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FEVER EPISODE DURING A DIALYSIS SESSION : PROTOCOL OF MANAGEMENT AND MICROBIOLOGICAL INVESTIGATION

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During the last week of July 2004, four patients hospitalised in our institution for an ambulatory haemodialysis session suffered severe fever. While, after the event, no infectious origin could be found out for 3 of them, a *Pseudomonas aeruginosa* bacteraemia was rapidly diagnosed for one of these patients. Because of the hydric feature of such an opportunistic pathogen, a contamination of the local network was suspected. Furthermore, during the week before the event, one of the handwash point had been evicted because of the presence of the same rod. Several samples were thus carried out among the patients present in the ward as well as in the environment. However, none was able to put in evidence any *Pseudomonas*.

Furthermore, the genomic analysis of the clinical and environmental (handwash point) demonstrated that there was no link between these two events. A posteriori, it was so decided that a management and an alarm protocols should be systematically performed in front of patients displaying fever during a dialysis session. Indeed, microbiological and endotoxin analyses of the osmotic water and the diluted infusion are thus actually carried out while blood culture is performed on the catheter and on another peripheral blood vessel. Furthermore, when positive samples are isolated from patients or/and environment, strains are immediately compared thanks to several methods (antibiotyping, serotyping, genomic analysis by RAPD and PFGE).

Though, this approach helped us in keeping the ward opened while a *Pseudomonas aeruginosa* epidemic was suspected and could be rejected thanks to rapid comparison of the strains.

This protocol setting up led us to better manage the infectious risk and to provide the physicians a quicker answer in front of a fever episode.

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EXPERIENCE OF CONTROL OF LEGIONELLA IN WATER NETWORKS OF A 220.000 M² BUILDING

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Background

It is always difficult to master the *Legionella* risk in a big building with multiple water networks and numerous supply points. The objective of this communication is to present the development of the results recorded in a situation of this type subsequent to the discovery - widely reported in the media - of a contamination in 2002.

Material and method

The building taken as an example is a construction of 220,000 m², built on 20 levels, comprising 1,150 offices (of which 670 are equipped with 'sanitation cabins' - i.e., a unit comprising shower, toilet and wash-basin), 40 meeting-rooms including one debating-chamber ('hemicycle') with 732 seats, 2 restaurants, 4 bars and a car-park with 1,200 places. The building comprises 9 water-supply networks, of which 4 are hot water for sanitary purposes, 4 are cold water for sanitary purposes and 1 is a botanical watering network. This summary does not include the technical networks.

Subsequent to the Legionella crisis, all the hot water sanitary networks were emptied, and only those serving the sanitation cabins were restored to function, albeit subject to a monitoring operation and an on-going risk analysis. Following this professional assessment, an extensive programme of work was carried out on the hot water and cold water networks over three years and more, mainly comprising these measures:

- The networks (hot water and cold water) were made into looped circuits;
- Installation of automatic discharge systems in the showers;
- Modification of water-distribution in the sanitary systems (shower, toilet, wash-basin) to bring them into series;
- Replacement of the pressure regulators and installation of one-way valves;
- Replacement of steel pressure boosters and pipes with stainless steel;
- Replacement of all the water distribution networks and valves in the basement;
- Regulation of the hydraulic equilibrium of the networks every 6 months.

In order to avoid stagnation of water in the 'dead branches' of the sanitation cabins water is drawn off manually on a weekly basis with measurement of the temperature in every rising column.

Results

The initial contamination of the hot water supplies was of the order of 450,000 UFC/l of *Legionella* sp in the hot water and 85,000 UFC/l of *Legionella* sp in the cold water, among which 4 groups of different 'pulsotypes' were identified, demonstrating the co-existence of several strains of *Legionella* with a majority of Lp 1, as well as Lp 12, Lp 7 and L. anisa.

Increasing the temperature of the networks, a sustained effort to search-out and eliminate 'dead branches' and contaminated zones, followed by intensive technical interventions, made it possible to effect a drastic reduction whereby, in 3 years, 100% of results were brought below a target-limit of 250 UFC/l on samples taken after 2 minutes of flow.

2002: 44% of the results < 50 UFC/l
(From 125 samples taken, 74 > 50 UFC/l)

2003: 96% of the results < 1000 UCL/l
(From 221 samples taken, 8 > 1000 UFC/l)

2004: 100% of the results < 250 UFC/l
(From 220 samples taken, 0 > 250 UFC/l)

However, in the new situation - in general more than satisfactory from the regulatory point of view - a biofilm analysis showed the presence of *Legionella* in the circulating hot water loop at 61° C, with, on the other hand, an absence of *Legionella* in small-diameter pipes for the distribution to the shower-cabins. *Legionella* analyses on samples taken from the initial water-jet from these pipes fairly regularly yield positive values with concentrations of the order of 700 to 55,000 UFC/l.

Conclusion

The health risk for the user is almost zero taking into account this low level of concentration for a volume of about one litre and the very good analytical results recorded after two minutes of flow, representing the water actually used for a shower. However, we must continue our efforts at improvement towards negative first-jet results.

The biofilm exists mainly in circulating sections, probably showing that by taking refuge there the *Legionella* organisms are able to adapt to the high temperature and poor conditions of survival.

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A NEW MODEL TO TEST EFFICIENCY OF CHLORINE DIOXIDE AND UV-C IRRADIATION ON BIOFILM REMOVAL AND PREVENTION

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Abstract: Biofilms as a source of pathogens may also pose a health risk via hospital water distribution systems. Bacteria released from biofilms can cause infections, particularly in elderly people, children and immunosuppressed patients. Because biofilms are difficult to eliminate, (i) it is crucial to prevent their formation by suppressing the level of planktonic bacteria in the water supply system, (ii) and a reliable and continuing disinfection of the water supply must be guaranteed. The purpose of this study was to show the differences in efficiency of chlorine dioxide and UV-C irradiation with regard to their capacity of removing existing biofilms and inhibiting the formation of new biofilms using a silicone tube model with running tap water from the hospital water distribution system. A permanent exposure to chlorine dioxide prevents formation of a new biofilm and does eliminate bacteria embedded in an existing two years-old biofilm within 70 days. UV irradiation is capable of preventing formation of new biofilms. It will not significantly change the level of viable bacteria in the biofilm.

The research shows that the silicone tube model is a useful tool to demonstrate the effect of different water treatments on biofilms under simulated worst case conditions.

Keywords

Biofilm, chlorine dioxide, UV-C irradiation, water distribution system, silicone tube model

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DETECTION AND QUANTIFICATION OF LEGIONELLAE IN HOSPITAL WATER SAMPLES BY QUANTITATIVE REAL-TIME PCR

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Background: *Legionella pneumophila* (LP) infection is normally acquired by inhalation or aspiration of contaminated water. Water systems of large buildings, such as hospitals, are often contaminated with legionellae and therefore represent a potential hazard to patients. For risk assessment of nosocomial *Legionella* infection, surveillance and rapid monitoring of suspected hospital water supplies is essential. As identification of LP takes three to ten days by conventional culture, several assays based on the polymerase chain reaction (PCR) have been evaluated. The advent of real time PCR technology has made possible reductions in analysis time.

Method: This study relates the use of tests designed for the iCycler™ iQ thermal cyler (Bio-Rad) for real-time PCR detection and quantification of LP (iQ-Check™ Quanti *Legionella pneumophila*) and *Legionella* spp. (iQ-Check™ Quanti *Legionella* spp.) in water samples after filtration. One hundred environmental water samples from various sites of the CHU Nancy were investigated by LP real-time PCR assay, of which 80 were also tested by *Legionella* spp. real-time PCR assay. In parallel conventional culture method was used.

Results: twenty water samples were found to be positive for LP by culture (nine were positive for LP serogroup 1, eleven for LP sero-

group 2-14). All twenty LP culture-positive samples were positive in the real time PCR assay for LP (detection limit (Ld) : 133 genomic units/L (GU/L)), of which 19 displayed results superior to the quantification limit (Lq) (Lq : 373 GU/L) : median PCR results (4200 GU/L [mean 4400 GU/L ; range 500 to 11000 GU/L]). Among these 20 LP culture-positive samples, data for *Legionella* spp. real time PCR assay were available for 15 water samples : median PCR results (15000 GU/L [mean 20000 GU/L ; range 900 to 70000 GU/L]).

Eighty water samples contained fewer viable LP cells than the detection limit (250 CFU/L) of the culture method, of which 20 were negative for LP real time PCR assay (with no inhibition of amplification), 19 were under the Ld, 9 between the Ld and Lq, and 32 displayed results superior to the Lq : median PCR results (2200 GU/L [mean 3100 GU/L ; range 500 to 10000 GU/L]). Among these 80 LP culture-negative samples, data for *Legionella* spp. real time PCR assay were available for 66 samples : two water samples had results superior to the upper quantification limit (UQL: 403783 GU/L) and required adapted dilution of DNA samples for precise quantification ; for the remaining 64 samples, 6 were negative (with no inhibition of amplification), 17 were under the Ld, 3 between the Ld and the Lq, and 38 displayed results superior to the Lq: median PCR results (4300 GU/L [mean 20000 GU/L; range 400 to 370000 GU/L]). For 20 water samples, results for both LP and *Legionella* spp. real time PCR assays were between the Lq and UQL: LP PCR assay: median PCR results (1900 GU/L [mean 2700 GU/L; range 500 to 11000 GU/L] ; *Legionella* spp. PCR assay: median PCR results (9000 GU/L [mean 30000 GU/L; range 900 to 370000 GU/L]). Although there was no correlation between these results, the association of both tests (iQ-Check™ Quanti *Legionella pneumophila* and iQ-Check™ Quanti *Legionella* spp.) leads to a better knowledge of hazards related to *Legionella* contamination in hospital water supplies.

Conclusion: These data support previous findings indicating that culture frequently underestimates the presence of LP in water samples. Thus, real time PCR assays may therefore be useful both for routine monitoring for *Legionella* contamination, and for rapid screening of large numbers of water samples during outbreak investigations, while the results of culture are still awaited.

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Non communiqué/Not transmitted

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WATER MICROFILTRATION : A PROCEDURE TO PREVENT PSEUDOMONAS AERUGINOSA INFECTION

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Pseudomonas aeruginosa is a major cause of pulmonary, urinary, wound and bloodstream nosocomial infections, especially in intensive care units (ICU). Taps and the moist environment surrounding them are easily contaminated and have been reported to be a source of nosocomial infections. The purpose of our study was to assess the impact of a systematic installation of water microfiltration on the incidence of *P. aeruginosa* infections in the ICU of a 600-bed surgery teaching hospital.

Over a five-year period, comprising 23611 patient-days and started by a 30-month no-filtration period, we numbered bloodstream, urinary- and pulmonary- *P. aeruginosa* infections which occurred in the ICU : 104 and 46 *P. aeruginosa* infections were found respectively during the no-filtration and the post-filtration periods, showing a significant decrease of the incidence of *P. aeruginosa* infections (8.7/1000 patient-days without filtration, 3.92 with filtration). Considering the only infections characterised with multisensitive *P. aeruginosa* isolates [more likely originating from water source of contamination rather than multiresistant isolates], the incidence decrease was again more marked (2.7/1000 patient-days without filtration, 0.5 with filtration). Our study demonstrated the significant positive impact of the water filtration in our ICU, and a noticeable effect over nosocomial infection prevention.

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EXPERIENCES WITH WATER SAFETY PLAN IN AN UNIVERSITY HOSPITAL OVER ONE YEAR INCLUDING PREVENTION OF BACTERIAL EMISSION FROM SINK DRAINS

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Due to the high number of immunosuppressed and other predisposed patients hospitals have to control the microbiological water quality. Water born nosocomial infections caused e.g. by *Legionella pneumophila* or *Pseudomonas aeruginosa* still are a common problem. Anaissie *et al.* recently estimated the number of fatal water-borne pneumonias acquired in U.S. hospitals caused by *Pseudomonas aeruginosa* at about 1400 cases per year. Each year severe *Legionella* outbreaks in health care facilities are reported worldwide, e.g. in summer 2003 in Frankfurt/Oder in Germany. The routes of infection could be identified by molecular subtyping methods and water taps became obvious as important source of nosocomial infections.

The underlying cause for the occurrence of pathogenic microorganisms in water pipes in buildings is the formation of biofilm. Biofilm can even be found not only in older but also in newly opened hospitals because the water stagnates for weeks or even months till the official opening.

At first we will report the strategy and management of such situation with a highly contaminated cold water system (i.e. *P. stutzeri* 103-4 cfu/100ml) after opening a new part of our hospital during normal clinical course of operation. First we had to choose a hopeful decontamination technique. In case of *Legionella* contamination of the hospital water system several systemic measures are recommended. Most frequently thermic shock (flushing water >70°C through all pipes over 3 min) is used for hot water systems. It has a rapid killing effect on planktonic bacteria but it is unable not destroy biofilms. UV radiation has only effects on planktonic bacteria passing the UV lamps but cannot reach the already established biofilm areas and needs special technique. Chlorine in concentrations according to European standards (German standard is 0.3%, in exceptional cases up to 0.6%) has sufficient effects on planktonic bacteria, but is also not suited to remove biofilm. Electrolytic disinfection concepts such as anodic oxidation are also based on the disinfecting effect of free chlorine. The effect on biofilm is controversially discussed. Therefore we selected the chlorine dioxide decontamination with a reaction time of 1 h of 20 ppm. This pro-

cedure decontaminated the water supply system completely inclusively tanks, washer disinfectors and other water bypasses. The key for the result was the following:

- Creation of general logistic with implementation of water task force (nurses, technical support, clinical management, external technical provider) guided by the head of hygiene institute
- immediate providing of special equipment (dosage pump, chemicals and devices)
- background information of water distribution in the building, to realize the follow up of opening, and consecutive closing (for reaction time) and re-opening (to clear water from chlorine dioxide and by-products) of all water taps (n=2237), toilets, washer disinfectors and other bypasses
- parallel information of all patients and staff about the sanitation and the necessity to avoid the use of the contaminated water for ingestion, hand washing and nursery
- after decontamination day to day control of the water quality following national standards with external control.

After the successful decontamination we have installed a water safety plan following the principle "search and destroy" based on an established and adapted HACCP concept. The most important measures are:

- concept for sample taking
- immediate response to any significant test result
- to fix the extend of measures depending on the degree of contamination and risk assessment with the following steps:
 1. cfu > 100 cfu/1 ml, but no pathogens: Cleaning of perlators, flooding of water system, control, if still positive, ClO2 decontamination
 2. *Pseudomonas* spp. in 100 ml: in risk areas POI filters and the same measures as above, in other wards only use of hot instead of cold water for nursering
 3. *Legionella* spp. in 100 ml: in risk areas POI filters and the same measures as above, shower excluded from use
- permanent heating level of 55 °C, additionally monthly thermo disinfection of hot water system
- identifying and eliminating resp. continuous use of non used end points of tap water
- installation of point of use (POU) filters in risk wards with immunodeficient patients, e.g. haematological-oncological units, transplant units, burned patient units, intensive care units as well as for the last washing cycle for endoscopic instruments.

Since implementation of the full concept we were able to avoid systemic contamination by chemical ways.

Further attention has to be focussed on lavatory sinks. They contain 105 to 1010 cfu/ml of bacteria, thereof about 103 to 106 cfu/ml proved to be gramnegative rods. In internal, surgical and neonatal intensive care, general and visceral surgery, oncology, and transplantation unit we measured the bacterial aerosol (n=257) 10 cm above the sinks during tap water running into the sink drain over 1 min. During the tap water running aerosols containing bacteria from the sink fluid were emitted into the surrounding area. Accordingly sink drains function as open bacterial reservoir. The higher was the microbial burden of the siphon fluid, the more bacteria were emitted into the air. Bacteria colonizing oncologic patients were found in the sinks and in aerosols around the basin of the patient room. Continuous thermo-disinfection in combination with low frequency vibration of the siphon prevented biofilm formation and eliminated siphons as bacterial reservoir.