

## Infection control by point-of-use water filtration in an intensive care unit – a Hungarian case study

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### ABSTRACT

Hospital tap water is a potential source of pathogenic bacteria associated with nosocomial infections. Infection control should include preventive measures to reduce the risk of waterborne infection. The efficiency of point-of-use water filters in infection control was assessed in the intensive care unit of a Hungarian hospital with long history of nosocomial *Pseudomonas aeruginosa* cases. All taps in the unit were fitted with disposable point-of-use filters. The incidence of nosocomial *P. aeruginosa* infections decreased from 2.71 to 0 cases/100 patient days when the filters were in place. Legionnaires' disease was not observed either during or outside the study period. Before the application of the filters, both *P. aeruginosa* and *Legionella* sp. were shown to colonize five of the seven taps. Filtration eliminated both bacteria completely, though secondary contamination was observed. Total genome restriction profiling of environmental and clinical *P. aeruginosa* isolates have shown the ubiquitous presence of a single genotype. The same genotype was detected in five of the seven previous nosocomial cases, which supports the assumption of water-derived infection. The results demonstrate that point-of-use filters are effective and cost-efficient measures in reducing health-care associated infections.

**Key words** | *Legionella* sp., nosocomial infection, point-of-use filter, *Pseudomonas aeruginosa*, tap water

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### INTRODUCTION

Nosocomial infection is one of the most severe risk factors in health care, affecting up to 10% of hospitalized patients in the developed countries (World Health Organization (WHO) 2002). Intensive care units (ICUs) are exceptionally hazardous areas for acquiring endogenous or exogenous bacterial infections (European Centre for Disease Prevention & Control (ECDC) 2012).

In Hungary, the incidence of Gram positive bacterial infections such as methicillin resistant *Staphylococcus aureus* has plateaued in the past decades, but Gram negative pathogens, especially multiresistant organisms are an increasing risk (Caini *et al.* 2013). Among these, *Klebsiella* sp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli* are reported most frequently in the National

Nosocomial Surveillance System (Hungarian National Center for Epidemiology (OEK) 2012). *P. aeruginosa* and *A. baumannii* had the highest mortality rate among hospital acquired infections (HAIs), reaching 57% in case of *P. aeruginosa* blood stream infections (Trethon *et al.* 2012). Nosocomial Legionnaires' disease had a similar mortality rate, though the number of reported cases was low (ECDC 2013). All of these pathogens – and many other bacteria involved in HAIs – were reported to persist in the water distribution system of hospitals. However, in Hungary, the role of drinking water as an infective source is considered insignificant compared to other transmission routes. The epidemiological investigation of HAIs – with the exception of Legionnaires' disease – usually does not involve water analysis.

World-wide, the most frequent water-related nosocomial outbreaks are associated with *P. aeruginosa* and *Legionella* sp. (Exner *et al.* 2005; O'Neill & Humphreys 2005; Trautmann *et al.* 2005). *P. aeruginosa* may cause a wide range of infections, including pneumonia, urinary tract, burn, wound or blood stream infections. *P. aeruginosa* forms biofilms on environmental surfaces, including health-care equipment, water system pipelines and tap faucets. Once colonized, it is almost impossible to eradicate them from the water system; a single strain was shown to persist for years in an ICU (Reuter *et al.* 2002). Long-term persisting endemic infections and outbreaks were reported from intensive care facilities world-wide (Bert *et al.* 1998; Blanc *et al.* 2004; Aumeran *et al.* 2007) and were often traced to potable water by epidemiological and genotyping evidence (Ferroni *et al.* 1998; Berthelot *et al.* 2001; Trautmann *et al.* 2001, 2006, 2008; Blanc *et al.* 2004; Muscarella 2004; Vallés *et al.* 2004; Cholley *et al.* 2008). The presence of chlorine or other disinfectants in the potable water may also give rise to multiresistant *P. aeruginosa* strains (Muscarella 2004).

*Legionella* species – particularly *L. pneumophila* – is an emerging water-related pathogen. Hospitalized, immunocompromised patients are especially susceptible; approximately 5 to 20% of all reported Legionnaires' disease cases are health-care associated, with high mortality rates (Exner *et al.* 2005, 2007; ECDC 2013). In the absence of preventive measures, legionellae often colonize the water distribution system of hospitals (Exner *et al.* 1993; Joly & Alary 1993; Kool *et al.* 1999; Stout *et al.* 2007). Unlike most countries in Europe, in Hungary there is no legal requirement on *Legionella* monitoring or risk management in hospitals.

The prevention of potable water-related infections in healthcare usually requires a complex risk management approach from engineering through disinfection to staff training. The aim is either to reduce the number of pathogenic organisms to below risk level or isolate particularly susceptible patients completely from the potable water distribution system. Systematic disinfection schemes such as thermal or chemical disinfection may achieve significant reduction, though not complete eradication of pathogens (Kim *et al.* 2002; Kusnetsov *et al.* 2003; Ortolano *et al.* 2005). Some of these methods raise further concerns,

such as corrosion or scalding risk (thermal disinfection) or toxicity in the case of chemical disinfection (e.g. monochloroamines, metal ions, or disinfection by-products) (Kim *et al.* 2002). Prevention of the passage of bacteria at the point-of-use provides a flexible option to protect high risk areas, such as hematological, perinatal or ICU without putting unnecessarily high constraints on the water quality of the entire system (Vonberg *et al.* 2005; Tu *et al.* 2007; Holmes *et al.* 2010).

The aim of this study was to assess the role of the water distribution system in endemic *P. aeruginosa* infections in a Hungarian ICU and efficiency of point-of-use filters in the control of waterborne nosocomial pathogens.

## METHODS

### Study setup

The study was undertaken in a 269-bed university teaching clinic in Budapest, Hungary over an 8-week period in 2008. The 5-storey building was built in 1970, the water system was renovated in 2007. The hot water is generated by an in-house steam-generator and a heat-exchanger. The hot water is stored in three linearly connected hot water storage tanks with 50 °C nominal water temperature. The water system is connected to the city main distribution network; there is no additional systematic water treatment or disinfection of cold or hot water in the hospital.

The study was carried out in a 12-bed ICU of the hospital. The ICU has four separate patient rooms, each with a hand-washing basin and two with additional instrument wash sinks, all equipped with non-thermostatic mixing taps. An additional water tap is located in a separate instrument washing room. All seven water outlets in the ICU were fitted with disposable 0.2 µm sterile point-of-use filters (Pall-Aquasafe AQ14F1S, Pall Medical, UK). The cleaning routine and hygiene practices of the ICU were unchanged during the study period.

Water quality was monitored for 1 week before the installation of the filters to provide a baseline. The first set of filters was applied to all seven taps, and replaced after 2 weeks in accordance with the manufacturer's instructions. In the second 2-week period, only five of the seven taps were

studied, two were out of order due to a leak in the drainage pipe.

### Patient data

The surveillance was carried out during the study period (4 weeks with point-of-use filters) and for 4 weeks immediately after the removal of the filters. The surveillance methodology was the protocol of the Hungarian National Nosocomial Surveillance System ICU Module (<https://www.oek.hu>).

### Water sampling and analysis

The water samples were collected according to standard methods (ISO 5667-5:2006; ISO 19458:2006). One liter samples were drawn in sterile glass flasks with 0.1% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Immediate and flushed cold and hot water samples were collected. The temperature of the flushed samples was measured in a separate flask and recorded.

The sampling was carried out weekly, starting 1 week before the application of the filters (Week -1). When filters were installed (Week 0) or replaced (Week 2), samples were taken both with and without the filters. Thus the taps were sampled four times (Week -1, Week 0, Week 2 and Week 4) without and five times (Week 0, Week 1, Week 2, Week 3, Week 4) with filters. The water pressure and the maximum flow rate were determined prior to the installation of the filters.

The water samples were analyzed for *P. aeruginosa*, *Legionella* sp. and heterotrophic plate count by standard culture technique (ISO 6222:1999; ISO 11731-2:2004; ISO 16266:2006).

### Human and environmental *P. aeruginosa* strains

*P. aeruginosa* strains were isolated from the ICU patients showing clinical symptoms (trachea discharge, wound discharge and abdominal wound drain were sampled). Asymptomatic colonization was not investigated. The isolates included in the study were randomly selected from all strains isolated in the period from 2 weeks before the application of the filters to 4 weeks after the removal of the filters. The environmental isolates were obtained from positive water samples ( $n = 76$ ).

### Typing of *P. aeruginosa* strains

The identification of the environmental and clinical isolates as *P. aeruginosa* was validated by biochemical characteristics (fluorescence on King B agar, acetamide hydrolysis and oxidase reaction).

The phage profile (Sjöberg & Lindberg 1968; Lindberg & Latta 1974) and the pyocin production (Govan & Gillies 1969) were determined for all environmental and human strains. Strains were O serotyped by slide agglutination (Bio-Rad, Richmond, California, USA). Serotyping was performed for all human isolates and selected environmental strains. The latter were chosen based on phage and pyocin type, to provide sufficient representation of all sampling points and dates. Phenogroups were defined based on the above results.

Beta-lactam (imipenem, meropenem, doripenem, piperacillin + tazobactam, ceftazidim, cefepime), fluoroquinolone (ciprofloxacin) and aminoglycoside (amikacin, gentamicin, tobramycin) antibiotic resistance profile of group representatives was determined. Total genome restriction pulse field gel electrophoresis (PFGE) with *SpeI* was used for the genotyping of the strains (Poh et al. 1992). Similarity was calculated using Fingerprinting II Informatix software, Version 3.0 (Bio-Rad, Richmond, California, USA) with Dice coefficient and unweighted pair group method with arithmetic mean (UPGMA) clustering.

### Typing of *Legionella* sp. strains

*Legionella* sp. strains were isolated from the positive GVPC (glycine, vancomycin, polymyxin B, cycloheximide amended buffered charcoal yeast extract agar) plates ( $n = 23$ ). Serological identification was carried out using both polyvalent and monovalent sera (Oxoid, Cambridge, UK and Denka Seiken, Tokyo, Japan, respectively). The antibiotic resistance profile was determined for all isolates (levofloxacin, erythromycin, tetracycline, rifampicin, ciprofloxacin, ofloxacin).

Repetitive consensus sequence (rep)-polymerase chain reaction (PCR) with ERIC1 and ERIC2 primers was used for the preliminary genotyping of the strains (Van Belkum et al. 1996). Similarity was calculated using FPQuest™ Software Version 4.5 (Bio-Rad, Richmond, California, USA) with Dice coefficient and UPGMA

clustering. The group representatives were characterized further with total genome restriction PFGE with *Sfi*I (Schoonmaker et al. 1992). Similarity was calculated using Fingerprinting II Informatix software, Version 3.0 (Bio-Rad, Richmond, California, USA) with Dice coefficient and UPGMA clustering.

## RESULTS

### Prevalence of nosocomial infections in the ICU

During the investigated period, *P. aeruginosa* infection was confirmed for 14 patients, 10 HAI and in 4 cases the infection already existed at the time of hospitalization. All of these occurred before the application or after the removal of the point-of-use filters in the ICU. When the filters were in place, no new *P. aeruginosa* infections were detected (Figure 1). The recorded patient care parameters for a 4-week period with and without filters are summarized in Table 1. The number of cases/100 patient days recorded in the period without filters (4 cases) was similar to the long-term prevalence data in this unit (2.71/100 patient days). Incidentally, newly admitted patients in the study period (with filters) did not carry *P. aeruginosa* infections either.

Legionnaires' disease cases were not reported either during or outside the study period.

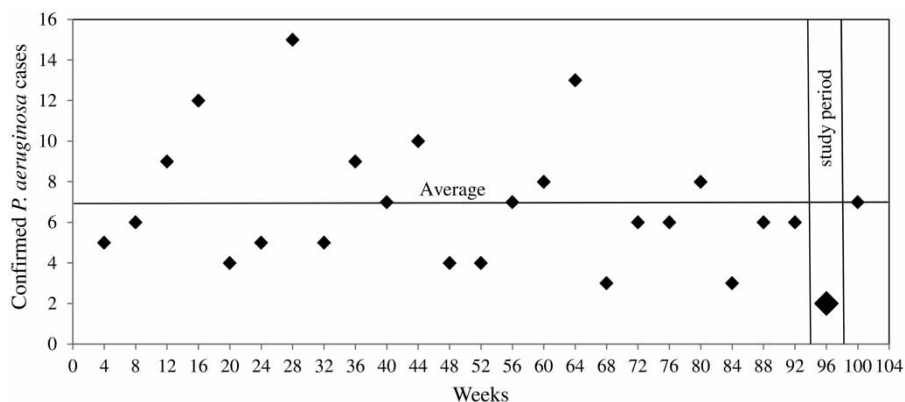
### Water samples

The water pressure was between 0.7 and 1.6 bar, the maximal flow was 10.8 to 22.2 L/min on the investigated taps. The values were within the limit for installation of the point-of-use filters. The temperature of the post flush cold water was generally 10–17 °C (46/47 samples); only one sample was above 20 °C (22 °C). The temperature of the flushed hot water was 40–45.5 °C.

Four sets of water samples were collected without filters: 1 week before the application of the filters (Week -1), immediately before the application (Week 0), when changing filters (Week 2) and after the removal of the second filter set (Week 4).

Four of the seven taps were positive for *P. aeruginosa* at every sampling time point in immediate and flushed samples as well (immediate sample titers are shown in Figure 2). The taps of the hand-washing basins in the two largest wards (taps 4 and 6) were the most heavily colonized. The instrument washing sinks in these wards (located next to the hand-washing basins) were less contaminated (taps 3 and 5). One tap had only a single positive immediate sample (tap 7), while two taps (tap 1 and 2) were negative at all samplings. One of these was the tap in the instrument washing room that is not directly in contact with the patients.

*Legionella* sp. was detected in five of the seven taps. Approximately half of the flushed hot water samples (11 of 24, 46%) were positive (Figure 3). The highest counts were detected on the tap in the instrument washing room (3200 CFU/100 mL, tap 1). The highest count in the patient



**Figure 1** | Number of *P. aeruginosa* confirmations in 4-week intervals between 11.04.2006 and 10.03.2008. In the study period, incidence (2) was lower than in any other interval in the above time frame, and both cases started before the study period.

wards was 280 CFU/100 mL. *Legionella* sp. prevalence and titer was lower in the cold water samples (6/24 positives, 2–30 CFU/100 mL).

The heterotrophic plate counts of the flushed samples were generally below the Hungarian public health limit

**Table 1** | Patient care statistics with and without the application of point-of use filters on water taps in the ICU. Study period was 4 weeks for each. The values recorded without filters are in accordance with the long-term averages in the ICU

	With filters	Without filters
Patient days	248	258
Mechanical ventilation days	160	189
Central venous catheter (CVC) days	188	214
Urinary catheter days	235	241
Patients	39	43
Number of confirmed nosocomial infections	4	4
Nosocomial infection (NS)/100 patient days	1.61	1.55
NS/100 ventilated days	2.50	2.12
NS/100 CVC days	2.13	1.87
NS/100 urinary catheter days	1.70	1.66
Legionnaires' disease cases	0	0
Patients positive for <i>P. aeruginosa</i>	0	7
<i>P. aeruginosa</i> /100 patient days	0.00	2.71
<i>P. aeruginosa</i> /100 ventilated days	0.00	3.70
<i>P. aeruginosa</i> /100 CVC days	0.00	3.27
<i>P. aeruginosa</i> /100 CVC days	0.00	2.90

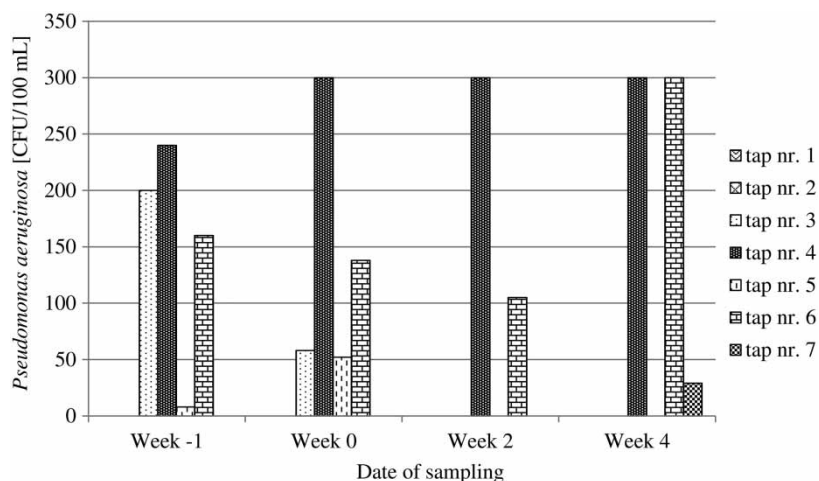
value (2/48 and 5/48 samples were non-compliant for colony count at 22 and 37 °C, respectively).

Five sets of samples were collected from the taps with point-of-use filters installed, immediately, 1 week and 2 weeks after the application of the first filter set (Week 0, 1 and 2), and 1 and 2 weeks after the application of the second filter set (Week 3 and 4). In the first round (Week 0, 1 and 2), all samples were negative for all investigated microbial parameters. The same applied for the first sampling with the second set of filters (Week 3). However, in the last sampling (Week 4), *P. aeruginosa* was detected from two of the five taps installed with filters in the immediate samples (>300 CFU/100 mL on tap 1 and 4). The heterotrophic plate counts were also high. Flushed hot and cold samples were negative. Immediately after the sampling, the filters were removed and the taps were resampled. Tap 1 was negative for *P. aeruginosa*, as it was at all previous samplings.

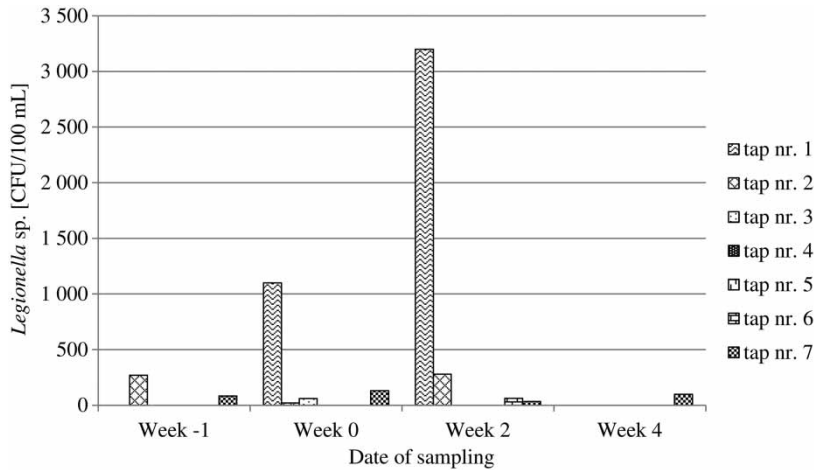
### Characterization of the *P. aeruginosa* strains

During the study period, 76 environmental and 9 human *P. aeruginosa* isolates were collected. Four different phage types were detected among the environmental strains, five types among the clinical isolates. Based on pyocin production, 10 and 2 types of environmental and clinical strains were differentiated, respectively (data not shown).

Serotyping resulted in three groups of the human strains and two among the environmental isolates; serotype O1



**Figure 2** | *P. aeruginosa* colony counts in tap water without filters (immediate samples). Taps were sampled 1 week and immediately before the application of the filters (Week –1 and 0), during the replacement (Week 2) and after the removal of the filters (Week 4). Taps 3 and 5 were out of order during from Week 2 to 4 and not sampled.



**Figure 3** | *Legionella* sp. colony counts in tap water without filters (flushed hot water samples). Taps were sampled 1 week and immediately before the application of the filters (Week -1 and 0), during the replacement (Week 2) and after the removal of the filters (Week 4). Taps 3 and 5 were out of order during from Week 2 to 4 and not sampled.

being the most abundant (78 and 52%, respectively) (Table 2).

The clinical isolates and selected environmental strains were characterized further with PFGE using *SpeI* total genome restriction. Previous studies have shown low correlation of PFGE clusters and serotypes (Trautmann et al. 2006). In the present case, there was complete overlap between the typing methods. Seven clusters were differentiated at 80% similarity; four clusters contained only clinical, two solely environmental isolates, while cluster Psa015 consisted of both (Figure 4). The clinical strains were more diverse. The strains from the patients with pre-existing *P. aeruginosa* infection were unique (Psa019, Psa020). Strains from the confirmed nosocomial cases belonged to the clusters Psa015 (5), Psa021 (1) and Psa022

(1). Most of the environmental strains belonged to the cluster Psa015 (76%). This strain has colonized the entire ICU and was persistent in time; it was recovered from all sampled taps and all sampling occasions. Cluster Psa016 consists of strains (five confirmed and three presumptive) isolated from taps 1 and 4 with filters in Week 4. The type was not isolated from any of the taps at the previous samplings.

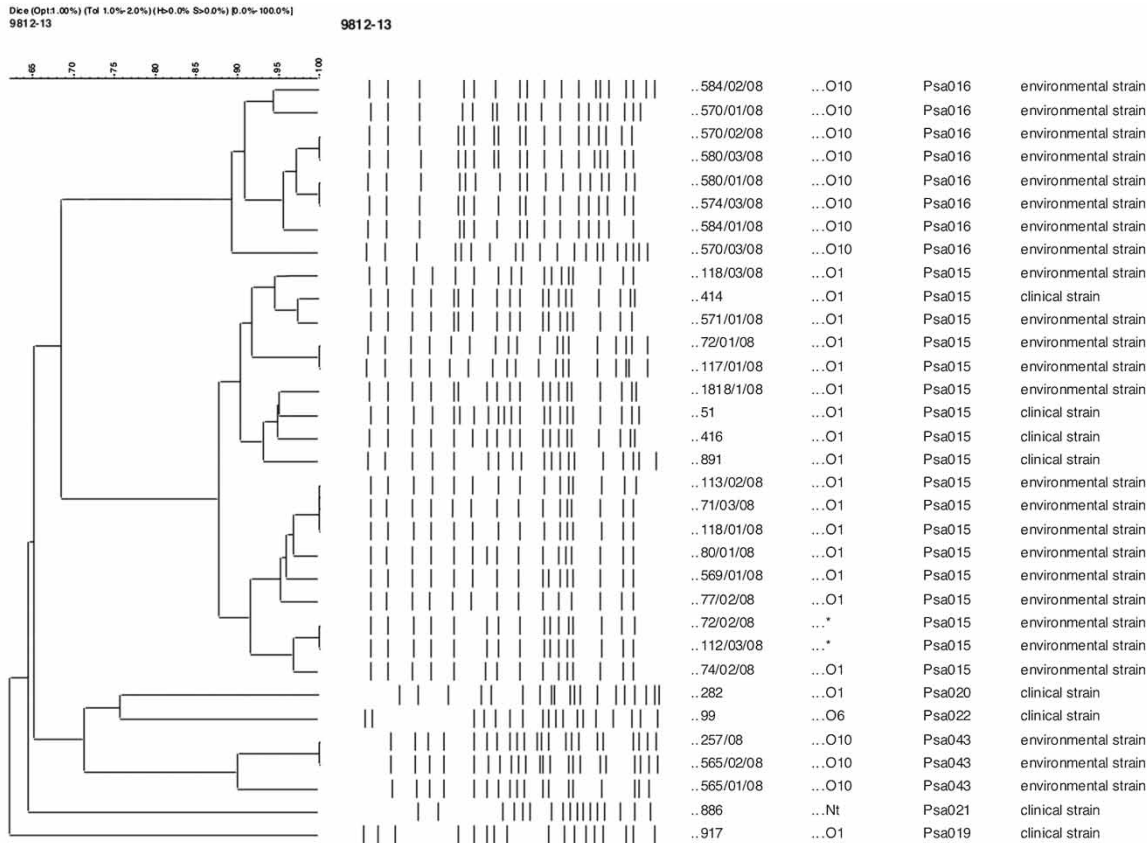
The majority of the environmental *P. aeruginosa* strains were sensitive to all tested antibiotics. The strains belonging to cluster Psa015 (both clinical and environmental) share a common antibiotic resistance profile: sensitive to beta-lactams and fluoroquinolone but resistant to aminoglycosides to various extents. The other (non-water-related) human isolates showed diverse resistance patterns, but none of them were multiresistant.

**Table 2** | Phenogroups of the clinical and environmental *P. aeruginosa* isolates based on phage type and serotyping

Phage type (selected strains)	Serotypes	Number of isolates		
		Human	Environmental	Total
A	O1	4	12	16
B	O10		9	9
C	O6	1		1
Other phage-types	O1	3	1	4
Non-typable	O1		1	1
	O10		4	4
	ONt	1		1

### Characterization of the *Legionella* sp. strains

From the water samples, 23 *Legionella* strains were isolated during the study. There were no clinical isolates. One isolate was identified as *L. pneumophila* sg. 1; 17 strains sg 3 and 5 *L. pneumophila* sg. 2-14 (non-agglutinating in monovalent sera). The strains were typed by rep-PCR with ERIC primers; the ERIC clusters were identical to the serogroups (Figure 5). The predominant type (*L. pneumophila* sg. 3, Cluster C, 17 strains) was detected on all positive taps. The non-agglutinating *L. pneumophila* sg. 2-14 strains ( $n = 5$ ) forming Cluster A were all isolated



**Figure 4** | Dendrogram of the PFGE profiles of clinical and human *P. aeruginosa* isolates. Total genome of the isolates was digested by restriction enzyme *SpeI* and fragments were separated by PFGE. Similarity was calculated using Fingerprinting II Informatix software, Version 3.0 (Bio-Rad, Richmond, California, USA) with Dice coefficient and UPGMA clustering.

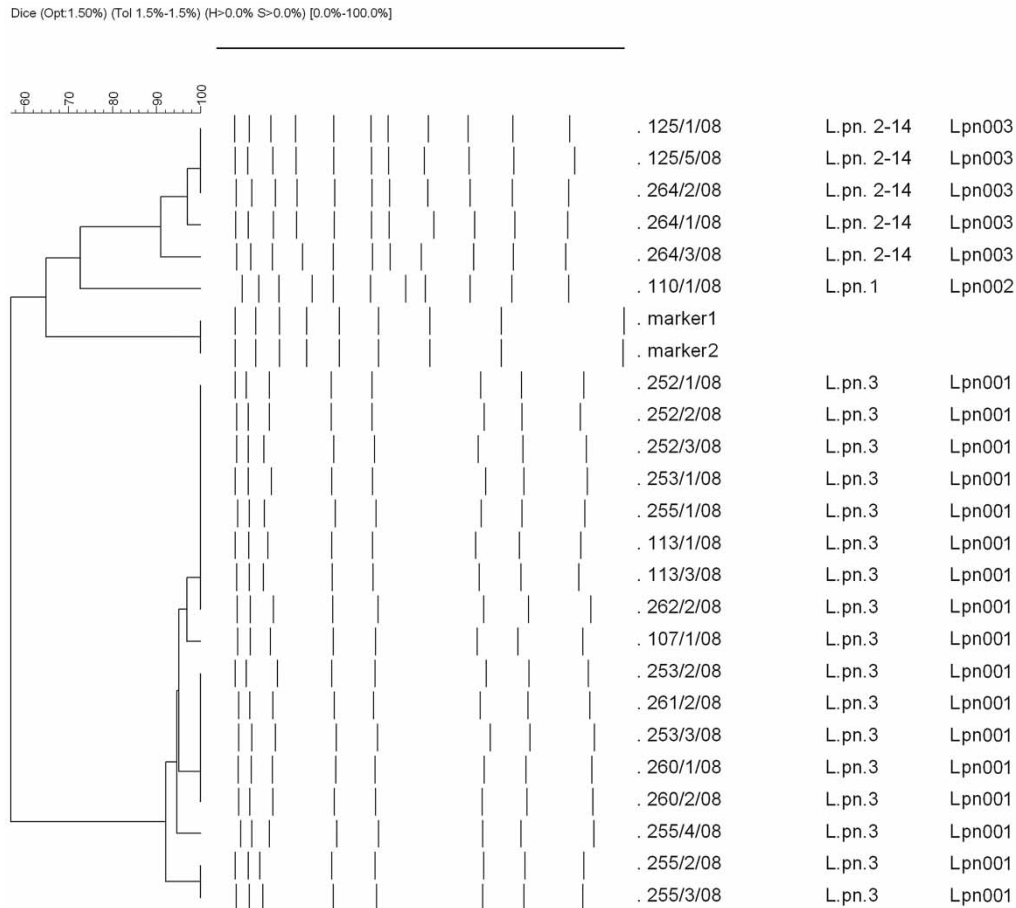
from tap 7. The single *L. pneumophila* sg. 1 isolate was isolated from tap 2; it had a unique ERIC profile.

Total genome restriction with *SfeI* and PFGE was performed on 16 selected strains. However, PFGE in this case provided lower resolution than the applied rep-PCR method, only the *L. pneumophila* sg. 1 isolate separated; the other strains formed a single cluster (data not shown).

## DISCUSSION

In a 2-year period, the only 4 weeks when there were no new cases of nosocomial *P. aeruginosa* infection in the ICU were when the filters were in place. Other patient care statistics, including the overall nosocomial infection rates were unchanged in the study period. This indicates that the use of the filters contributed to the prevention of infections.

*P. aeruginosa* strain typing also supported the hypothesis of water-related infections: the majority of the nosocomial clinical isolates (5/7) and the dominant waterborne strain were identical by all typing methods. Previous studies also indicated the presence of multiple genotypes on the taps of hospital wards, and the dominant type was persistent in time (Trautmann et al. 2006). Both clinical and water isolates belonging to the dominant group were resistant to aminoglycosides, and sensitive to other antibiotics. Amikacin is frequently the first choice in the treatment of *Pseudomonas* infections, and this practice might contribute to the prolonged nosocomial endemia in the ICU. Other water isolates were sensitive to all tested antibiotics. Multiresistant strains were not isolated from clinical or environmental samples. The investigation of non-symptomatic colonization could have revealed further diversity of the strains present in the ICU, but this was not the focus of the present study.



**Figure 5** | Dendrogram of the environmental legionellae strains based on their ERIC-PCR profiles. Similarity was calculated using FPQuest™ Software Version 4.5 (Bio-Rad, Richmond, California, USA) with Dice coefficient and UPGMA clustering.

*P. aeruginosa* was present in three of the four investigated wards. Previously, the hospital was not aware of the *P. aeruginosa* contamination of the water system. The observed colonization status unquestionably poses a health risk; distribution systems with much lower colonization rates (Trautmann et al. 2001) and minimal colony counts (Blanc et al. 2004) were linked to endemic *P. aeruginosa* infections or outbreaks in ICUs.

Legionnaires' disease was not diagnosed either during or outside the study period, though legionellae were present in the water distribution system, and the hot water temperature was in the optimal range for their proliferation. The detected *Legionella* concentration is above the limit of intervention defined for hospitals in most EU countries (Exner et al. 2005). In Hungary, there is currently no regulation for *Legionella* monitoring or risk assessment in hospitals

or other drinking water systems. However, though the hospital has never reported Legionnaires' disease cases, the positive water analysis data may raise awareness and facilitate the diagnosis of previously potentially overlooked infections (Stout et al. 2007).

Point-of-use filters were capable of the complete elimination bacteria, including both investigated pathogens. However, the positive results for *P. aeruginosa* on two taps with filters in Week 4 indicates that secondary (retrograde) contamination of the water outlets is also possible. The strain isolated from the filters was a unique type, not isolated on any other occasion, confirming the hypothesis of external contamination. Though filter housing material incorporates silver which acts as a bacteriostatic, it will not provide a foul-safe barrier to retrograde contamination (Vonberg et al. 2005). The source of the contamination was



not identified and there was no similarity to clinical isolates either.

In conclusion, point-of-use filters were found to be an effective method for the control of water-derived nosocomial *P. aeruginosa* infections. Cost-analysis comparing antibiotic expenditure and expenses of regular filter replacement also proved filters to be cost-efficient means of infection prevention. They also provide protection against other waterborne pathogens, including *Legionella*. This is an important additional benefit as in the absence of recognized infections, there are no targeted interventions against these organisms.

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