila SG1 was detected from the outlet water of a bathtub spout and a bath sponge. In addition to *L. pneumophila*, Legionella anisa, Legionella busanensis, Legionella rowbothamii, Legionella sainthelensi, and other Legionella spp. were also detected. Use of amoebic co-culture effectively increased legionellae and Legionella DNA detection rates from all sample types. A logistic regression analysis revealed that the heterotrophic plate count is significantly related to Legionella contamination, and residual chlorine concentration had an inverse relationship with Legionella contamination.

Conclusions

There is a risk of legionellosis from exposure to Legionella spp. in a variety of aquatic environments in residential houses. Water systems, including taps, showers, toilets, washing machines, and aquariums, may be sources of Legionella spp. transmission.

P5. Prevalence of Legionella pneumophila in patients hospitalized: twelve years of experience

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Objectives

The aim was to evaluate the prevalence of the infection by Legionella pneumophila (Lp) in patients hospitalized in University Polyclinic Hospital of Modena in a period of twelve years.

Methods

Between 2003-2015 in the Microbiology and Virology Unit were performed 16458 tests on 13580 clinical samples from 8548 patients admitted for Lp suspected infection. In particular, 1818 (13.4%) respiratory specimens were subjected to culture and serotyped, 8884 (65.4%) urine samples were tested for urinary antigen (UAT) and 2878 (21.2%) sera for IgM and IgG assays. Out of 8548 patients, 4949 (57.9%) were male and 3599 (42.1%) were female.

Results

Overall, 15807/16458 (96.1%) test were negative, 484/16458 (2.9%) were positive and 167/16458 (1%) were borderline. On a total of 1818 respiratory samples, 1755 (96.5%) were culture negative and 63 (3.5%) culture positive for Legionella. The immunochromatographic method has identified Lp sg1 in 61 (98.8%) and Lp sg 2-15 in 2 (1.2%) samples. The latex agglutination test has identified Lp sg 11 in 2010 and Lp sg12 in 2011. No other species has been found. The UAT has revealed 203/8884 (2.3%) positive and 8616/8884 (97%) negative and 65/8884 (0.7%) borderline samples. IgM and IgG were positive respectively in 93/2878 (3.2%) and in 125/2878 (4.4%) and negative in 2753/2878 (95.7%) and in 2683/2878 (93.2%), borderline in 32/2878 (1.1%) and 70/2878 (2.4%).

The patients with at least one test positive were 321, among these 4 (1.3%) were positive in all tests. 241 (75%) were positive in only one test of which 116 (48.1%) in UAT, 18 (7.5%) in culture, 37 (15.4%) only in IgM, 70 (29%) only in IgG. 76 patients (23.7%) were positive in two tests of which 6 (7.9%) in IgM and UAT, 9 (11.9%) in IgG and UAT, 20 (26.3%) in IgM/IgG (seroconversion), 29 (38.2%) in UAT and culture, 1 (1.3%) in culture and IgM, 2 (2.6%) in culture, UAT and IgM, 2 (2.6%) in culture, UAT and IgG, 7 (9.2%) in IgM/IgG (seroconversion) and UAT.

Conclusions

The data show that the infection affects more males (67.6%) than female (32.4%) with a male/female ratio of 2.1 and a mean age of 62.6 years in men and 69.0 in women. Lp mainly affects men aged from 48.4 to 76.5 years and women aged from 52.6 to 82.0. Moreover, the results obtained in this study show that all tests available for Legionella identification should be used.

P6. Evaluation of bactericidal activity of nine biocides against *Legionella pneumophila* serogroup 1

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Objectives

Legionella pneumophila is the aetiological agent of Legionnaires' disease. This bacterium is able to colonize human made systems as cooling towers, air conditioning conductions or water pipes. *Legionella* can multiply into these systems, and aerosols contaminated with *Legionella* generated in these water facilities, can be inhaled by human beings and cause legionellosis. The aim of this study is to evaluate the antimicrobial activity of nine biocides commonly used to treat water systems against planktonic *Legionella* spp.

Methods

The anti-*Legionella* activity was evaluated following the instructions of the european regulations EN 13623:2010. The assay method chosen was the dilution-neutralization method. *Legionella pneumophila* serogroup 1 ATCC 33152 (Philadelphia) and *Legionella pneumophila* serogroup 1 strain Lens (CIP 108286) were tested. The oxidizing biocides evaluated were: bromochloro-5,5-dimethylimidazolidine-2,4-dione (Adic 512), calcium hypochlorite (Adic 513), trichloroisocyanuric acid (Adic 516), peracetic acid (H-90) and sodium hypochlorite. As non-oxidizing biocides we evaluated: 2,2-dibromo-3-nitrilopropionamide (Adicida 141), mixture of didecyl dimethyl ammonium and propan-2-ol (Adicida 182), mixture of 5-chloromethyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (Adicida W-128), tetrakis (hydroxymethyl) phosphonium sulfate (Adiclene 1375). Sodium hypochlorite was supplied by Nacar and other biocides were supplied by Adiquímica S.A.

Results

The biocide concentrations that reduced 4 log₁₀ the initial inoculum (0.5 McFarland) of *Legionella pneumophila* ATCC 33152 and ATCC LENS were respectively: 0.5 and 2.5 ppm for Adic 512, 0.198 and 0.66 ppm for Adic 513, 1 and 2 ppm for Adic 516, 1 and 10 ppm for H-90, 2 and 4 ppm for sodium hypochlorite, 2.5 and 1.25 ppm for Adicida 141, 1.25 and 2.5 ppm for Adicida 182, 1.25 and 25 ppm for Adicida W-128, and 0.5 and 1 ppm for Adiclene 1375. The required concentration to get bactericidal activity with the biocide was 2 to 50 fold lower than the recommended concentrations to be used.

Conclusions

The biocide concentrations necessary to reduce 4 log₁₀ the initial inoculum of *Legionella pneumophila* ATCC 33152 and *Legionella pneumophila* serogroup 1 strain Lens (CIP 108286) were in all cases lower than the concentrations recommended by suppliers. For all of the biocides, except 2,2-dibromo-3-nitrilopropionamide, the bactericidal activity was higher for strain Lens (CIP 108286) than for ATCC 33152.

P7. *Legionella* bacteria in water systems of hospitals - criteria of a case study research design for risk management in the built environment

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Objectives

An ongoing research project addresses duty holders and facility managers in hospitals with portfolios of services on built environments regarding water systems and *Legionella*. The aim of this project is to systematically uncover the present situation of *Legionella* prevention in water systems in selected healthcare (HC) organisations in different countries. It contributes to working out a 'reference system' guiding people responsible in HC organisations to identify, understand and properly take action for prevention. Merely complying with existing legislation and guidance not necessarily means that a system is safe or operates gapless. A lived understanding and deep knowledge of the necessities of the own organisation is essential for all participants who fulfil duties and contribute to hygiene and prevention.

Theory Hospitals represent complex organisations. The complexity is made up of the organisational structure as well as the technical systems and challenging service demands. Thus duty holders act within a highly interdisciplinary field, which is also affected by existing regulations and organisational barriers. Activities regarding Risk Management of *Legionella* in water systems include hazard analysis, systematic prevention activities and interdisciplinary work. HC facilities (hospitals) can be part of an Facility Management (FM) portfolio.

Methods

The stakeholder theory provides a suitable theoretical framework to analyse the relation between policy issues, responsibilities and activities of people being involved in processes serving for hygiene and prevention. The theory explains how to identify and engage with stakeholders for group effort, mutual dependence and legitimacy. This research focuses on activities of different stakeholders of FM / Facility Services (FS) in hospitals serving on Risk Management and *Legionella* prevention of water systems. The theoretical drive of this research is inductive, based on a qualitative approach of data collection.

Results

Preliminary results determine a selection of three countries to participate in the study, each presenting an item-cluster for the analysis. For the selection of HC organisations each item is subjected to certain inclusion and exclusion criteria. Decision criteria were for example the existence of a health system in a country, including different types of hospitals (providers e.g. 'public', 'charitable' or 'privately'). Furthermore the size groups (number of beds) and the age of a building complex were considered. The results exemplarily present the organisational structure (organigrams, job descriptions, hierarchy levels, boards and teams), different legislative frames regarding water safety and *Legionella* prevention, the types of operating water systems and the existence of related services and processes (inhouse / outsourced) that can be ascribed FM / FS or to which links of collaboration are recognised, seen from the FM / FS perspective.

Conclusions

Deeper analysis will follow on for each hospital representing a case. It will work out further endeavour on managing risks properly according to organisation's specific necessities. For this, data collections of field phase I and II will provide comprehensive data.

P8. Characteristics of Travel Associated Legionnaires Disease in travellers from Europe to South Africa (1996 – 2015)

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Objectives

The main objective of this study is to analyze trends of Travel Associated Legionnaires' disease (TALD) in South Africa (SA).

Methods

SA obtains information of TALD cases from the European Legionella Disease Surveillance network (ELDSNet) supplied by TESSy (data repository of the European surveillance system at European Centre for Disease Prevention and Control [ECDC]).

Results

Twenty-nine TALD cases made 86 accommodation site visits, identifying four clusters. Out of the four clusters, one was a complex cluster involving six different accommodation sites. During the study period (1996-2015), 75 site notifications were reported during SA summer and 10 in winter. There were six accommodation sites with >1 notification in >2 year interval.

There were 21 males and 8 females with a ratio 2.1 : 1 and the age range was 48 – 88 years, with a median age of 64 years. Ten cases survived, 2 died and outcome was reported as unknown for 17 cases. Diagnosis was made using the following laboratory tests: urinary antigen (24), single high titre (3), four-fold rise in titre (1), PCR (2), culture (1). During the years 2005 – 2013 an average of 1,433,875 tourists came from Europe per year (total = 12, 904, 879). The breakdown for the top 5 countries follows: UK (442,523), Germany (304,090), France (134, 840), Netherlands (122,299), Italy (67,790). The TALD cases originated from these countries: UK (13), Netherlands (11), Norway (2), Denmark (1), Austria (1), Ireland (1). Sequence based typing was carried out on environmental strains from three clusters. Eight strains were typed, seven were *L. pneumophila* serogroup 1. These were ST 1014 (one strain), ST 1015 (one strain), ST 1 (4 strains) and ST 211 (one strain). One strain was *L. pneumophila* serogroup 2 -14 (ST 1317). ST 1 is a globally common strain, ST 1014 & 1015 are unique, ST 211 has been linked to cases from Canada, France, Spain, Saudi Arabia, and Japan. In the ESGLI database a single environmental strain belonging to ST 1317 is from Japan (*L. pneumophila* serogroup 7).

Conclusions

Numbers of reported TALD clusters in SA were low (4 over 19 years; SA population in 2015, 54 million) while TALD clusters in EU (population in 2015, 508 million) average 106 per year. More in-depth studies are needed to fully understand this difference.

P9. LegioType AS-1: Rapid microarray-based genotyping of *Legionella pneumophila* Sg1 isolates

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Objectives

Reliable typing of clinical and environmental *L. pneumophila* isolates is necessary in routine testing as well as in outbreak situations. Sequence based typing (SBT) and monoclonal antibody (mAb)-based subgrouping reach more and more its limits as gold standard typing methods. The urge for new typing methods is ongoing – with strong focus on whole genome sequencing (WGS). Although WGS became more affordable during the last decade, this technique is not yet applicable for all routine diagnostic laboratories. We focused therefore on a method with similar properties regarding handling, speed and analysis as SBT and mAb-subgrouping but with increased discriminatory power.

Methods

In total, 97 probes of different variants of LPS-biosynthesis genes, variable elements and the genes *pilE* and *neuA* were spotted in quadruplicate on DNA microarrays that were mounted onto ArrayStrips (Alere Technologies, Jena, Germany). Linear multiplex target amplification facilitated the simultaneous generation of a high number of biotin labeled amplicons. An optimized protocol was established based on the HybridizationPlus Kit (Alere) to hybridize amplicons to the probes. Arrays were read out on the ArrayMate reading device (Alere). Raw data signal intensities were normalized and converted into a 97-ternary hybridization profile (HP) categorizing signals as positive (POS), negative (NEG) or ambiguous (AMB).

Results

Stability and reproducibility of the microarray-based assay was evaluated by repeated testing 103 *L. pneumophila* isolates including 80 reference strains of the EUL strain collection in independent experiments. Additional 46 sets of related isolates were tested. At first, HPs of valid experiments were divided into lag-1+ and lag-1- profiles due to the clinical relevance of lag-1+ isolates. Analysis of HPs revealed similarities $\geq 94\%$ as good indication to define isolates as related to each other, thus putatively belonging, i.e., to the same clone. HPs were summarized into clonal complexes (CC) or singletons based on MLST data. Additional 600 *L. pneumophila* Sg1 isolates were tested. Thereby, we were able to identify 27 CC with 85 sub-complexes and 22 singletons. The index of discrimination for the strains used in this study is 0.961 and thereby slightly higher than SBT (0.948).

Conclusions

Starting with colonies on plates, a single run can be completed within five hours. A user-friendly software was implemented into the ArrayMate reading device that assigns the HP-similarity score and the corresponding CC, singleton or sub-complex to the isolate, based on all internal validation and calculation steps.

P10. How long have Legionella culture media to be incubated?

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Objectives

The now valid ISO 11731 and ISO 11731-2 "Water quality – Enumeration of Legionella" require a final examination of plates after an incubation period of 10 days. According to a draft of ISO 11731 from 2015 it is up to the test laboratory if culture media are examined finally after 7 or 10 days of incubation. To decide this question in our laboratory in all water samples cultivated for Legionella the plates were examined and evaluated after 7 and 10 days in 2015.

Methods

16,386 water samples (15,948 samples of warm and cold drinking water, 209 samples from swimming baths and 229 water samples for wet cooling towers) were processed according to ISO 11731-2. 100 ml and 10 ml were filtered through 0.45 µm membrane filters which were directly placed on GVPC-media. Additionally 0.5 ml were directly plated both on GVPC and BCYE α media. Plates were examined both after 7 and 10 days of incubation. Legionella species were identified serologically and grouped in *L. pneumophila* serogroup (sg.) 1, sg. 2-14 and non-pneumophila Legionella. The latter were determined by MALDI-TOF mass spectrometry.

Results

Of the 15,948 samples of drinking water in 1,214 *L. pneumophila* sg. 1, in 1,291 *L. pneumophila* sg. 2-14 and in 319 non-pneumophila Legionella were detectable. Only in 72 samples (0.45 %, 95% Confidence interval: 0.36%; 0.57%) there were differences in colony counts between the examinations of day 7 and 10. In non-pneumophila Legionella colony counts raised most frequently (n=38), namely in *L. anisa* (n=31), *L. rubrilucens* (n=5), *L. erythra* (n=1) and *L. bozemanii* (n=1). In samples with *L. pneumophila* sg. 1 and *L. pneumophila* sg. 2-14 colony counts increased only 17 times each. Only in 4 samples colony counts of *L. pneumophila* sg. 1 raised significantly (0.70, 10.50, 10.170, 600.1200) and those of *L. pneumophila* sg. 2-14 only twice (6.410, 10.50). Of 229 water samples from wet cooling towers in 67 Legionella were detected and only in one case (0.44%, 95% confidence interval: 0.08%; 2.43%) there were differences in colony counts between the examinations of day 7 and 10 where 10 CFU/100 ml of *L. pneumophila* were detected only after 10 days. In samples from swimming baths 27 times Legionella were detected but in no case differences in colony counts were observed between day 7 and 10.

Conclusions

Concentrations of Legionella pneumophila in water samples are more critical than of non-pneumophila Legionella. Only in 6 of 15,948 samples of drinking water (0.04%) the final examination of plates after 7 days would have led to a less critical assessment of a single sample. Hot and cold water installations cannot be sufficiently evaluated based on results of single specimens but results of several specimens are necessary. Therefore the danger is minimal, that an installation is wrongly assessed as safe if Legionella plates are examined finally after 7 days of incubation only.

P11. Free-living Amoebae and Legionella in different microbiological complexity water systems

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Objectives

Legionella is ubiquitous in aquatic environment, where it is living in symbiosis with Amoebae. Protozoa have an important role in the transmission of bacteria from environment to man: Legionella virulence is enhanced by Amoeba, propagation, resistance and survival are increased. Aim of the study was to evaluate role of free-living amoebae in different microbiological complexity water systems and correlation between Legionella detection by culture and quantitative real-time PCR (qPCR).

Methods

1849 water samples were examined: 1673 from hospital water supplies, 57 from dental units, 63 from cooling towers (CT), 56 from air conditioning systems (UTA). Legionella culture was quantitatively determined. Aliquots of direct, heat-treated and acid-treated concentrates by filtration were plated on BCYE, BMPA and MWY media. After incubation at 37°C up to 15 days, suspected colonies were serologically typed and quantified. qPCR for Legionella spp. and *L. pneumophila* was performed from 231 samples (1 liter) and DNA extraction by Aquadien (Bio-Rad). Amoeba culture was performed from 1769 samples (100 mL) on Non Nutrient Agar plates precoated with *Escherichia coli* incubated at 25°C and 37°C.

Results

Legionella was isolated from 29.3% of hospital water, 17.5% dental unit, 22.2% of CT samples. UTAs were negative for Legionella, but elevated heterotrophic bacteria contamination was found. *L. pneumophila* serogroups 1, 3, 6, 8 and 2-15, *L. bozemanii*, *L. anisa*, *L. brunensis*, Legionella spp. and other fluorescent Legionella spp. were found. Amoeba was positive in 15.4% samples: 9.6% hospital water, 85.7% CT, 45.6% dental unit, 71.4% UTA. 5.4% samples were both positive for Legionella and Amoeba, 10% only positive for Amoeba, 22.3% only for Legionella. Amoeba occurrence (34.2%) was significantly higher in water samples with Legionella count >103 cfu/L and in cold water. Amoeba (but not Legionella) was found in 71.4% UTA, 85.7% CT and 47.4% dental unit samples. Amoeba isolation rate was higher at 25°C. Weak correlation between Legionella culture and qPCR values was observed, with GU/L higher than cfu/L. Higher percentage of Amoeba isolation was noted in samples positive for Legionella culture (p<0.01) and Legionella qPCR (p<0.01). Better correlation between culture and *L. pneumophila* qPCR was found in amoeba negative samples.

Conclusions

In our study Legionella isolation rate was higher than Amoeba from hospital water supplies, while Amoeba was found in higher percentage from UTA, CT and dental unit water samples. Legionella qPCR values were higher than those of Legionella culture, better correlation was observed in hospital hot water supplies, than in more complex systems of cooling towers and dental units, more influenced by free-living Amoebae colonization.

P12. Characterization for clinical *Legionella* species by *Legionella* Reference Center in Japan

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Objectives

We surveyed clinical isolates from patients with legionellosis in Japan in order to clarify their characteristics.

Methods

Legionella Reference Center in Japan collected 435 *Legionella* clinical isolates between February 2000 and March 2016 including 17 representative isolates from corresponding outbreaks. As for *Legionella pneumophila*, serogroups and STs were determined.

Results

The collections comprised 425 *L. pneumophila* isolates and ten *Legionella* species isolates: *Legionella bozemanii*, *Legionella dumoffii*, *Legionella feeleeii*, *Legionella longbeachae*, *Legionella londiniensis*, and *Legionella rubrilucens*. Among 425 *L. pneumophila* isolates, 371 isolates were SG1 (87%) of which 90% were lag-1 positive and other isolates were distributed throughout SG2-15 except for SG7 and SG11. A total of 187 STs of which 112 STs were submitted to the SBT Database as new STs, including 125 singletons, were found (index of discrimination (IOD): 0.984). Major STs were ST23 (n=34), ST120 (21), ST138 (21), and ST1 (12). ST23 was the most prevalent ST and the most causative ST in outbreaks in Japan like in Europe. ST138 was detected in only Japan and caused small-scale outbreaks.

The minimum spanning tree analysis of SG1 environmental isolates showed nine major clonal complexes previously (ESGLI 2015): three B groups, two C groups and three S groups, which included major environmental isolates each, were derived from bath water (B), cooling towers (C), and soil and puddles (S); and U group included various environmental isolates. ST138 belongs to the B group and 19 of ST138 strains (90%) were suspected or confirmed to infect humans through bath water. On the other hand, ST23 and ST120 belong to the S group, and ST1 belongs to the C group, and 11 of ST23 (32%), 14 of ST120 strains (24%), and one of ST1 strains (9%) were suspected or confirmed to infect humans through bath water. ST23 and ST120 strains had been isolated from puddles, but rarely detected from other environments. Meanwhile ST138 and ST1 strains had been isolated frequently from bath water and cooling tower water, respectively.

Conclusions

Clinical isolates with legionellosis in Japan were highly diverse but particular some STs were isolated frequently and were suggested to relate with their infectious sources.

P13. Detection of *Legionella pneumophila* and *Streptococcus pneumoniae* urinary antigens during the largest German outbreak of Legionnaires' disease in Warstein

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Objectives

In the summer of 2013, the city Warstein had to deal with the largest outbreak of Legionnaires' disease (LD) in Germany (78 laboratory-confirmed cases, including one fatality) till now. The epidemic strain which could be isolated from seven LD patients as well as from environmental sources including two cooling towers, one sewage plant and one river water was identified as *Legionella pneumophila*, serogroup 1, subtype Knoxville, sequence type 345 [Maisa et al., Euro Surveill. 2015]. For the laboratory diagnosis of LD the *L. pneumophila* urinary antigen was detected using two commercial assays. In addition *Legionella* antigen-negative samples were retrospectively analyzed for *Streptococcus pneumoniae* antigen.

Methods

A total of 508 urine samples of 315 persons suffering from pneumoniae during the LD outbreak (1 August to 6 September 2013) living in or around Warstein was analyzed using the Binax *Legionella* Urinary Antigen Enzyme Immunoassay (Binax EIA; Alere) according to manufacturer's instructions. Of these, 473 urines were also tested by the Sofia *Legionella* Fluorescence Immunoassay (FIA; Quidel). Positive samples were retested after heating for 5 min at 95°C. *Legionella* antigen-negative samples confirmed by the results of both tests were checked for pneumococcal infection by using the immunochromatographic BinaxNow *Streptococcus pneumoniae* Antigen CARD (Alere). As recommended by the manufacturer the samples were incubated for 15 min. Additionally, the results were recorded after a prolonged incubation time of 60 min.

Results

Taken together, the Binax EIA revealed 99 *Legionella* antigen-positive samples corresponding to 62 patients. By means of the FIA 66 of 68 *Legionella* antigen-positive samples could be confirmed. However, after heating the samples 60 remained positive indicating unspecific reaction for the 6 samples. Interestingly, 10 of 338 *Legionella* antigen-negative urines were found to be positive based on the FIA results even after heating the samples. To detect pneumococcal pneumonia 216 *Legionella* antigen-negative urines were tested up to now by the respective BinaxNow assay. The analysis revealed 8 *Streptococcus* antigen-positive urines after prolonged incubation of 60 min.

Conclusions

For the detection of the *Legionella* urinary antigen during the outbreak in Warstein the commonly used Binax EIA as well as the FIA were suitable for the rapid diagnosis of LD. As expected, the sensitivity could be increased by urine concentration. Since we also identified pneumococcal infections by the detection of *S. pneumoniae* antigen LD can be excluded at least for these patients. Hence, testing for pneumococcal pneumoniae during LD outbreaks is recommended.

P14. Emergence of a *Legionella pneumophila* subsp. non-pneumophila ST701 clone

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Objectives

Among nearly 60 species of the genus *Legionella*, the species *Legionella pneumophila* is responsible for more than 90 % of the Legionnaires' diseases (LD). It is subdivided into three subspecies: *L. pneumophila* subsp. *pneumophila*, *L. pneumophila* subsp. *fraseri* and *L. pneumophila* subsp. *pascullei*. The subspecies mostly involved in infections is *L. pneumophila* subsp. *pneumophila*. However in absence of an easy tool allowing to identify the three subspecies in the diagnostic laboratories, the role of the two other subspecies in LD is largely unknown. In a previous comparative genomic analysis we observed that ST701 strains were evolutionary closer to the subspecies *fraseri* and *pascullei* than to the subspecies *pneumophila*. Interestingly, the legionellosis cases involving ST701 strains seem to be emerging in France: only one case was recorded between 2006 and 2008, then 15 cases between 2009 and 2011 and 25 cases between 2012 and 2014. The objectives of our study are to better characterize the ST701 strains and to determine if these strains really belong to a non-pneumophila subspecies.

Methods

A phylogenetic analysis was performed using the sequences of the 45 single copy ribosomal proteins of 80 genomes of *Legionella*, *Tatlockia*, *Fluoribacter* and *Coxiella* that have been extracted from the RiboDB database and aligned with the MAFFT software. The 45 multiple sequence alignments have been cured with the Gblocks tool available into SeaView to suppress poorly aligned positions and divergent regions. They then have been concatenated into a single multiple sequence alignment. The selection of the best-fit evolutionary model was carried out using ProtTest.

According to the AIC (Akaike Information Criterion) test, the model that best fit our data is the LG (Le and Gascuel) model with correction by the Gamma distribution with four categories for substitution rates, use of the invariant sites and use of the empirical amino-acid frequencies (LG + I4 + I + F). We used this model and a maximum likelihood method to reconstruct the phylogenetic tree and assess the branch support by computing the aLRT (approximate Likelihood Ratio Test) thanks to the PhyML software.

Results

The reconstructed phylogenetic tree shows that the *L. pneumophila* subsp. *fraseri* and *L. pneumophila* subsp. *pascullei* strains form a well supported clade among which the strains ST701 are nested. Among this clade, the strains of the *pascullei* subspecies form a monophyletic group and the two ST701 strains are clustered near to the *fraseri* subspecies strains.

Conclusions

These results suggest for the first time that the ST701 strains could be an emerging clone of *L. pneumophila* subsp. non-pneumophila.

P15. Why a positive *Legionella* urinary antigen test result may not be the final answer: the role of the National *Legionella* Reference Laboratory

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Background

Urinary antigen testing is the most frequent method for diagnosing *Legionella* infection in hospital laboratories across England and Wales. The National *Legionella* Reference Laboratory (NLRL) offers a validation service on all urinary antigen positive specimens referred to it from hospital laboratories. This NLRL function may help identify spurious results that cannot be confirmed after boiling. This analysis was to determine the proportion of referred positive cases in which *Legionella* infection could not be confirmed. Objective To determine the impact of NLRL 'positive urinary antigen test' on the inclusion of Legionnaires' disease cases to national surveillance.

Methods

Cases of legionellosis, positive by urinary antigen testing, in referring laboratories, with symptoms onsets between 01 January 2010 and 08 February 2016, were identified in residents of England and Wales, from the National Enhanced *Legionella* Surveillance Scheme (NELSS). The number of cases with urine specimens referred to the NLRL were extracted from the NELSS database. The numbers and proportions of these cases which tested negative at the NLRL were also determined from the database.

Results

There were 2516 cases with an eligible onset date, recorded in the NELSS database with an initial positive urinary antigen test in a referring laboratory. Of these, 1444 (57%) had urine specimens submitted to the NLRL. At the NLRL, 91 (6.3%) of referred samples could not be confirmed.

Conclusions

Although the proportion of cases which tested negative on urinary antigen testing at the NLRL was low, this NLRL function allowed urine sampled that could not be confirmed as positive to be excluded from further unnecessary public health investigations, thereby conserving limited public health resources. This highlights just one of the essential contributions that a NLRL service for *Legionella* makes to frontline public health services.

Less than two thirds of cases which tested positive in local hospital laboratories during the study period are referred to the NLRL; therefore a greater number of cases in which *Legionella* infection may not be confirmed may be undetected; local hospitals need to be encouraged to refer such specimens to the NLRL.

P16. Molecular typing and monoclonal subgrouping of Legionella pneumophila serogroup 1 clinical strains in Piemonte, Italy

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Objectives

Molecular typing for discriminating different bacterial isolates is an essential epidemiological tool in prevention and control of Legionella infections. Monoclonal antibody (MAb) reactivity pattern due to presence or absence of the lag-1 gene is also a very useful tool. The aim of the study is molecular typing by sequence-based typing (SBT) and MAb subgrouping of Legionella pneumophila serogroup 1 (Lp1) clinical isolates in a Regional Reference Laboratory (Torino, Piemonte, Italy).

Methods

65 Lp1 clinical isolates were analyzed. The strains were from sporadic cases of community, nosocomial and travel associated Legionnaires' disease. One strain from a respiratory sample of each patient was considered in this study. Environmental isolates epidemiological linked to clinical strains from 13 legionellosis cases were also analyzed. All these strains were genotyped by sequence-based typing (SBT) analysis following EWGLI/ESGLI protocol for epidemiological investigations. Genomic DNA was extracted by using Instagene Matrix and the supernatant was used as a DNA template. The primers lag-F and lag-R were used for PCR amplification of the lag-1 gene (584 bp amplification product). MAb subgrouping was performed using Dresden Panel.

Results

SBT analysis revealed 22 different sequence types (STs) and 7 new STs. ST23 (9 strains), ST435 (7 strains) and ST1 (5 strains) were most frequently isolated as observed worldwide. ST37 and ST42 were found for 4 strains, ST20, ST62, ST82 for 3 strains, followed by ST16, ST259, ST751, ST701, ST733 for 2 strains. The other strains were different STs. MAb typing results showed a prevalence of MAb 3/1 positive isolates (96.9%) with the Philadelphia subgroup representing 55.4%, followed by Benidorm (16.9%), Knoxville (12.3%) and Allentown/France (12.3%). The remaining 3.1% were MAb 3/1 negative, Olda subgroup. Strains of Pontiac group were all lag-1 positive. Clinical and environmental epidemiological related strains belonging to Philadelphia (7 isolates), France/Allentown (2), Knoxville (2) and Benidorm (1) were lag-1 positive in 11 cases. In a case study related environmental strains were both Olda subtype and lag-1 negative. In another legionellosis case clinical strain was Benidorm, lag-1 positive, while the environmental isolate was Olda, lag-1 negative.

The results of genotyping of these strains showed 9 different STs: 4 strains were ST23, 2 strains ST751 and the other strains were of different STs. A helpful analysis of data from SBT, MAb subgrouping is provided. From EWGLI database 6 STs are not yet reported in Italy.

Conclusions

This study describes the first Lp1 strains database in Piemonte, providing the phenotypic and genotypic characterization of clinical strains and molecular epidemiology data useful for epidemiological investigations.

P17. Development of a new system for the detection of Legionella pneumophila in water samples

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Legionella pneumophila inhabits natural and manmade aquatic environments as drinking water systems, cooling towers, swimming pools and artificial supply systems. The presence of this bacterium needs to be controlled in order to avoid high levels of colonisation. The conventional method (culture) for L. pneumophila environmental detection is tedious and often involves the use of complex growth media, a validation plaque growth in 10 days and expertise staff. Recently, some detection systems have been commercialized. However, they have some limitations such as no detection of all serogroups of L. pneumophila and manual protocols difficult to automate. Then, there is a need of a specific and fast method that could be applied for the Legionella control.

Objective To develop a new system to detect Legionella pneumophila in water samples following a simple protocol in order to detect the presence of these bacteria with high specificity. This protocol needs to be cheap and has to be performed in hours. Moreover, the system must identify all the serogroups of L. pneumophila but not species that coexist with this bacterium. **Methods** 1) Validation of a set of commercial L. pneumophila antibodies in ELISA in front of 33 different strains (L. pneumophila and non-L. pneumophila) in order to select a suitable antibody that specifically recognised all serogroups of L. pneumophila. 2) Development of a new method including capture by filtration and a subsequent immunodetection in the same membrane.

Results

A sensible antibody for L. pneumophila with a high specificity was found although one interference was observed. The optimization of this process has allowed the capture of L. pneumophila and subsequently the detection by targeted antibodies against this bacterium with a detection limit of 100 cells/ml (samples of 30 ml) in around 2 hours. The detection limit is being further improved.

Conclusions

We have developed a detection systems with potential applications in environmental surveillance. The combination of a specific and sensitive antibody against all Legionella pneumophila with the filtration and detection of the sample in the same membrane have allowed the automation of the process. An automated system that does not require qualified personnel with good sensitivity and specificity should greatly improve the current control of the bacteria and therefore should significantly reduce Legionnaires' disease cases and hospitalizations outgoings. The automation of the process is currently being developed in order to obtain a portable and on-site device.

P18. First survey and identification of *Legionella pneumophila* in tropical countries: environmental isolates from Cameroon, Cambodia and Senegal

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Objectives

From May 2009 to June 2010, 635 water samples (Hot Water System and Cooling Tower) from hotels and hospitals in Cameroon (212), Cambodia (216) and Senegal (207) were taken in the framework of a multicentre project to gain knowledge on the presence, distribution and molecular characteristics of *Legionella* strains in tropical areas. Among these, 43% of the samples from hotels and 23% from hospitals were positive for *Legionella* by culture. In total 234 strains were isolated. Here we describe the molecular characteristics of these environmental *L. pneumophila* strains from tropical countries that are developing their LD surveillance network.

Methods

Among the 234 *Legionella* strains isolated, 173 were *L. pneumophila* (Senegal, n=17, Cameroon; n=49 and Cambodia, n=107). Their genomes were sequenced by the Nextera XT Paired-end 150bp read length technology, using a NextSeq 500 sequencer (Illumina®). Sequences were assembled using the SPAdes assembler and then the sequences of the 7 genes used to define the sequence type (ST) were extracted from the assemblies excepted for *mompS* as different copies may exist. Thus the *mompS* genes were PCR amplified and re-sequenced using the Sanger technology. To analyse phylogenetic relationships the whole genome assemblies were compared using the *parsnp* software.

Results

The 173 strains (88 serogroup (sg) 1 and 85 sg2-14) belonged to 33 different STs including 20 STs newly described in this study. ST1 and closely related STs isolated represent between 38 and 59% of all tested *L. pneumophila* strains. ST1 was the only ST identified in all 3 countries. ST1 related STs belonged to *L. pneumophila* sg1, sg4 and sg7 and those isolated from hospital water networks accounted for 11, 33 and 52% in Cambodia, Senegal and Cameroon, respectively. Furthermore, ST59 strains were isolated in Cambodia, ST68 and ST80 in Cameroon. The ST1 and related isolates from the three countries differed from the Paris reference genome by 21 to 8098 SNPs. The less divergent strains were those isolated in Cameroon, the more divergent ones in Cambodia. Whole genome analyses identified 6 subgroups within ST1, almost all geographically linked to their country.

Conclusions

L. pneumophila is present in hospital and hotel water systems in tropical countries, although it was thought that this bacterium is mainly present in moderate climates. Similar to the Northern hemisphere an important proportion of *L. pneumophila* in tropical countries belongs to ST1 and related STs.

P19. Impact of urine samples concentration on Legionnaires' disease diagnosis with BinaxNOW® *Legionella* urinary test: a prospective study

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Objectives

Legionella pneumophila serogroup 1 antigen detection in urine samples (US) is widely used for the early diagnosis of Legionnaires' disease (LD). Previously published studies showed an increase of sensitivity of BinaxNOW® *Legionella* urinary antigen card (UAC; Alere, USA) after concentration of US but most of them were realised on frozen US. The aim of the present study was to evaluate the impact of fresh US concentration on BinaxNOW® *Legionella* UAC performance.

Methods

13 French laboratories included US between April 2015 and March 2016. US were included if BinaxNOW® UAC was positive on concentrated US and confirmed positive after heating. Concentration was performed by centrifugal ultrafiltration (Amicon Ultra-4; Millipore Corporation). The test was then repeat with BinaxNOW® UAC on nonconcentrated US. US with sufficient volume, as well as respiratory samples or *Legionella* strain isolated from it when available were sent to the French Reference Centre for *Legionella*.

Discrepant result was defined as a positive result on concentrated US, confirmed positive after heating and found negative on nonconcentrated US. All discrepant results were analysed further using the Binax® *Legionella* EIA (Alere, USA). A form was filled in for each patient concerned by discrepant result indicating risk factors for LD, patient's medicines and factors that may impact US dilution. Results From April 2015 to March 2016, 13 centres included 90 US. The French Reference Centre received 86 of this 90 US and 32 *Legionella* strains were obtained by sputum culture. A total of 8 US from 3 centres showed discrepant results. They were all confirmed positive by using the EIA. No *Legionella* strain was isolated for these 8 cases. Among them, 4 patients received fluid replacement, 1 was usually treated with diuretic medicines and 1 received both.

Conclusions

This study shows the effect of US concentration on LD diagnosis. No lack of specificity was detected with concentration. As pointed out by the instructions for use (IFU) of the BinaxNOW® UAC, the test has not been evaluated on diuretic urine, so dilution of urine by patient's medicines may explain lack of sensitivity in 6 of the 8 discrepant non-concentrated samples. Excluding the 6 off-label diuretic samples, 2 LD samples were diagnosed with concentration only. Furthermore, as these 8 patients came from 3 of the 13 centres, we can also hypothesize that the sensitivity of BinaxNOW® UAC could depend on particular *Legionella* strains. Unfortunately, no *Legionella* strain was isolated from these patients.

P20. Evaluation of Legionella K-set®, A Lateral flow test for the detection of Legionella pneumophila serogroup 1 antigens in urine samples

A. Souche, A. Ranc, G. Descours, C. Ginevra, G. Lina, S. Jarraud, L. Beraud, France

Objectives

Legionella pneumophila serogroup 1 antigen detection in urine samples (US) is widely used for the early diagnosis of Legionnaires' disease (LD). The aim of this study was to evaluate the Legionella K-set® test (Coris BioConcept, Gembloux, Belgium) in comparison with the BinaxNOW® Legionella Urinary Antigen Card (UAC) (Alere, Scarborough, Maine, USA).

Methods

A total of 250 US were tested, including 200 prospective US submitted for Legionella urinary antigen detection, and 50 frozen US from patients with confirmed LD. Performances of Legionella K-set® test were evaluated and compared to the BinaxNOW® UAC, both realised on concentrated US. Both tests were strictly read at 15 min as recommended by manufacturers. Legionella K-set® test was read by a technician (visual reading) and by the Coris BioConcept Strip reader prototype (automatic reading). US yielding a positive result was retested after heating. Any US yielding discordant result was tested by a third test, the Binax® Legionella EIA.

Results

Legionella K-set® Visual reading Automatic reading - + - + BinaxNOW® - 200 02000 + 1a 49 2a,b48. Total 2014920248 Table 1: Visual reading and automatic reading results of Legionella K-set® test compared to BinaxNOW® UAC. a: One positive sample was negative with the Legionella K-set® test. Both readings were negative. A new reading performed at 3 hours remained negative by automatic reading but was weakly positive by visual reading. b; One sample was negative by automatic reading but it was weakly positive by visual reading. A new automatic reading realised after 1 hour became positive. After heating, all positive US were confirmed, with the exception of three of them, which were not interpretable by automatic reading because of lack or weak control strip. These errors were due to migration defect but the sample strips were strongly positive.

Conclusions

Regarding our results, Legionella K-set® on concentrated US detected 49 out of 50 positive US. Among 200 prospective US, no difference were noticed in comparison to BinaxNOW® UAC. The heat treatment leads to 3 migration defects without impacting the final result. The automatic reading allows traceability with a test's picture recording and reduces the operator dependant variability, providing an intensity strips ratio. Automatic reading shows similar results than visual reading and the only two differences noticed could be resolved by a cut-off decrease. In conclusion, performances of Legionella K-set® test in concentrated US are similar to the ones of BinaxNOW® UAC.

P21. McFarland methodology for quantification of Legionella spp.

T. Graells, E. Jiménez, E. Padilla, P. Pérez, Spain

Objectives

The purposes of this study were, first of all, to check the number of viable and culturable cells of different Legionella strains using the well-known McFarland methodology as a constant. And, secondly, how Legionella strains are affected by temperature using this constant.

Methods

First, suspensions of different Legionella strains using 1McFarland were inoculated, after dilution, on buffered charcoal yeast agar supplemented with α -ketoglutarate (BCYE α) agar plates.

The quantitative seeding allows the counting of individual colony forming units(CFU). Then the concentration in CFU/ml was calculated. Legionella strains tested were:

- Legionella pneumophila serogroup 1 ATCC 33152
- Legionella pneumophila serogroup 4 ATCC 33156
- Legionella micdadei CECT 7760 After that, suspensions of each strain using 1 McFarland were subjected to temperature treatment during 30 minutes.

The range of temperatures tested were from 20°C to 70°C. The samples were inoculated, after dilution, in BCYE α agar and quantitative seeding allowed the calculation of CFU/ml.

Results

Assuming that 1 McFarland contains approximately 3*10⁸ CFU/ml, and using it as a constant, the number of Legionella strains tested were variable in agar plates. The number of culturable and viable Legionella pneumophila serogroup 1 ATCC 33152 cells and Legionella pneumophila serogroup 4 ATCC 33156 had an average of 106 CFU/ml. Legionella micdadei CECT 7760 had an average of 107 CFU/ml. The number of culturable and viable cells of all strains hardly showed differences between 20°C and 50°C. Above 50°C, countings were lower while the temperature increased. Above 60°C Legionella strains showed no growth

Conclusions

Legionella strains tested had lower quantification in CFU/ml using the culture which is the gold standard method and the McFarland methodology. Recovery of Legionella strains using the culture is difficult. This decrease in number is more pronounced in Legionella pneumophila strains. This feature should be considered in methodologies which uses McFarland such as antibiotic susceptibility tests. Temperature increase is an important factor to reduce bacteria concentration in water related systems.

P22. Antibiotic susceptibility of environmental strains and clinical strain of *Legionella* spp. from a hospital nearby Barcelona (Spain)

T. Graells, E. Jiménez, E. Padilla, P. Pérez, Spain

Objectives

The most important goal of this study is to describe the antibiotic susceptibility using the minimum inhibitory concentration (MIC) of different *Legionella* strains isolated from a hospital nearby Barcelona (Spain).

Methods

Antibiotic susceptibility of 12 environmental strains and one clinical strain was tested using ϵ -test strips on buffered charcoal yeast agar supplemented with α -ketoglutarate (BCYE α). The antibiotics tested were: ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, azythromycin, doxycycline, cefotaxime and amoxicillin-clavulanic. The minimum inhibitory concentration (MIC) was read after 2 days of incubation at 36°C in an humidified atmosphere with 5% CO₂. After that, MIC₅₀ and MIC₉₀ were calculated. As a control, *Staphylococcus aureus* ATCC 29213 was used in BCYE α and Mueller-Hinton (MH) agar. Antibiotic susceptibility of this strain was read after 24 hours of incubation at 37°C. Moreover, *Legionella pneumophila* serogroup 1 ATCC 33152, *Legionella pneumophila* serogroup 4 ATCC 33156 and *Legionella micdadei* CECT 7760 were also tested. The 12 environmental strains were isolated from a hospital water system, 10 were identified as *Legionella pneumophila* serogroups 2-14. The other 2 were identified as *Legionella* spp.

The clinical strain was isolated from the lower respiratory tract of a patient hospitalized in the intensive care unit (ICU) and it was identified as *Legionella pneumophila* serogroup 1. Comparison between the MIC₅₀ of environmental strains and the MIC of the clinical strain was performed.

Results

The MIC 50 in μ g/ml (MIC range) of the antibiotics tested in environmental strains were: Ciprofloxacin: 0.038(0.038-1.5) Levofloxacin: 0.125 (0.094-1) Moxifloxacin: 0.5 (0.25-1.5) Erythromycin: 0.094 (<0.016-0.19) Azythromycin: <0.016 (<0.016-0.064) Cefotaxime: 0.19 (<0.016-0.5) Amocillin-clavulanic: 0.047 (<0.016-0.125) Doxycycline: 2 (0.75-4) The MIC in μ g/ml of the antibiotics tested in the clinical strain *Legionella pneumophila* serogroup 1 were: Ciprofloxacin: 1 Levofloxacin: 0.19 Moxifloxacin: 0.75 Erythromycin: 0.094 Azythromycin: 0.125 Cefotaxime: 0.25 Amocillin-clavulanic: 0.047 Doxycycline: 3

Conclusions

According to our results, levofloxacin and azythromycin had the lowest MIC of quinolones and macrolides, respectively, in environmental strains. In general, the clinical strain isolated had higher MIC values in comparison with the MIC₅₀ of the environmental ones. Due to the low recovery of clinical strains, environmental strains isolated from structures with antimicrobial pressure such as hospitals could be used to monitor the possible emergence of antibiotic resistance in *Legionella* spp.

P23. Detection of *Legionella pneumophila* urinary antigen by the rapid lateral flow ImmuView® kit

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Objectives

The ImmuView® *S. pneumoniae* and *L. pneumophila* urinary antigen test (UAT) was compared against two enzyme immunoassays (EIA) from Trinity Biotech® and Binax® for the detection of *Legionella* urinary antigen and the Binax Now immuno-chromatographic test (ICT) for *Streptococci*.

Methods

In total, 554 routine and *Legionella* previously positive urines were tested. Seventy five of the urines were retrospective samples that were previously positive in the Trinity Biotech® Bartels and Binax® *Legionella* EIA and had been stored at -20°C and eight were prospective routine *Legionella* EIA positives. The remainder of tests (471) were routine urines sent for atypical pneumonia screening. Of the 83 *Legionella* positives, 27 were also positive for *Legionella* culture and 12 were *Legionella* serology positive.

Results

The overall sensitivity for the *Legionella* antigen component of the ImmuView® test was 84% but this increased to 89% when the invalid group was removed. Specificity was 100% as no false positives were detected.

Conclusions

ICT and UAT both lateral flow assays are known to be less sensitive than EIA (1) however with a sensitivity of 89% the ImmuView® is an excellent rapid assay for the detection of *Legionella* antigen in urine. The test also detects pneumococcal antigen. The duality of the ImmuView® makes it a unique addition to the UAT market and could provide clinicians with additional information regarding treatment and ultimately patient outcome.

1. Carmen Guerrero, Carmen M. Toldos, Genoveva Yagüe, Cristobal Ramirez, Tomás Rodríguez and Manuel Segovia 2004. Comparison of Diagnostic Sensitivities of Three Assays (Bartels Enzyme Immunoassay [EIA], Biotest EIA, and Binax NOW Immunochromatographic Test) for Detection of *Legionella pneumophila* Serogroup 1 Antigen in Urine. *J Clin Micro* 42:467-468

P24. Genotypic diversity of *L. pneumophila* in a hospital water supply: comparison between 2 areas

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Objectives

To study and to compare the genotypic diversity of *L. pneumophila* in a hospital over a 3 year period.

Methods

We studied 4 wards from two independent water distribution systems (WDS). A, B, C belonged to WDS-1 and D to WDS-2. The disinfection program included copper-silver ionization since 1999 and heat shock treatments 4 times yearly. Molecular typing by PFGE and SBT was performed on 323 isolates selected on spatial and temporal criteria (128 points analysed).

Results

Legionella was detected by culture in 131 (%) out of 620 environmental samples. Differences in the positive points were observed between wards (A: 17% (24/200); B: 6.7 % (5/75); C: 55.6 % (60/108); D: 13.9% (44/237), $p < 0.01$). *L. pneumophila* non-sg.1 was recovered in most of the samples (97/131). *L. pneumophila* sg. 1 isolates were divided into 10 PFGE patterns and 5 ST types, while *L. pneumophila* non-sg.1 isolates were divided into 13 PFGE patterns and 3 ST types. The distribution of PFGE and ST were different between both WDS-1 and WDS-2. The ST813 (51% isolates) followed by ST42 (28%) and ST2011 (17%) were predominant in WDS-1 while the predominant ST in WDS-2 was ST813 (83%).

Although the historically ST42 associated with clinical cases were found in 22% of isolates analysed, only 1.85% of isolates (6/323) showed the PFGE-A_ST42 related with historically clinical cases (identified in WDS-1).

Conclusions

The circulation and persistence in the hospital environment of 23 PFGE types corresponding to 8 ST was observed during the 3-year period. The diversity of *L. pneumophila* PFGE types was higher in comparison with sequence types. The no identification of nosocomial cases could be explained by the no detection of PFGE-A_ST42 in WDS-2, accounting for most of inpatient areas. Our results reinforce the use of discriminatory typing methods such as PFGE into the surveillance programme. In addition, Whole Genome Sequencing characterization of ST42 with different PFGE patterns could explain the association of Legionella with clinical cases.

P25. Impact of disinfection strategies in environmental legionellosis in a pediatric consulting room

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Introduction

A new pediatric consulting ward was opened in our hospital on January 2012. The unit had a high hot water (HW) Legionella colonization rate from the beginning.

Objectives

To describe the impact on environmental Legionella colonization of the different disinfection systems and shock treatments implemented in this area.

Methods

Site: A new pediatric consulting room in a 600-bed university hospital. -Study period: from December 2011 to March 2014. -Disinfection system: Period I (December 2011-Agost 2013): copper-silver ionization; Period II (September 2013-March 2014): chlorine dioxide. -Shock treatments in both periods: 3 heat-flushing yearly. -Design: 10 HW peripheral points were sampled before and 15 days after each shock treatment and at the end of the study (108 samples). Water temperature was tested in each sampling and maintenance system events were recorded.

Results

Period I: 70.5% positivity for Legionella without differences between before and after heat-flushing (74.4% vs 66.7%, p ns), HW median temperature 53.8 °C, median Legionella inocula 487 CFU/L. Usual events. Period II: 13.3% positivity for Legionella without differences between before and after heat-flushing (10% vs 20%, p ns), HW median temperature 53.6 °C, median Legionella inocula 150 CFU/L. Substantial increase in connection water leaks.

Conclusions

Chlorine dioxide was successful in controlling a persistent high Legionella hot water contamination in an area where copper-silver ionization and heat-flushing had failed. At the same time a substantial increase in maintenance events was observed leading to come back to copper-silver ionization and to remove HW faucets as a definitive solution.

P26. Presence of Legionella and high-throughput profiling of bacterial communities in the water distribution system of a university building before and after disinfection

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Objectives

Tap water contamination with Legionella represents an environmental challenge and a public health threat. Despite the improvements in Legionella monitoring, a deeper understanding of the microbial ecology of building plumbing is needed. We studied the effects of disinfection with silver hydrogen peroxide on Legionella and bacterial communities in the water distribution system of a building of the University of Perugia (Italy).

Methods

Cold and hot water samples were taken, before and after flushing, from three taps, together with samples from the hot water production unit (heat exchanger) and three points on the distribution system (network inlet, tank, water softener). Sampling was repeated one week, one and four months after disinfection. Legionella was monitored by standard methods and identified by agglutination test and mip gene sequencing. Bacterial communities were characterized with culture-independent techniques, namely qPCR, PCR-DGGE and Illumina Sequencing of 16S rRNA.

Results

Legionella contamination (10000 CFU/L) was found at taps and in the hot water production unit, despite this was off at the time of sampling. Legionella was absent in cold water after flushing, as well as in the common part of the distribution system, indicating that the contamination was limited to the hot water system and likely originated inside the heat exchanger. Microbial communities were very different between cold and hot water samples, with the former being more similar to those in the common part of the distribution system and the latter similar to those in the heat exchanger. One week after disinfection and turning on hot water production, Legionella was absent in all samples. After one month, Legionella was found again in hot water samples, though at lower levels (100-1000 CFU/L) than before treatment. After four months the contamination raised up to 100000 CFU/L, overreaching the levels before the disinfection. Noticeably, the hot water temperature during the day ranged between 22 and 48°C. The disinfection treatment had only a limited effect on bacterial quantity and diversity and, as before treatment, cold and hot water showed different microbial communities, with the latter being strongly influenced by the heat exchanger.

Conclusions

We found Legionella in the hot water distribution system of a university building. The contamination originated inside the heat exchanger, where also a distinct bacterial community was established, affecting those found in hot water at the tap. Silver hydrogen peroxide treatment was effective in controlling Legionella only in the short-term and showed limited effects on bacterial communities

P27. Clonal distribution of Legionella pneumophila macrolides specific efflux pump

C. Ginevra, J. Chastang, C. Massip, G. Descours, C. Gilbert, S. Jarraud, France

Objectives

To assess the distribution in Legionella pneumophila of the efflux pump targeting specifically macrolides that have been previously identified in the reference strain L. pneumophila Paris CIP107629.

Methods

The presence of the 2 specific genes of the efflux pump was assessed by a specific real-time PCR assay on a collection of 443 L. pneumophila isolates representing 105 different STs. Primers specific of a region overlapping the 2 genes coding the pump were design to detect the presence of both genes. The presence of the 2 specific genes was also investigated in 142 L. pneumophila genomes (136 sg1 and 6 non-sg1) from public (15) and personal (127) databases.

Results

The genes of the efflux pump were detected in 130 out of the 585 L. pneumophila tested. Regarding to the ST distribution, the efflux pump seems to be present in 3 specific clades. The first one includes ST1 and closely related isolates, the second one includes ST701/259 and closely related isolates, the third one includes ST1335 and closely related isolates. The 2 last clades belong to non-pneumophila subspecies.

Conclusions

The macrolides specific efflux pump is not present in all L. pneumophila isolates. Interestingly, this efflux pump seems to be specific of 3 different L. pneumophila clades: 1 belonging to L. pneumophila subspecies pneumophila (associated to ST1 and closely related isolates) and 2 belonging to L. pneumophila subspecies non-pneumophila.

P28. Fast and reliable screening of *Legionella* spp. and identification of *L. pneumophila* in water by real-time PCR including live/dead discrimination

F. Priller, S. Helbig, B. Junge, C. Grönewald, K. Berghof-Jäger, Germany

Objectives

Rising numbers of legionellosis outbreaks all over the world show the high demand of diagnostic tools to monitor water systems such as drinking water systems, cooling towers, air conditioning systems, fountains, and whirlpools. Purpose was the development of a quantitative multiplex real-time PCR assay for the simultaneous detection of *Legionella* genus (*L. spp*) and *Legionella pneumophila* in water. In addition, *L. pneumophila* serogroup 1 detection is targeted as this serogroup accounts for more than 90% of all legionellosis infections. An internal control was included to control PCR inhibition. As special feature, the PCR assay was designed to allow discrimination between live and dead cells (so called viability PCR), in contrast to conventional PCR assays which always detect DNA from live and dead cells. Furthermore, the determined quantity of *Legionella* cells by the assay gives rapid information about the need for action, when compared to national alert levels for *Legionella* spp. and helps to monitor effectiveness of disinfection methods within water systems.

Methods

In order to develop the PCR assay, primers and probes were designed for each target (*Legionella* genus, *L. pneumophila* and *L. pneumophila* serogroup 1), the PCR was established and compared for single- and multiplex assay performance. Specificity (inclusivity/exclusivity) was tested with DNA extracts. Sample matrix compatibility, sensitivity and viability PCR were tested with genomic DNA as well as spiked water samples. Different filtration protocols (membrane filters with different diameter, pore size, material) were tested for various water samples. Live/dead discrimination and subsequent DNA preparation were conducted either using the filter directly or with cells rinsed from the filter.

Results

Optimization of the mix components (e.g. concentrations of primers, probes, nucleotides, ions and Taq polymerase) was necessary to reach high efficiency for quantification purposes as well as to be stable against inhibiting substances in the water. Specificity (inclusivity and exclusivity) was tested with a large panel of *Legionella* species as well as close relatives and bacteria of the same habitat. For each target, no false-negative or false-positive results were obtained for all tested strains. The sensitivity was at least 10 genomic equivalents (GE) per reaction for DNA extracts and 10 cells per 100 ml filtrated sample of different water matrices and with different filtration protocols.

The sample preparation includes a live/dead discrimination step, which efficiently removes DNA of at least 103 cfu/ml dead *Legionella*.

Conclusions

100 % specificity and high sensitivity meet the demands of testing laboratories. As water samples upon disinfection treatment are often contaminated with dead cells of *Legionella*, live/dead discrimination step prevents false positive results which may be encountered with other PCR methods. Comparison studies of cell numbers obtained by culture-based detection (ISO 11731) and by our foodproof® *Legionella* Detection LyoKit in various water samples and systems are planned in order to assess the predictive value as alternative to the labour-intensive and slow culture method. In conclusion, this novel diagnostic tool for *Legionella* detection provides a new approach with viability PCR to improve surveillance in water systems and prevention of new infections.

P29. NF validation of a fast real-time PCR method for the quantification of *Legionella* spp. and *Legionella pneumophila* in “clean” water samples

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

Objectives

According to standard methods (NF T90-431 and ISO 11731), *Legionella* is typically isolated from water by filtration followed by heat or acid treatment then plating onto GVPC agar. The main drawbacks of this cultural method are linked to its long time to result (up to 12 days) and its lack of sensitivity as only culturable cells are detected. Moreover, the presence of background organisms may interfere with *Legionella* growth, leading to an underestimation of the real number of *Legionella* in the sample.

As an alternative to growth on agar, two standard for detection and quantification using real-time PCR were established: NF T90-471 and ISO 12869 standards. Alternative qPCR-based methods, certified by AFNOR Certification against these standards, 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. In order to reduce the hands on time, a new protocol was developed and validated using the GeneDisc® PCR technology from Pall Corporation for *Legionella* quantification in “clean” water samples (e.g. tap, hot tubs, showerheads, whirlpools and spas, and public fountains waters).

Methods

Briefly, 100 mL to 1 L of clean water sample were filtered through polycarbonate membrane (0.4 µm). The membrane was directly transferred in a lysis tube (Extraction Pack Environment 3, Pall) to perform mechanical cell lysis by sonication and heating. Lysate containing DNA released from cells is either directly analysed by real-time PCR using the GeneDisc Plate for *Legionella* DUO (Pall) or DNA is concentrated with the Nanosep® centrifugal device 30K (Pall) 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 (199 % > recovery > 25 %).

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 with a simplified protocol reducing hands on time by 67 %.

P30. CYTO-WATER: An integrated and portable platform for rapid detection and quantification of Legionella in environmental waters

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Objectives

Waterborne diseases are illnesses caused by bacteria that are present in contaminated water sources. Waterborne diseases can manifest as either food poisoning or pneumonia, depending on the microorganism involved. Legionella causes a disease that manifests as a pneumonia called legionellosis, commonly known as Legionnaires' disease that has symptoms such as cough, fever, and chills. Legionella is found naturally in water sources and can contaminate large, contained water supplies such as hot tubs, cooling towers, and large air-conditioning supply units. New rapid methods for waterborne monitoring microorganisms could substitute the labor-intensive and time-consuming methods currently employed for the detection of microorganisms in the laboratory. The main objective of CYTO-WATER project is to deploy a new platform for on line monitoring of microorganisms in industrial and environmental water samples.

Methods

The CYTO-WATER platform will integrate technologies available to the consortium partners: an automated liquid concentration module that includes Celltrap® concentrating filters, a microfluidic system where microorganisms of the concentrated sample will be labelled into in order to be detected and measured by a newly designed fluorescence image cytometer whose readings will be recorded and processed by a Global Control System. Each component will be validated individually to make sure that they meet market specifications. The whole integrated platform will be validated using natural samples and with spiked samples with known concentrations of Legionella, using reference materials. The CYTO-WATER platform validation will include a comparison with the conventional method employed in the laboratory (culture isolation) and also with quantitative PCR. The final objective is to accredit the method under ISO 17025, in order to be easy acceptable as alternative method for fast results.

Results

The autonomy of CYTO-WATER platform is four samples. The automated water concentration module adaptation is finished and the concentrator integration is finished as well. A microfluidic cartridge where labelling of microorganisms and measuring steps will be performed into was designed and manufactured. Several specific fluorescence markers for Legionella were evaluated in order to adapt the device to the requirements for the system. The Cytometry Reader is able to detect and quantify different concentrations of microorganisms, from 108 to 1 CFU/mL both for non-fluorescent samples and for samples labelled with fluorescence markers.

Conclusions

The automated concentration module, microfluidic cartridge for sample preparation and image reader will be integrated resulting in the CYTO-WATER system. The platform will be a low cost solution and will be designed as a portable device suitable for on-site applications. The system will avoid sampling, transport and manual concentration in the laboratory and will enable on-site detection of possible bacterial contamination in a reduced timeframe with decision making capability. The CYTO-WATER platform will be adapted to uses in other applications such as potable waters monitoring.

P31. Legionella as a lower respiratory pathogen in India

S. Ponnoussamy, D.K. Kandy, India

One hundred patients of lower respiratory tract infection (LRTI) were prospectively studied over 2 years to find out if Legionella is a causative agent in these patients. In addition, 50 environmental samples and 50 age and sex matched controls were studied. Culture and direct fluorescent antibody testing (DFA) of respiratory tract secretions, and serodiagnosis by indirect immunofluorescence (IIF) and ELISA, were employed to detect Legionella. Respiratory tract secretions from all patients were negative for Legionella on culture and DFA. Low antibody titers to Legionella were observed in 21 patients and these could be attributed to cross reaction with other gram-negative bacteria. All environmental samples and controls tested negative for Legionella. Legionella does not seem to be an important lower respiratory tract pathogen in this part of the country and empirical addition of erythromycin to treatment regimens for pneumonia is not warranted in our setting.

P32. High prevalence of Legionella in water systems of merchant vessels

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

Objectives

Over 50,000 non-passenger merchant vessels (NPMVs) operate in the world, crewed by more than 1 million seafarers. Outbreaks of waterborne illness associated with passenger vessels are well known and significant progress has been made in controlling these through the introduction of water safety plans. However, there is a paucity of information pertaining to the risk from NPMVs particularly with regards to Legionella. This retrospective study examined the microbial quality of potable water on board NPMVs docking in UK ports.

Methods

Water samples for microbial analysis were collected from 550 vessels docked at eight UK ports between 2013 and 2016. Samples were collected according to BS7592:2008 for Legionella and Health Protection Agency (now PHE) guidelines for potable water parameters. Galley taps were most frequently sampled for potable water analysis and showers for Legionella. Samples were analysed for aerobic colony count (ACC), total coliforms, faecal indicators (*E. coli* and enterococci) and Legionella according to standard methods. A proportion of Legionella pneumophila sg-1 isolates were subjected to sequence-based typing.

Results

Coliforms and faecal indicators showed an improvement in the contamination rate of potable water (9.4% of vessels failed) compared to previous studies, however, the majority of vessels (58.3%) were positive for Legionella spp. with 28% of samples greater than the UK upper action limit of 1000 CFU/L. Cabin and hospital showers were frequently positive. Legionella pneumophila sg1 was isolated from 46% of vessels and sequence based typing revealed they include strains of clinical importance and are distributed across the globe.

Conclusions

These data raise significant concerns about the management of Legionella on board NPMVs and suggest a revision of current guidance is required to prompt improved control. The high rate of Legionella isolation combined with the international transit of vessels raises the possibility that merchant vessels may transfer Legionella around the globe. This warrants further investigation.

P33. Legionnaires' Disease in Ireland 2005-2013: How Complete is Reporting?

T. Mitchell, J. O'Donnell, D. O'Flanagan, Ireland

Objectives

Legionnaires' disease is a severe and sometimes fatal respiratory disease caused by infection with gram negative Legionella bacteria. Found in freshwater and soil, these bacteria contaminate man-made water systems and spread by inhalation of contaminated aerosols or occasionally by aspiration of contaminated water. This study aims to evaluate the effectiveness of the Irish national infectious diseases surveillance system to capture the incidence of Legionnaires' disease in Ireland and to determine if it reflected the rate of hospitalisations annually which are due to this disease.

Methods

To evaluate the effectiveness of the national computerised infectious disease reporting (CIDR) surveillance system to capture the incidence of Legionnaires' disease in Ireland, we analysed two data sources: CIDR and hospital in-patient activity (HIPE) data (from hospitalised patients following discharge) accessed via Health Intelligence Ireland. Data between 2005 and 2013 were analysed by age, gender, setting and serogroup using inter-censal population estimates.

Results

Most (59.8%) of the cases reported in Ireland between 2005 and 2013 were travel-related and 91.2% were associated with Legionella pneumophila, serogroup 1. The mean Irish annual notification rate was 2.51 cases per million population, which is much lower than the European gold standard rate of 20 per million (1). This rate and the mean annual hospitalisation rate (2.30 hospital discharges per million population) compared favourably. Similar age profiles were found, with the highest age-specific incidence rate of notifications in the 75-79 year age group and of hospitalisations in the 70-74 year age group. The male:female ratio was 1.5:1 for Legionnaires' disease notifications and 1.46:1 for hospitalisations.

Conclusions

Our study shows that cases being reported to CIDR reflect hospital activity which is re-assuring, but the possibility of under-detection of Legionnaires' disease as a cause of community acquired pneumonia in Ireland needs to be further explored.

1. National Guidelines for the Control of Legionellosis in Ireland, 2009. Health Protection Surveillance Centre.

Meet and Greet at Hortus Botanicus

The Hortus Botanicus Amsterdam is one of the oldest botanic gardens in the world. Today, there are more than 4,000 plant species growing in the garden and greenhouses. The Hortus is located in the Plantage district on the edge of the hectic center of Amsterdam. Behind the 300-year-old gates, however, the bustle of the city seems to disappear.

Originally, the Hortus was a medicinal herb garden, founded in 1638 by the Amsterdam City Council. In those days, herbs were of vital importance as the basis of medicines and the city had just experienced a plague epidemic. Doctors and pharmacists trained in the preparation of prescriptions at the Hortus.

Thanks to the ships of the Dutch East India Company (Verenigde Oost-Indische Compagnie, VOC), the Hortus expanded quickly in the 17th and 18th centuries. The VOC ships did not only bring herbs and spices, but also exotic ornamental plants. In fact, a few of the Hortus' 'crown jewels' date from this period, e.g. the 300-year-old Eastern Cape giant cycad.

Address: Hortus, Plantage Middenlaan 2a, Amsterdam

Conference dinner at the St. Olof Chapel

Just standing in front of the St. Olof Chapel there is little to reveal its long and fascinating history. When it caught fire in 1966, the ensuing conflagration seemed to mark the definite end of the historic St. Olof Chapel. However, the Barbizon Palace did not want that one of Amsterdam's oldest buildings, dating back to around 1440, would disappear.

So it was decided to restore the old building to its former glory, with all modern comforts and amenities. By doing so the old chapel was converted into the ideal location for dinners. The St. Olof Chapel opened its doors in its new form in January 1993.

Address: St Olof Chapel, Prins Hendrikkade 59, Amsterdam

Experience Amsterdam

As Amsterdam is a surprisingly compact city with excellent public transport, travelling around is quick and easy. Amsterdam is the ideal place for planning an extra informal meeting with your colleagues! What about arranging to meet up in one of the 51 museums, many of them within walking distance of the city centre, or in one of the 25 beautiful old courtyards, 1215 pavement cafés or 55 theatres. After a busy day you may appreciate a relaxing stroll back to one of the 350 excellent hotels to take your mind of things.

The Netherlands' capital city has been a centre of creativity and culture for centuries. From its humble beginnings as a 13th-century fishing village to its current role as a major hub for business, tourism and culture, Amsterdam has a strong tradition as a centre of culture and commerce. During the 17th-century (Golden Age), the city became famous as the leading hub for trade and art; in the 1960s, it emerged as Europe's magical centre. People have made the city unique; it has a highly individual, spirited and young-at-heart character. Amsterdammers are known for their friendly, tolerant and cosmopolitan nature; that's why not too surprisingly, most speak at least two languages (English is almost always one of them). With inhabitants from 177 different cultures, Amsterdam is one of the top three most diverse cities in the world.

Amsterdam has 51 museums, with a wide range of permanent collections and temporary exhibitions. The largest and most renowned institutions include: the Van Gogh Museum (world's largest collection of the artist's masterpieces), the Rijksmuseum (works of the 17th-century Dutch masters Rembrandt and Vermeer), the Stedelijk Museum (world-renowned bastion of modern and contemporary art), the Hermitage Amsterdam, the Anne Frank House, the baroque Royal Palace on the Dam square and the Rembrandthuis. There are also a surprising number of smaller museums, which exhibit a great diversity of art and collections in Amsterdam. The recent popularity of photography and multimedia has also flourished in Amsterdam.

Dining out in Amsterdam is as varied as its multicultural population; as a result of 177 nationalities of people living in Amsterdam, there is a wealth of international cuisine to choose from. Experience the exotic flavours of the Indonesian rice table, a specialty here, or dine in a genuine Bedouin tent. From authentic Spanish tapas and award-winning Japanese food to organic-focused fusion cuisine and Michelin-star French dishes. It is a global gastronomic scene in Amsterdam. So if your delegates are homesick for their own style of cooking or are in the mood for a culinary adventure, they will not be disappointed by the menu in Amsterdam. Many of the restaurants can accommodate groups and banquets and there are plenty of smaller establishments for guests looking for a more relaxing dining experience to end the day.

Amsterdam's name originates from a dam on the Amstel River. The city's nickname, Mokum, is derived from the Hebrew word Makom, which means place. The 165 canals that the city boasts have earned it another title: 'Venice of the North'. Amsterdam actually has more canals than Venice, more bridges than Paris and nearly 7,000 monumental buildings. There are almost as many bicycles as inhabitants in Amsterdam. Some claim the bicycles outnumber the population. The city is home to 600,000 bicycles and 750,000 inhabitants. There is even a bicycle parking garage near Amsterdam Central Station. Amsterdam has a great variety of bars, nightclubs, cafés and amazing events. There are 1215 cafés and bars, 1250 restaurants, 61 cinemas and 36 discotheques. As far as accommodation goes, there are 350 hotels and 5 campsites in Amsterdam. Shopaholics are in for a treat, as there are 21 markets and 6179 shops. You might be interested to know that 6100 animals live in Artis Zoo, there are 110 canal barges with panoramic glass roofs and saloon boats, 28 parks planted with 600,000 flower bulbs.

Sponsors

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Water gives life to people, animals and plants and is a necessity for industry to maintain production. Water is very useful when heating and cooling buildings and is also used to drain off waste products. Anywhere where water is a coveted resource or needs to be drained away, Grundfos plays a central role.

Extensive knowhow and intensive research and product development allow us to develop new, trend-setting products, which meet the ever-increasing requirements of customers and society for improved energy efficiency and reduced impact on the environment.

Our well-educated and committed employees are the most important resource in the Grundfos Group. Therefore, we strive to offer them further training and to create a challenging environment that promotes the development of new products with an increased utility value.

Grundfos is a globally responsible company. All over the world, we strive to create and strengthen lasting ties with employees and business partners as well as the communities in which we operate. The Group's global nature is our customers' guarantee for continuous and easy access to pumps, spare parts and service.

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The Grundfos Group is owned by the Poul Due Jensen Foundation, whose primary purpose is to expand and develop the Group. Reinvestment of earnings ensures that the Grundfos Group remains an independent company.

Gold

SSI Diagnostica



SSI Diagnostica has been an independent business unit under Statens Serum Institut since 1998. In March 2016 Adelis Equity Partners acquired the company with a planned takeover date of 1. September 2016 after final approval from the Danish authorities.

SSI Diagnostica develops, produces and sells in vitro diagnostica for clinical microbiology, veterinary diagnostics, food, environmental and hygiene control to the home market as well as abroad. Today the company employs around 100 people. Our products are sold worldwide and encompass antisera, diagnostic kits and culture media from our advanced production facilities based on the latest knowhow as well as blood products from our farm Hvidesten. We strive to supply products of a very high quality and the development, production and sales of in vitro diagnostics are quality assured and certified in accordance with ISO 9001 and ISO 13485. Products that meet the definitions in Council Directive 98/79/EC of 27 October 1998 on in vitro diagnostic medical devices are CE labelled.

Silver

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Bio-Rad produces tests for food and water safety, including real-time PCR test kits for detection of key pathogens, culture media for nutritive enrichment and RAPID chromogenic media with easy colony identification for detection of pathogens and enumeration of quality indicators.

We also provides instrument options for both low and high volume users, including our iQ-Check® Prep automation system.

Alere



Alere believes that when diagnosing and monitoring health conditions, Knowing now matters™. Alere delivers on this vision by providing reliable and actionable information through rapid diagnostic tests, enhancing clinical and economic health outcomes globally. Headquartered in Waltham, Mass.,

Alere focuses on rapid diagnostics for infectious disease, cardiometabolic disease and toxicology. For more information on Alere, please visit

Rqmicro AG



rqmicro AG, based in Zurich, Switzerland, develops and markets proprietary reagents and innovative instruments for microbial tests in water and food. Our vision is that consumers worldwide benefit from water and food which is free from microbiological contamination. A crucial step towards realizing that vision is to detect contamination in a quick and accurate way. That is why our interdisciplinary team develops ultrafast microbiological solutions on the basis of immunomagnetic separation, microfluidics and flow cytometry. rqmicro's CellStream instrument and test kits enable your lab to detect specific pathogens with standard flow cytometers. Water labs benefit from our newly available reagents and

kits to detect Legionella pneumophila SG1 with a flow cytometer within 1-2 hours instead of 12 - 14 days using standard methods. Contact us discuss how our technology integrates into your lab workflows and to schedule a product demo.

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Pall Medical is a leading supplier of filtration, separation, and purification products in the global healthcare market. Pall's portfolio includes advanced microporous materials and devices with proven performance in applications such as point-of-use water filtration. Pall's water filtration technologies increase water distribution efficiency and provide barriers against waterborne particles and microorganisms.

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IDEXX Water is a global provider of water testing solutions that deliver easy, rapid, accurate and cost-effective information on water quality to laboratories and public utilities around the world.

Headquartered in Maine USA, IDEXX helps to protect water quality for an estimated two billion people every day, with tests accepted or approved in more than 40 countries around the world.

The IDEXX portfolio includes products that identify the presence of E.coli, and coliforms enterococci and opportunistic pathogens such as Pseudomonas aeruginosa, Cryptosporidium and Giardia. The ease-of-use and simplicity of the IDEXX tests mean that they can be very easily performed in environments where the use of a laboratory may be impractical.