Examinations on the cultivated bacteria from the drinking water system of a healthcare building

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Abstract. The aim of our research was the identification of nosocomial pathogens found in the internal drinking water network of healthcare facilities which can cause infections in hospitals. In this study, the composition of bacterial communities from the internal water network of a health institution in Győr (Hungary) was examined using standard culture-based methods. Identification of the bacterial isolates was performed using Analytical Profile Index (API). Members of 13 bacterial taxa were recovered from a multi-storey healthcare institution. 10 species were identified via API20E and API20NE testing, out of which isolates of genera *Aeromonas*, *Pseudomonas* and *Sphingomonas* were found in the highest proportion on different media. These bacteria can cause nosocomial infections in clinical environments, leading to serious illnesses mainly in patients, as they may have multiresistance genes. In addition, *Legionella* species were also identified in the water samples, which are also known to be nosocomial pathogens, since they can be spread with aerosols in hospital environments and can cause severe respiratory diseases in immunocompromised individuals.

1 Introduction

Nosocomial infections occur in hospitals and healthcare services. These infections can be transmitted in many ways. The direct aerosol-transmission can happen during showering or bathing, with room humidifiers, from cooling towers and even while drinking. Contaminated objects like towels and bed linen can also spread the disease. In most cases the origin of the disease cannot be identified, however it can be reduced by following appropriate hygienic procedures [1].

National drinking water regulations must follow standards and guidelines in order to help the drinking water supply systems in surveying chemical and microbial contaminants. At the time of sampling, 201/2001 (X.25.) Government Decree on drinking water quality requirements and order of inspection was in force in Hungary. (The currently valid legislation is 5/2023 (I. 12.) Government decree on drinking water quality requirements and order of inspection.) The law regulates the chemical and microbiological parameters to be tested at

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consumer points, including the frequency of sampling, components to be tested, health limit values, and applicable standard methods (which are indicated in subsection 2.1 of this paper). There is no separate legislation in Hungary for the quality control of drinking water networks in healthcare institutions.

For microbial contaminants, total colony counts and colony count of coliforms, *Escherichia coli*, *Enterococci*, *Pseudomonas aeruginosa* and *Clostridium perfringens* have to be assessed by standard culture-based methods. In addition, the detection and enumeration of *Legionella* are also worth to be carried out in the water systems of hospitals and health services. Sometimes target bacteria cannot be detected in the water samples; meanwhile several nosocomial pathogens can hide in the background microbiota, which contaminates the water distribution systems.

With our research we would like to draw attention to the importance of the microbial condition of the internal drinking water system of a healthcare institution and its effects on the human health, as the bacterial colonies derived from the water system are able to carry multiresistance genes. Thus, the standard antibiotic therapy can be ineffective on the patients' health. The aims of this study are the following: (i) Gain information on the bacterial communities of internal drinking water system in a multi-storey healthcare institution, using standard culture-based methods. (ii) Identification of bacterial isolates, using analytical profile indices, based on biochemical tests. (iii) Characterization of antibiotic profiles of the presumably pathogenic isolates using disk diffusion method.

2 Material and methods

2.1 Sampling and cultivation

The samples for this research originated from the drinking water system of a healthcare institution in Győr, Hungary. The samples were taken in August 2020. Selection of sampling sites was based on the permission of hospital management. Sample 1 reflected the water arriving into the building from the main distribution system. This sample was collected after running the water for 5 minutes. Samples 2, 3 and 4 were collected from the tap in the bedpan-washing room of three different departments (Department 2, Department 3, Department 4). In these rooms water was used only by the staff and mainly for bedpan cleaning purpose. The bedpan-washing rooms are consistently among the most contaminated areas within the departments, making them highly likely locations for finding pathogens from previous nosocomial epidemics and/or potential sources of reinfection. It is worth noting that those responsible for washing the bedpans are often non-medically qualified staff, leading to insufficient emphasis on disinfection. The samples marked “A” (2A, 3A, 4A) were collected before running the water, while samples marked “B” (2B, 3B, 4B) were collected after running the water for 5 minutes. 500-500 ml water samples were collected in sterile bottles in each sampling point on one occasion. The samples were stored at 4-8 °C until processing within 24 hours.

To obtain bacterial isolates from the water samples, standard culture methods were used with different selective and non-selective culture media.

The total colony count gives important information on the microbial background contamination of the drinking water, however, the colony count does not give information on whether the samples contain human pathogens or not. To obtain the total colony count, non-selective Yeast-extract agar (Merck) was used. During the processing 1 ml of the water sample was added to the medium, and the agar plates were incubated at 22°C and 37°C for 48-72 hours (ISO 6222).

To confirm the presence of *Coliform* bacteria (members of *Enterobacteriaceae* family), Tergitol-7 agar and Chromogenic-coliform agar (Biolab) were used. Following the standard methods, 100 ml of water samples were filtered through 0.45µm gridded membrane (Merck-Millipore) filter onto the agar plates (ISO 9308-1), and incubated, following the standard. To confirm *Pseudomonads* the samples were membrane filtered onto Pseudomonas-CN-selective agar (Merck, ISO 16266), finally to confirm *Enterococci*, the samples were membrane filtered onto Slanetz-Enterococcus-selective agar plates (Biolab, ISO 7899-2), and incubated according to the standard protocols.

After incubation, different shaped and pigmented bacterium colonies were picked up from these selective media and transferred through Columbia agar plates. Columbia agar is a non-selective nutrient medium on which heterotrophic bacteria can easily grow. Based on our experience, it has been successful in cultivating species that do not thrive or grow very slowly on other nutrient media such as Yeast-extract agar. The isolates were incubated at 37°C for 24-48 hours and stored at 4-8°C for further investigations.

The isolates were identified using Analytical Profile Index (API) tests according to the manufacturer's instructions (Biomérieux, API20E REF 20 100 / 20 160; API20NE, REF 20 050).

The presence of *Legionella* bacteria was also tested with membrane filtration method using Legionella-selective GVPC agar (BioRad). 10 ml of samples were filtered through 0.45µm black, gridded membrane filter (Merck-Millipore) and incubated at 37°C for 7 days, following the standard method (ISO 11731-2). The presumably *Legionella* colonies were confirmed with cysteine auxotrophy on BCYE-biplate agar (BioRad).

2.2 Taxonomic identification of isolates using API tests

For taxonomic classification Analytical Profile Index (API) was used containing biochemical test strips and an online database [2]. Two different API tests were used in this research: API20E test (for identification of the members of *Enterobacteriaceae* and their closely related taxa with similar biochemical properties) was used to identify isolates derived from Tergitol-7 agar plates and Chromogenic-coliform agar plates, while the API20NE (for identification of Gram-negative non-fermentative rod-shaped bacteria) was used to identify isolates from Yeast-extract agar plates and Pseudomonas-CN-selective agar plates. We were considering using API20Strep tests in order to identify *Enterococci*, but colony form units on the Enterococcus selective agar plates were not detected in our water samples.

All the test strips contained 20 microtubes with dehydrated substrates, in which different bacterial metabolic reactions were indicated by colour changes. Following the user's guideline, the API20E strips were incubated at 37°C for 24 hours and the API20NE strips at 30°C for 24 hours. After incubation, the evaluation of test results was performed following the manufacturer's protocol. Based on the colour reactions, 7-digit biochemical profiles were uploaded to the online platform and our isolates were identified with the API-database [3]. The match between our data and the closest related taxon was shown in percentages by the software. Profile similarities above 80% resulted in successful identifications, while similarities below 80% were noted as uncertain or unsuccessful identifications.

2.3 Antibiotic resistance profiles of the isolates

Examination of isolates for antibiotic resistance was accomplished according to the EUCAST protocol [4]. In this research, variable antibiotics used to treat serious bacterial infections in hospitals (ciprofloxacin, trimethoprim-sulfamethoxazole (TMP-SMX), imipenem, meropenem, cefpodoxime) were selected. The antibiotic resistance profiles of the most frequent isolates with high level of API identification values were studied. The selected

isolates were incubated at 36°C for 24 hours on growth medium and 0.5 McFarland suspensions were made. Approximately 200 µl suspension was plated on the surface of Mueller-Hinton agar plates, and the antibiotic disks were placed on. These agar plates were incubated at 36°C for 24 hours. The results were evaluated using the EUCAST Clinical Breakpoint Tables [4].

3 Results and discussion

3.1 Total colony counts of the water samples

The first sampling point, where the water was coming directly from the main distribution system contained the smallest number of colony form units (3-10 cfu/ml). After that, the number of the bacterial colonies increased in the internal sampling points (Table 1). The highest colony counts were found in the warmest water samples (4A and 4B), where the temperature of the tap water was 20.5°C. Our results showed that the total colony count of heterotrophic bacteria after running the tap water were less in all sampling points than in the samples taken before running. The results indicated that heterotrophic bacteria (and presumably opportunistic pathogens as well) could find the possibilities in the internal drinking water distribution system for microbial growth.

Table 1. Results of culturing on Yeast-extract agar.

Sampling point		Number of colonies 37 °C/48 h [cfu/ml]	Number of colonies 22 °C/72 h [cfu/ml]	Water temperature [°C]
1	Main internal pipe	3	10	15.9
2A	Department 2	140	200	15.7
2B		80	50	15.7
3A	Department 3	10	35	16.2
3B		10	10	16.2
4A	Department 4	>1000	>1000	20.5
4B		250	250	20.5

3.2 Cultivation of legionella from the water samples

The presence of *Legionella* was confirmed in three samples (Table 2).

Table 2. Results of confirmed *Legionella* colony counts.

Sampling point		Number of confirmed *Legionella* cultures [cfu/10 ml]	Water temperature [°C]
1	Main internal pipe	5	15.9
2A	Department 2	130	15.7
2B		0	15.7
3A	Department 3	90	16.2
3B		0	16.2
4A	Department 4	0	20.5
4B		0	20.5

First was the main distribution sampling point (Sample 1), despite the fact that the water temperature (15.9°C) was not favourable for these species. In addition, an order of magnitude higher concentration were measured from the water before running the water at the sampling

points 2A and 3A, respectively. The presence of *Legionella* species, however, could not be retrieved in the same sampling points from the fresh tap water. Our results indicated that the incoming water system was infected with *Legionella*, and it could proliferate in the internal drinking water system of the building. Therefore, we assume that the health risk of using stagnant tap waters can be much higher than using fresh tap waters in the building.

Legionella survey for hospitals is important since out of the identified taxa these are the most likely to cause nosocomial infections mainly affecting airways and lungs of patients. The presence of *Legionella pneumophila* in water samples was investigated at the Tokyo Medical University Hospital [5]. The research was conducted between 2015 and 2018. Samples were collected regularly from both water networks and cooling towers and they were cultured periodically. A total of 1439 samples were obtained from wards, restrooms, waste rooms and fountains, out of which 19 tested positive for *L. pneumophila*. Even though they attempted to stop the spreading of Legionella in the water networks of the institution by chlorination and raising the temperature, the problem persisted for 3 years. Research in Poland also showed exceed count of *Legionella pneumophila* in the hot water supply system of hospitals [6, 7]. In Hungary, 20% of Legionnaires' disease cases are related to healthcare. In 2015, twenty-three hospitals were investigated and 90% of their warm water networks were colonised by *Legionella* [8]. The measured values were over the public health threshold. This might have occurred because of the low temperature of the hot water in use (45 °C), which was probably due to the unsuitable circulation. Another factor raising the risk can be the age of the building; however, the latest water networks can be colonized by pathogenic bacteria as well. Research in Tehran city identified *Legionella* in 38 cases (38%) out of 100 samples collected from toilet faucets and showers in eight hospitals [9].

3.3 Identifications of the isolates using API20E tests

Altogether 14 isolates were identified using API20E tests, and all of them were successfully identified at genus or species level (Table 3).

Table 3. Results of identification using API20E.

Isolate	Origin (sampling point, medium)	The closest related taxon	ID (%)
1 TF	Main internal pipe, Tergitol-agar	*Pasteurella pneumotropica*	91.2
1 TSS		*Aeromonas hydrophila*	95.5
2B TS	Department 2 Tergitol-agar	*Aeromonas hydrophila*	99.0
2B TSZ		*Chryseobacterium sp.*	55.5
2B TNZ		*Vibrionaceae sp.*	47.7
2B TSZZ		*Vibrionaceae sp.*	86.7
2B TBZ		*Aeromonas hydrophila*	98.4
2B CR	Department 2 Coliform-agar	*Aeromonas hydrophila*	95.7
2B CLS		*Ochrobactrum anthropi*	97.9
3A TNS	Department 3 Tergitol-agar	*Aeromonas hydrophila*	95.7
3A TKS		*Aeromonas hydrophila*	95.6
3B TZS		*Aeromonas hydrophila*	95.7
3B CR	Department 3 Coliform-agar	*Aeromonas hydrophila*	94.5
4B CNS	Department 4 Coliform-agar	*Pseudomonas fluorescens*	94.0

ID % marked the match between our data, and the closest related taxon was shown in percentages by the Apiweb© software. The identified isolates grouped in 6 different taxa, and *Aeromonas hydrophila* occurred in the largest proportion with 8 isolates. The other 6

isolates belonged to different taxa: *Ochrobactrum anthropi*, *Pasteurella pneumotropica* and *Pseudomonas fluorescens* were detected with single isolates, respectively, and three identifications resulted uncertain relations (one isolate to the genus *Chryseobacterium* and two isolates to the family *Vibrionaceae*).

Representatives of genus *Aeromonas* are capable of the colonization in the water distribution networks and the formation of biofilms, which make them hard to eliminate by chlorination or by heating up the water distribution system. They can be found in aquatic environments worldwide including drinking water and sewage water [10]. *Aeromonas hydrophila* is a water-borne bacterium, and widely distributed in the environment. This organism is a recognized as causative agent of diarrhoea and an opportunist pathogen in immunocompromised patients [11]. *Aeromonas hydrophila* is a significant pathogen to consider in nosocomial infections. A retrospective study was conducted at a tertiary university hospital with 1000 beds in Jeddah, Saudi Arabia. In total, 24 patients were found to have *Aeromonas hydrophila*-positive cultures between 2015 and 2022. Seventy-five percent of them had hospital-acquired infections, and the 30-day mortality rate was almost 21% [12]. *Aeromonas salmonicida* causes infections primarily on the skin of fish. As one of the most crucial primary pathogens in salmonids, it is responsible for substantial economic losses in the global aquaculture industry, particularly in salmonid farming [13]. It is rare in humans but it can get into the body by the consumption of contaminated food and cause diarrhoea [14]. Several recent studies have provided evidence on its zoonotic potential [15-17].

Pasteurella pneumotropica occurs in the oral cavities of many animals for example dogs and cats and for the bacterial microbiome on the surface of the pharynx, but bacteraemia in humans due to *P. pneumotropica* is extremely rare [18]. Most *Pasteurella pneumotropica* infections reported in humans typically involve the skin and soft tissues, often occurring after an animal bite, scratch, or lick on an open wound [19].

Ochrobactrum anthropi rarely infects human bodies, but due to its robust survival abilities, it has been known to cause nosocomial and opportunistic infections and might be quite common in immunocompromised hosts [20]. It can occur in the bloodstream of immunodeficient patients and can cause septicaemia [21].

3.4 Identifications of the isolates using API20NE tests

Using API20NE tests, 25 isolates were identified. The isolates grouped in 9 different taxa. 18 of them (78%) were successfully identified in species level (Table 4). From the isolates, *Pseudomonas stutzeri* and *Sphingomonas paucimobilis* were represented in the largest numbers (7 and 4 isolates, respectively). In addition, two isolates were identified as *Brevundimonas vesicularis*. *Aeromonas salmonicida*, *Pseudomonas fluorescens*, *Vibrio parahaemolitycus* and *Mannheimia haemolytica* were represented by single isolates, respectively.

Two *Pasteurella* isolates resulted low similarity rates, so they could be identified only at genus level. Another five other isolates resulted unidentifiable biochemical profile according to the API tests, since they did not belong to the non-fermentative, Gram-negative bacterial group.

Pseudomonas fluorescens can cause acute diseases in the human body. The most common origin of the infection is in the bloodstream, mainly affecting immunocompromised patients. *Pseudomonas fluorescens* spread from a contaminated drinking water dispenser in a bone marrow transplant unit was reported at a teaching hospital in the United Kingdom [22]. There was a sharp increase in the isolation of *P. fluorescens* from weekly pharyngeal surveillance swabs over a 1-month period. Nine out of 41 (22%) hematology inpatients were identified as being colonized with a meropenem-resistant strain of *P. fluorescens*. Outside of mammals,

this bacterium can survive in different environments, for example in soils, surface of plants, shower heads, walls [23].

Table 4. Results of identification using API20NE.

Isolate	Origin (sampling point, medium)	The closest related taxon	ID (%)
1 CF	Main internal pipe Coliform-agar	*Pseudomonas stutzeri*	89.7
1 PF	Main internal pipe Pseudomonas-agar	*Pseudomonas stutzeri*	89.7
1 PS		*Brevundimonas vesicularis*	99.5
1 PB		*Pseudomonas stutzeri*	83.4
2A CS	Department 2 Coliform-agar	*Pseudomonas stutzeri*	89.7
2A PS	Department 2 Pseudomonas-agar	*Sphingomonas paucimobilis*	88.8
2A PSZB		*Pseudomonas fluorescens*	99.9
2B PNR		*Pseudomonas stutzeri*	92.3
3A PN	Department 3 Pseudomonas-agar	*Vibrio parahaemolitycus*	83.9
3A PK		*Pseudomonas stutzeri*	89.7
3A PNS		*Pasteurella sp.*	73.3
3A PHR		*Pseudomonas stutzeri*	89.7
4A PS	Department 4 Pseudomonas-agar	*Pasteurella sp.*	73.3
4B PF		*Aeromonas hydrophila*	99.9
4B PS		unidentifiable profile	-
1 TSZ37 S	Main internal pipe Yeast-extract-agar	*Mannheimia haemolytica*	96.3
2A TSZ37 SF	Department 2 Yeast-extract-agar	*Brevundimonas vesicularis*	98.4
2A TSZ37 S		*Aeromonas salmonicida*	85.5
3A TSZ37 S	Department 3 Yeast-extract-agar	unidentifiable profile	-
3A TSZ22 S		*Sphingomonas paucimobilis*	98.2
3A TSZ22 HS		unidentifiable profile	-
4A TSZ22 S	Department 4 Yeast-extract-agar	*Sphingomonas paucimobilis*	99.9
4A TSZ37 S		unidentifiable profile	-
4B TSZ37 S		unidentifiable profile	-
4B TSZ22 S		*Sphingomonas paucimobilis*	99.9

Pseudomonas stutzeri is a denitrifying bacterium, which is widely prevalent in the environment; in addition, it has been isolated as an opportunistic human pathogen [24]. This bacterium had been isolated from hospital environments, occasionally [25]. The illness caused by the bacterium is dangerous to individuals in bad health, patients with serious underlying conditions or those, who had undergone surgery [26]. Reported cases of *P. stutzeri* infections are primarily sporadic, with very few outbreaks attributed to the contamination of intravenous fluids [27], water systems used for haemodialysis [28], or soap used to prepare skin for intravenous insertions [29]. Previously, *Sphingomonas paucimobilis* and *Brevundimonas vesicularis* belonged to the genus *Pseudomonas* [30, 31]. *Sphingomonas paucimobilis* was often isolated from hospital equipment and from water samples. Originally, it was not regarded as a pathogen, but since then it has been reported as the causative agent of different infections. While it may not be highly virulent or linked to severe life-threatening infections at present, its developing resistance patterns and broader range of infections must be taken seriously [32]. Like most bacteria, *Sphingomonas paucimobilis* and *Brevundimonas vesicularis* are dangerous to immunocompromised patients or to those with underlying health conditions [30].

Brevundimonas vesicularis can be found everywhere in the environment, however, it is rarely isolated as a pathogen. It has been reported in a study that the presence of

Brevundimonas vesicularis in a previously healthy individual was shown using API 20 NE test, and it caused pneumonia in the host [31]. Another case study reports peritonitis caused by *Brevundimonas vesicularis* in a patient on continuous cycling peritoneal dialysis [33].

Vibrio parahaemolyticus can be found in marine environments and it can infect the host in different ways. The disease caused by *V. parahaemolyticus* is linked to three main clinical manifestations: gastroenteritis, wound infections, and septicemia. Most commonly it gets into the stomach by the consumption of raw or improperly prepared sea food causing stomach- and intestine inflammation which can be accompanied by high fever. The contact of open wounds and salt water can lead to wound infections [34]. The United States reports about 45,000 cases of food-borne illness related to *V. Parahaemolyticus* infections annually. This presents a significant public health concern as the incidence continues to rise despite control measures. This trend is attributed to the influence of climate change on pathogen abundance and distribution [35]. In South Africa, during three months of sampling, 378 probable Vibrio isolates were recovered from hospital wastewater and community wastewater effluents. A total of 270 isolates belonging to the Vibrio genus were confirmed, including *V. parahaemolyticus* (9.1%), which were isolated from secondary hospital wastewater effluent [36].

In the case of our two isolates, the identification was not successful at species level, but that they belong to the *Pasteurella* genus. Members of the family *Pasteurellaceae* are Gram negative bacteria, some of which are important pathogens, as well as some being found in the bacterial communities of animal and human mucosa. *Mannheimia haemolytica* belongs to the latter category, which is responsible for the respiratory illnesses of cattle and other ruminants [37]. It can be found in the nasopharynx of dogs, cats, horses, and birds. It can cause a disease if it is present with other stress factors. It primarily affects animals but can occur in humans after contact with animal saliva, for example as a consequence of bites [38].

3.5 Distribution of the identified isolates

According to the API20E and API20NE results, altogether 34 isolates were identified, and they grouped in 13 taxa (Figure 1). From those taxa, aeromonads, pseudomonads and sphingomonads were the most common. They appeared in the stagnant tap water and in the running fresh water of the internal sampling points (Table 2, Table 3). So we assume that aeromonads, pseudomonads and sphingomonads may colonize the whole drinking water distribution system of the building.

We revealed that aeromonads and pseudomonads may have multiresistance genes, and presumable, that they are able to transfer those to another species in contact points like in taps, in bath rooms or in shower rooms. Although, *Sphingomonas paucimobilis* isolates were susceptible to all antibiotics (see Section 3.6), other studies described that this bacterium is a causative microbial agent of healthcare associated infections, since it can cause primary bacteremia, intravascular catheter infections, urinary tract infection and pneumonia [39, 40].

In the study of Spinks et al. [41] thermal inactivation analyses were carried out on members of *Enterobacteriaceae*, pseudomonads, aeromonads and *Enterococci*. Their results suggested that the water temperature range from 55 to 65°C was critical for effective elimination, so we can suppose that most opportunistic pathogens we revealed may also be inactivated at that high temperature, so heating the water in the networks may make unviable of harmful microorganisms. Adding chlorine and applying filters can also be effective. In addition, many bacteria, including *Legionella*, which were also revealed, form biofilms in stagnant water system. This can be avoided by the continuous use of water. Finally, replacing the taps can also help the institution to remove many presumably nosocomial pathogens we described in this study.

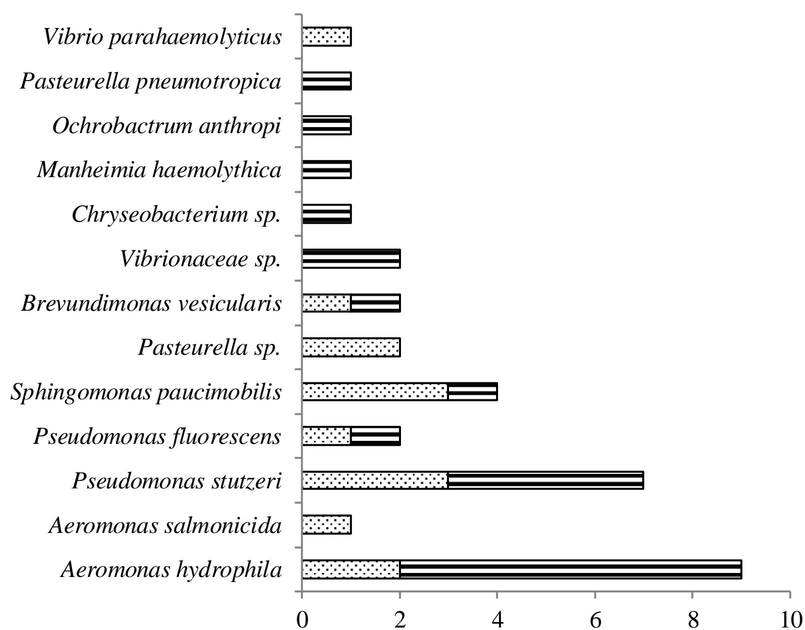

Fig. 1. Distribution of the identified taxa derived from the stagnant tap water samples (dotted) and the running fresh tap water samples (striped).

3.6 Antibiotic resistance of isolates

The antibiotic resistance profiles of 14 isolates were tested (Table 5-7). These isolates were members of the genera *Pseudomonas*, *Aeromonas* and *Sphingomonas*, and they were found in all 3 sampled departments of the healthcare building, indicating, that they widely colonized the drinking water supply system. From these isolates we found variable antibiotic resistant phenotypes for quinolones, carbapenems and cephalosporins, which could be associated with nosocomial infections. Our results showed that 2 ESBL (extended spectrum beta-lactamase enzyme-producing) and AmpC-beta-lactamase-producing *Pseudomonas fluorescens* isolates were found in 2 sampling points (Table 5).

Table 5. Antibiotic resistance characteristics of *Pseudomonas* isolates (P.stu: *P. stutzeri*, P.flour: *P. fluorescens*, R: resistant, S: susceptible).

Antibiotic disc test	**P.stu (1 CF)**	**P.stu (2A CS)**	**P.fluor (2A PSZB)**	**P.stu (3A PK)**	**P.fluor (4B CNS)**
Ciprofloxacin 5μg	S	S	R	S	S
Imipenem 10 μg	S	S	R	S	S
Meropenem 10 μg	S	S	R	S	S
Cefpodoxime 10 μg	S	S	R	S	R
Cefpodoxime 10 μg + Clavulanic acid	S	S	R	S	R
Cefpodoxime 10 μg + Cloxacillin	S	S	R	S	R
Cefpodoxime 10 μg + Clavulanic acid + Cloxacillin	S	S	R	S	R

The “2A PSZB” showed multiresistance for quinolones, carbapenems and cephalosporins, while, the “4B CNS” isolate showed resistance to cephalosporins, however it was susceptible

to quinolones and carbapenems. We had three *P. stutzeri* isolates from 3 different sampling points, which were susceptible to these antibiotics. Presumably, *P. fluorescens* isolates from the drinking water distribution system were capable of transferring multiresistance genes from other species in the hospital environment and producing antibiotic resistance phenotypes.

Among the aeromonads, isolate “4B PF” showed antibiotic resistance for quinolones and cephalosporins, and produced AmpC beta-lactamase, while other *Aeromonas* isolates were susceptible to all antibiotics (Table 6).

Table 6. Antibiotic resistance characteristics of *Aeromonas* isolates (A.hyd: *Aeromonas hydrophila*, R: resistant, S: susceptible).

Antibiotic disc test	**A.hyd (1 TSS)**	**A.hyd (2B TS)**	**A.hyd (3A TNS)**	**A.hyd (3B TZS)**	**A.hyd (4B PF)**
Ciprofloxacin 5 µg	S	S	S	S	R
TMP-SMX 1.25-23.75 µg	S	S	S	S	S
Cefpodoxime 10 µg	S	S	S	S	R
Cefpodoxime 10 µg + Clavulanic acid	S	S	S	S	R
Cefpodoxime 10 µg + Cloxacillin	S	S	S	S	S
Cefpodoxime 10 µg + Clavulanic acid + Cloxacillin	S	S	S	S	S

Each *Sphingomonas paucimobilis* isolate was susceptible to all antibiotics (Table 7). According to the results, *S. paucimobilis* might be a low-virulence bacterium, and most antibiotic therapies can be effective as it did not seem to transfer antibiotic-resistant genes, in our case.

Table 7. Antibiotic resistance characteristics of *Sphingomonas* isolates (S.pau: *S. paucimobilis*, R: resistant, S: susceptible).

Antibiotic disc test	**S.pau (2A PS)**	**S.pau (3A TSZ22S)**	**S.pau (4A TSZ22S)**	**S.pau (4B TSZ22S)**
Ciprofloxacin 5 µg	S	S	S	S
Imipenem 10 µg	S	S	S	S
TMP-SMX 1.25-23.75 µg	S	S	S	S
Cefpodoxime 10 µg	S	S	S	S
Cefpodoxime 10 µg + Clavulanic acid	S	S	S	S
Cefpodoxime 10 µg + Cloxacillin	S	S	S	S
Cefpodoxime 10 µg + Clavulanic acid + Cloxacillin	S	S	S	S

4 Conclusions

The most important implications of our work are the followings. Even if target bacteria (*Enterobacteriaceae*, *P. aeruginosa*, *Enterococci*) which indicate water quality change were not present in the water sample, the background microbiota may contain human pathogens that may lead to health problems, when multiplicate in the drinking water supply system. Stagnant water means significant danger due to the possibility of biofilm formation, which serves as primary source of pollution. Therefore, it is worth to check the internal drinking water supply systems using additional methods eg. API tests, antibiotic tests, where the

background microbiota is regularly detected in high amount by the conventional standard and routine methods.

13 different taxa were identified via API20E and API20NE testing, out of which *Aeromonas hydrophila* and *Pseudomonas fluorescens* were described as multidrug-resistant bacteria, as they showed resistance to several antibiotics, which are used in hospitals. Both *A. hydrophila* and *P. fluorescens* may cause nosocomial infections in the hospital environment, leading to serious illnesses with inappropriate use of antibiotics.

Members of the genus *Legionella* were also identified in the water samples. These can spread with aerosols in hospital environments and can cause severe respiratory diseases in weak individuals.

The bacterial colonization of the drinking water could be reduced using chlorination and heating up the water up to 65°C, in order to eliminate germs in the distribution system. In addition, removal of the infected taps and install new ones can reduce the bacterial contamination of the tap water. On the other hand, water temperature above 65°C would only be usable in departments where patients can control the risk of scalding themselves. This excludes children's and psychiatric departments, as well as other wards due to the presence of elderly individuals, including those with dementia. For this reason, hot water is rather not used anywhere. Heating water, which is serve as cold water is also not feasible. Continuous disinfection is impractical due to cost and potential biofilm issues.

While it would be feasible to pinpoint which department is affected, continuous control and checking of the polluted areas is impossible due to the poor financial situation of the health sector. Endpoint filters present a potentially effective and reasonable solution, albeit an expensive one that requires maintenance for complete effectiveness. If costs are a concern for the institution, critical departments such as anaesthesiology and intensive care or haematology have to be prioritized for implementation.

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