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ORIGINAL ARTICLE

Co-existence of *Legionella* and other Gram-negative bacteria in potable water from various rural and urban sources

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Abstract

A total of 320 potable water samples were collected from various rural and urban sources located in the Lublin region of eastern Poland. They comprised: 55 samples of treated (chlorinated) tap water from rural dwellings distributed by the municipal water supply system (MWSS), 111 samples of treated tap water from urban dwellings distributed by the MWSS, 45 samples of untreated well water from household wells and 109 samples from private water supply systems (PWSS) distributing untreated well water. Water samples were examined for the presence and species composition of *Legionella*, *Yersinia*, Gram-negative bacteria belonging to family Enterobacteriaceae (GNB-E) and Gram-negative bacteria not belonging to the family Enterobacteriaceae (GNB-NE), by filtering through cellulose filters and culture on respectively GVPC, CIN, EMB and tryptic soya agar media. The occurrence of *Legionella* in the samples taken from the outlets of the urban MWSS was high (77.5%), and significantly greater compared to frequencies noted in rural MWSS (7.3%), and samples of well water from household wells (28.9%) and PWSS (13.8%) ($p<0.001$). Strains *L. pneumophila* serogroups 2-14, *L. pneumophila* serogroup 1 and *Legionella* spp. (species other than *L. pneumophila*) formed respectively 64.3%, 17.5%, and 18.2% of total isolates from urban MWSS, 100%, 0, and 0 of those from rural MWSS, 69.2%, 7.7%, and 23.1% of those from household wells, and 66.7%, 0, and 33.3% of those from PWSS. The concentration of *Legionella* strains in the positive samples from urban MWSS exceeded the threshold limit value of 100 cfu/100 ml in 86.1%, while in the other sources this value was not exceeded. No *Yersinia* strains were isolated from the examined water samples. Altogether 8 species or genera of Gram-negative bacteria belonging to Enterobacteriaceae family (GNB-E) and 10 species or genera of Gram-negative bacteria not belonging to the Enterobacteriaceae family (GNB-NE) were found in the examined samples. In the MWSS samples, an inverse relationship was found between *Legionella* and GNB-E and the numbers of *Enterobacter* spp. and *Serratia* spp. strains were significantly more common in the samples without *Legionella*. By contrast, in the PWSS samples, the numbers of *Enterobacter* spp., *Klebsiella* spp. and *Salmonella* spp. were distinctly and significantly greater ($p<0.01$-$p<0.001$) in the samples containing *Legionella*. Among GNB-NE, *Pseudomonas aeruginosa* strains occurred significantly more frequently in samples containing *Legionella* (for MWSS and well water separately $p<0.05$, for total samples $p<0.001$). Similarly, strains of *Flavobacterium breve* and *Xanthomonas* spp. occurred significantly more often in the samples with *Legionella*, while the numbers of *Aeromonas* spp. and *Vibrio* spp. strains were significantly greater in the samples not containing *Legionella*. In conclusion, a health risk could be associated with exposure to the water from urban MWSS because of the high prevalence and concentration of *Legionella*, and with exposure to well water from PWSS because of the correlation of occurrence of *Legionella* and potentially pathogenic Enterobacteriaceae strains, and the possibility of synergistic effects. The adverse effects could be also due to the significant correlation of *Legionella* and *Pseudomonas aeruginosa* that occured in water from various sources.

Key words

Gram-negative bacteria, *Legionella*, Enterobacteriaceae, Non-Enterobacteriaceae, correlation, potable water, farms, wells, water supply systems

INTRODUCTION

Legionella pneumophila and related species are fastidious Gram-negative bacteria, developing mostly in natural and anthropogenic aquatic environments, which in humans may cause atypical pneumonia (legionellosis, legionnaires' disease) or flu-like illness [1-3]. People become infected with *Legionella* mostly by the inhalation of bacteria-laden aerosol droplets, less often by the oral route through drinking water and through traumatized skin or mucous membranes [3]. According to Stout *et al.* [4] potable water supplies that harbour *Legionella pneumophila* are an important source of community-acquired legionnaires' disease.

The growth of *Legionella* in water depends on interaction with the other microorganisms such as protozoa, heterotrophic bacteria, green algae and cyanobacteria living in the same environment [5]. The most important relationships are: intracellular growth of *Legionella* in free-living amoebae and its growth and/or survival in biofilm matrices used as a shelter and source of nutrients [6]. A number of other microorganisms in biofilm, including heterotrophic bacteria, may promote the growth of *Legionella* by excreting extracellular compounds as carbon and energy sources [1, 2, 5, 7, 8]. Besides stimulatory effects, in the cases of some bacteria, inhibitory effects are also possible [5].

The aim of the present study, designed as a continuation of earlier research [3, 9], was to determine the degree of contamination with *Legionella* of potable water from various rural and urban sources, and to assess relationships between the presence of *Legionella* and other heterotrophic Gram-negative bacteria belonging and not belonging to the family Enterobacteriaceae.

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MATERIALS AND METHODS

Samples of water. A total of 320 samples of water used as a potable water for consumption were taken in the years 2007-2010 during summer months (June-August) from various places located in the Lublin province of eastern Poland. Water samples were taken into sterile glass bottles with a volume of 700 ml at the following sites:

- 45 samples of well water were taken directly (with use of a pail) from 45 private household wells located on farms in 6 villages.
- 109 samples of well water were collected from taps of 109 private water supply systems (PWSS) conducting untreated and unheated water from household wells to outlets within farm buildings located in 11 villages.
- 55 samples were taken on 55 farms in 3 villages from cold-water taps of the municipal water supply system (MWSS) distributing treated (chlorinated) groundwater, pumped from a depth of 40-100 m. The taps were equipped with aerators or other endings for better outflow of water.
- 111 samples were collected in the years 2007-2010, during the period May-November, in the city of Lublin (eastern Poland), from hot-water taps of the municipal water supply system (MWSS) distributing treated (chlorinated) groundwater, pumped from a depth of 40-100 m. The taps were equipped with aerators or other endings for better outflow of water.

Processing of samples. Water samples were examined for the presence of the following Gram-negative bacteria (GNB): (a) *Legionella*; (b) *Yersinia*; (c) non-fastidious Gram-negative bacteria belonging to the Enterobacteriaceae family (GNB-E); (d) non-fastidious Gram-negative bacteria not belonging to the Enterobacteriaceae family (GNB-NE). For the recovery of *Legionella*, water samples of 300 ml volume were filtered through cellulose filters (pores 0.45 μm, Millipore Corporation, Billerica, MA, USA). Filters were washed for 10 min in acid buffer (pH 2.2), then rinsed in Ringer solution (Merck, Darmstadt, Germany) and finally placed on isolation agar medium. For recovery of *Yersinia*, GNB-E, and GNB-NE, water samples of 100 ml volume each were filtered through cellulose filters (pores 0.45 μm, Millipore, USA), and finally placed on the appropriate isolation agar medium.

Isolation and identification of *Legionella* strains. The buffered charcoal yeast extract (BCYE) agar medium supplemented with the Growth Supplement SR 110 A and the Selective GVPC Supplement SR 152 E (Oxoid, Basingstoke, Hampshire, England) [10-13] was used for isolation of *Legionella* (further referred to as GVPC medium). Inoculated agar plates were incubated for 7 days at 37°C with an everyday check of growth. Colonies of Gram-negative bacteria grown after 4-7 days were isolated and examined for ability to grow on media with and without cysteine. Strains unable to grow on media without cysteine were considered as suspected *Legionella* strains. The isolates were determined to the species and serogroup level with the use of the Legionella Latex Test Kit (Oxoid, Basingstoke, Hampshire, England) which enables, on the basis of microagglutination with latex particles sensitised with specific rabbit antibodies, a separate identification of *Legionella pneumophila* serogroup 1, *Legionella pneumophila* serogroups 2-14, and *Legionella* spp. (a complex group including: *L. longbeache* serogroups 1 and 2, *L. bozemanii* serogroups 1 and 2, *L. dumoffii, L. gormanii, L. jordanis, L. micdadei* and *L. anisa*) [11]. Only isolates giving positive reaction in the latex test were considered as strains of *Legionella*.

Isolation and identification of *Yersinia* strains. The *Yersinia* selective CIN agar medium (Cefsulodin Irgasan® Novobiocin agar) with mannitol [10] (Merck, Darmstadt, Germany) was used for isolation of *Yersinia*. Inoculated agar plates were incubated for 24 hrs at 37°C. Isolates suspected to be *Yersinia* were identified with the microtest API Systems 20E (bioMérieux, Marcy l'Etoile, France).

Isolation and identification of GNB-E. The eosin methylene blue (EMB) agar (Merck, Darmstadt, Germany) was used for isolation of bacteria of the Enterobacteriaceae family. Inoculated agar plates were incubated for 24 hrs at 37°C. The grown colonies were counted and differentiated and the isolates identified to species or genus level with the microtest API Systems 20E (bioMérieux, Marcy l'Etoile, France).

Isolation and identification of GNB-NE. The tryptic soya agar (bioMérieux, Marcy l'Etoile, France) was used for isolation of bacteria not belonging to the Enterobacteriaceae family. Inoculated agar plates were incubated for 24 hrs at 37°C. The grown colonies were counted and differentiated and the isolates identified to species or genus level with the microtest API Systems NE (bioMérieux, Marcy l'Etoile, France).

Statistical analysis. The data were analysed by Student's t-test. The value $p<0.05$ was considered significant.

RESULTS

Prevalence and concentration of *Legionella* in potable water from various sources. The occurrence of *Legionella* in the samples taken from the outlets of the urban municipal water supply system (MWSS) was high (77.5%) and significantly greater compared to the occurrence noted in the rural MWSS, and in the samples of well water from household wells and from the private water supply system (PWSS) ($p<0.001$) (Tab. 1). In total samples, the most common were isolates belonging to the *Legionella pneumophila* serogroups 2-14, found in 81 samples (73.0%) from urban MWSS, in 4 samples (7.3%) from rural MWSS, in 9 samples (20.0%) from household wells, and in 10 samples (9.2%) from the PWSS distributing well water. Isolates belonging to *Legionella pneumophila* serogroup 1 were found in 22 samples (19.8%) from urban MWSS and in one sample (2.2%) from household wells. Strains classified as *Legionella* spp. (species other than *L. pneumophila*) were found in 23 (20.7%) samples from urban MWSS, in 3 samples (6.7%) from household wells and in 5 samples (4.6%) from PWSS. With regard to only positive samples, strains *L. pneumophila* of serogroups 2-14, *L. pneumophila* serogroup 1 and *Legionella* spp. formed respectively 64.3%, 17.5%, and 18.2% of total isolates from urban MWSS, 100%, 0, and 0 of those from rural MWSS, 69.2%, 7.7%, and 23.1% of those from household wells, and 66.7%, 0, and 33.3% of those from PWSS (Tab. 1).

Table 1. Occurrence of *Legionella* in various sources of potable water

		Positive samples (number, percent of total samples)						
Legionella group Source of potable water		*L .pneum.* 1	*L .pneum.* 2-14	*L .pneum.* 1 + + *L .pneum.* 2-14	*Leg.* spp.	*L .pneum.* 2-14 + + *Leg.* spp.	*L .pneum.* 1 + + *L .pneum.* 2-14 + + *Leg.* spp.	Total positive
Municipal water supply systems								
Urban	N=111	0	52 (46.9%)	11 (9.9%)	5 (4.5%)	7 (6.3%)	11 (9.9%)	86 (77.5%)[a]
Rural	N=55	0	4 (7.3%)	0	0	0	0	4 (7.3%)[b]
Well water								
Household wells	N=45	1 (2.2%)	9 (20.0%)	0	3 (6.7%)	0	0	13 (28.9%)[b]
Private supply systems	N=109	0	10 (9.2%)	0	5 (4.6%)	0	0	15 (13.8%)[b]
Total	N=320	1 (0.3%)	75 (23.4%)	11 (3.5%)	13 (4.0%)	7 (2.2%)	11 (3.5%)	118 (36.9%)

Explanation: *L .pneum.* 1: samples containing only *Legionella pneumophila* serogroup 1; *L .pneum.* 2-14: samples containing only *Legionella pneumophila* serogroups 2-14; *L .pneum.* 1 + *L .pneum.* 2-14: samples containing *L. pneumophila* serogroup 1 and *L. pneumophila* serogroups 2-14; *Leg.* spp.: samples containing only *Legionella* species other than *L. pneumophila*; *L .pneum.* 2-14 + *Leg.* spp.: samples containing *L. pneumophila* serogroups 2-14 and *Legionella* species other than *L. pneumophila*; *L .pneum.* 1 + *L .pneum.* 2-14 + *Leg.* spp.: samples containing *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14 and *Legionella* species other than *L. pneumophila*.

[a] Out of 86 positive samples, the concentration of *Legionella* in the water was below 100 cfu/100 ml in 12 (13.9%), 100-200 cfu/100 ml in 45 (52.4%), 200-300 cfu/100 ml in 18 (20.9%), and above 300 cfu/100 ml in 11 (12.8%).

[b] the concentration of *Legionella* in the water was in all the samples below 100 cfu/100 ml.

Regarding the concentration of *Legionella* strains in water, the most adverse situation was noted in urban MWSS, where in only 13.9% of positive samples it was below the threshold limit value of 100 cfu/100 ml [14], while in 52.4% it was within the range 100-200 cfu/100 ml, and in 33.7% of samples it was above 200 cfu/100 ml. By contrast, in all samples of water taken from rural MWSS, household wells and PWSS, the concentration of *Legionella* was below 100 cfu/100 ml (Tab 1).

Correlation of *Legionella* with other Gram-negative species. No *Yersinia* strains were isolated from the examined water samples. Altogether, 8 species or genera of Gram-negative bacteria belonging to the Enterobacteriaceae family (GNB-E) were identified. *Enterobacter* spp., *Klebsiella* spp. and *Serratia* spp. were isolated most often (Tab. 2). The numbers of GNB-E isolates from well water (household wells, PWSS) were significantly greater compared to isolates from MWSS (101 *vs.* 24 strains, p<0.001). No GNB-E strains were isolated from urban MWSS (Tab. 2). Interesting relationships between the prevalence of *Legionella* and GNB-E were found. While in MWSS samples an inverse relationship was found between these 2 groups and the numbers of *Enterobacter* spp. and *Serratia* spp. strains were significantly more common in the samples without *Legionella*, in the PWSS samples the numbers of *Enterobacter* spp., *Klebsiella* spp. and *Salmonella* spp. were distinctly and significantly greater (p<0.01-p<0.001) in the samples containing *Legionella* (Tab. 2).

Altogether, 10 species or genera of Gram-negative bacteria not belonging to the Enterobacteriaceae family (GNB-NE) were identified. *Pseudomonas* spp., *Aeromonas* spp., *Acinetobacter* spp., and *Chryseomonas* spp. were isolated most

Table 2. **C**o-existence of *Legionella* with Gram-negative bacteria belonging to Enterobacteriaceae family in various sources of potable water

Source	Municipal water supply systems						Well water						Total water	
Species	Urban		Rural		Total		Household wells		Private supply		Total		sources	
	Leg + N=86	Leg (-) N=25	Leg + N=4	Leg (-) N=51	Leg + N=90	Leg (-) N=76	Leg + N=13	Leg (-) N=32	Leg + N=15	Leg (-) N=94	Leg + N=28	Leg (-) N=126	Leg + N=118	Leg (-) N=202
Citrobacter spp.	0	0	1 (25.0%)	5 (9.8%)	1 (1.1%)	5 (6.6%)	1 (7.7%)	2 (6.3%)	0	0	1 (3.6%)	2 (1.6%)	2 (1.7%)	7 (3.5%)
Escherichia coli	0	0	0	0	0	0	0	4 (12.5%)	0	3 (3.2%)	0	7 (5.6%)	0	7 (3.5%)*
Enterobacter spp.	0	0	0	8 (15.7%)	0	8 (10.5%)**	1 (7.7%)	10 (31.3%)	9 (60.0%)+++	13 (13.8%)	10 (35.7%)+	23 (18.3%)	10 (8.5%)	31 (15.3%)
Hafnia spp.	0	0	0	0	0	0	0	0	0	1 (1.1%)	0	1 (0.8%)	0	1 (0.5%)
Klebsiella spp.	0	0	0	0	0	0	3 (23.1%)	11 (34.4%)	7 (46.7%)++	15 (16.0%)	10 (35.7%)	26 (20.6%)	10 (15.2%)	26 (12.9%)
Pantoea spp.	0	0	0	2 (3.9%)	0	2 (2.6%)	0	1 (3.1%)	1 (6.7%)	3 (3.2%)	1 (3.6%)	4 (3.2%)	1 (0.8%)	6 (2.9%)
Salmonella spp.	0	0	0	0	0	0	0	2 (6.3%)	2 (13.3%)+++	0	2 (7.1%)	2 (1.6%)	2 (1.7%)	2 (1.0%)
Serratia spp.	0	0	1 (25.0%)	7 (13.7%)	1 (1.1%)	7 (9.2%)*	0	0	5 (33.3%)	6 (6.4%)	5 (17.9%)+	7 (5.6%)	6 (5.1%)	14 (6.9%)

Explanation: Leg + = *Legionella* present; Leg (-) = *Legionella* absent; N=number of samples; in individual fields numbers of positive samples and frequencies of occurrence are given; *-** prevalence of bacterial species significantly greater in the samples without *Legionella*, *p<0.05, **p<0.01; +-+++ prevalence of bacterial species significantly greater in the samples with *Legionella*, +p<0.05, ++p<0.01, +++p<0.001.

Table 3. Co-existence of *Legionella* with Gram-negative bacteria not belonging to Enterobacteriaceae family in various sources of potable water

Source	Municipal water supply systems						Well water						Total water	
Species	Urban		Rural		Total		Household wells		Private supply		Total		sources	
	Leg + N=86	Leg (-) N=25	Leg + N=4	Leg (-) N=51	Leg + N=90	Leg (-) N=76	Leg + N=13	Leg (-) N=32	Leg + N=15	Leg (-) N=94	Leg + N=28	Leg (-) N=126	Leg + N=118	Leg (-) N=202
Acinetobacter spp.	4 (4.7%)	4 (16.0%)	3 (75.0%)+++	4 (7.8%)	7 (7.8%)	8 (10.5%)	3 (23.1%)	12 (37.5%)	0	5 (5.3%)	3 (10.7%	17 (13.5%)	10 (8.5%)	25 (12.3%)
Aeromonas spp.	4 (4.7%)	0	1 (25.0%)	13 (25.5%)	5 (5.6%)	13 (17.1%)*	0	1 (3.1%)	5 (33.3%)	26 (27.7%)	5 (17.9%)	27 (21.4%)	10 (8.5%)	40 (19.8%)**
Chryseomonas spp.	10 (11.6%)	2 (8.0%)	0	1 (2.0%)	10 (11.1%)	3 (3.9%)	1 (7.7%)	2 (6.3%)	2 (13.3%)	10 (10.6%)	3 (10.7%	12 (9.5%)	13 (11.0%)	15 (7.4%)
Flavimonas spp.	5 (5.8%)	0	0	0	5 (5.6%)	0	0	0	0	4 (4.3%)	0	4 (3.2%)	5 (4.2%)	4 (1.9%)
Flavobacterium breve	12 (13.9%)	4 (16.0%)	1 (25.0%)	13 (25.5%)	13 (14.4%)	7 (9.2%)	0	0	1 (6.7%)	4 (4.3%)	1 (3.6%)	4 (3.2%)	14 (11.9%)+	11 (5.3%)
Pseudomonas aeruginosa	17 (19.7%)	4 (16.0%)	0	1 (2.0%)	17 (18.9%)+	5 (6.6%)	0	1 (3.1%)	1 (6.7%)+	0	1 (3.6%)	1 (0.8%)	18 (15.2%)+++	6 (3.0%)
Pseudomonas spp.	34 (40.0%)	11 (44.0%)	3 (75.0%)	17 (33.3%)	37 (41.1%)	28 (36.8%)	1 (7.7%)	12 (37.5%)	7 (46.7%)	27 (28.7%)	8 (28.6%)	39 (30.9%)	45 (38.1%)	67 (33.7%)
Stenotrophomonas spp.	0	0	0	0	0	0	1 (7.7%)	1 (3.1%)	0	1 (1.1%)	1 (3.6%)	2 (1.6%)	1 (0.8%)	2 (1.0%)
Vibrio spp.	0	0	0	0	0	0	1 (7.7%)	1 (3.1%)	2 (13.3%)	18 (19.1%)	3 (10.7%	19 (15.1%)	3 (2.5%)	19 (9.4%)*
Xanthomonas spp.	8 (9.3%)	1 (4.0%)	0	2 (3.9%)	8 (8.9%)	3 (3.9%)	0	0	0	0	0	0	8 (6.8%)+	3 (1.5%)

Explanation: Leg + = *Legionella* present; Leg (-) = *Legionella* absent; N=number of samples; in individual fields numbers of positive samples and frequencies of occurrence are given; *-** prevalence of bacterial species significantly greater in the samples without *Legionella*, *p<0.05, **p<0.01; +-+++ prevalence of bacterial species significantly greater in the samples with *Legionella*, +p<0.05, ++p<0.01, +++p<0.001.

often (Tab. 3). There was no significant difference between the numbers of GNB-NE strains isolated from well water and from MWSS (150 *vs.* 156 strains, p>0.05). *Pseudomonas aeruginosa* strains occurred significantly more frequently in samples containing *Legionella* (for MWSS and well water separately p<0.05, for total samples p<0.001) (Tab. 3). Similarly, strains of *Flavobacterium breve* and *Xanthomonas* spp. occurred significantly more often in the samples with *Legionella* (p<0.05), while the numbers of *Aeromonas* spp. and *Vibrio* spp. strains were significantly greater in the samples not containing *Legionella* (p<0.05) (Tab. 3).

According to the Polish regulations [14], 7 samples (2.2% of the total) did not meet hygienic standards because of the presence of *Escherichia coli,* and 24 samples (7.5%) because of the presence of *Pseudomonas aeruginosa*.

DISCUSSION

The prevalence and concentration of *Legionella* in the outlets of the urban municipal water supply system (MWSS) distributing hot, chlorinated water, was significantly greater compared to the examined rural water sources. The levels of *Legionella* exceeded the Polish threshold limit value of 100 cfu/100 ml in 66.7% of total samples (86.1% positive) [14]. The risk is enhanced by the fact that in 19.8% of the samples there occurred the most virulent strains of *Legionella pneumophila* serogroup 1. The presented results represent a greater hazard compared to the samples of tap water from urban MWSS examined in our earlier work [3], which showed a *Legionella* prevalence lower by over 25%, and did not contain the strains of *Legionella pneumophila* serogroup 1. It is possible that the presence of aerators or other endings in the taps or other outlets was more common in this study and thus favoured proliferation of legionellae.

The prevalence of *Legionella* in the outlets of the rural MWSS recorded in the present study was very low, which could be partly explained by the fact that the system distributed only cold water. It was significantly lower compared to our earlier work, while the prevalence of *Legionella* in well water from household wells and private water supply systems (PWSS) was similar [3].

Yersinia, the other fastidious Gram-negative bacterium transmitted by water, was not found in the present study in the well and MWSS water samples, in contrast to earlier Polish papers reporting the isolation of *Yersinia* strains from well water in a rural environment [11, 15].

The prevalence of Gram-negative bacteria belonging to the family Enterobacteriaceae (GNB-E) in well water samples was high and similar to that of Gram-negative bacteria not belonging to the family Enterobacteriaceae (GNB-NE). By contrast, in water samples from the outlets of urban MWSS, the GNB-E were absent, and in the samples from rural MWSS occurred with a low frequency. Most probably this it was due to the great vulnerability to chlorination, and imperfect adaptation to persist and proliferate in specific ecological niches of MWSS, such as aerated taps.

It is noteworthy that the prevalence of some potentially pathogenic GNB-E strains (*Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp.) was especially high in well water from a private water supply system (PWSS) and showed a highly significant correlation with *Legionella*. This phenomenon, which creates a potential risk to people drinking well water from PWSS, may be due to the distribution of not-chlorinated water, and possibly also to the composition and construction of the PWSS forming a specific niche favourable for both groups of pathogens.

In contrast to GNB-E, the isolation frequency of Gram-negative bacteria not belonging to the Enterobacteriaceae (GNB-NE) in water samples from urban and rural sources was similar. Among them, the most common were strains identified as *Pseudomonas* spp. The prevalence of these strains was higher in the water samples containing *Legionella*, but the correlation was not significant. By contrast, a highly significant correlation was found between the occurrence of *Legionella* and *Pseudomonas aeruginosa,* which creates a potential hazard because of the possibility of synergistic action of these 2 pathogens. A significant correlation was also noted between the occurrence of *Legionella* and that of *Flavobacterium breve* and *Xanthomonas* spp. On the contrary, the isolation frequency of *Aeromonas* spp. and *Vibrio* spp. was smaller from the samples containing *Legionella*.

Our results are in accordance with those obtained by Moritz *et al.* [7] and Murga *et al.* [8] who found an integration of *Legionella pneumophila* and *Pseudomonas aeruginosa* in biofilms, and with those obtained by Cotuk *et al.* [5] who found experimentally that the cultures of *Pseudomonas* stimulated the growth of *L. pneumophila*, while the cultures of *Aeromonas* inhibited this growth. The correlation between *Flavobacterium breve* and *Legionella* stated by us, was earlier reported by Wadowsky and Yee [16], who noticed a satellite growth of *Legionella pneumophila* around colonies of *Flavobacterium breve*. In general, our results support the views of other authors who have reported the stimulative effects of *Pseudomonas* and *Flavobacterium* strains on growth and/or survival of *Legionella* [1-3, 5, 7, 8, 17-19]. These results could well be explained by the fact that both Pseudomonadaceae and *Legionella* grow well in similar aerobic niches constituted by water aerators. Stojek [20] found a much greater isolation frequency of Gram-negative bacteria in water from indoor taps equipped with aerators or other endings than in outdoor taps without these gadgets (80.0% vs. 44.4%).

By contrast, the presented study does not confirm the inverse correlation between *Legionella* and *Pseudomonas aeruginosa* in shower water samples that was reported by Leoni *et al.* [21], or the suppression of growth of *Legionella* by *Pseudomonas aeruginosa* reported by Kimura *et al.* [22].

CONCLUSIONS

In conclusion, a health risk could be associated with exposure to water from the urban MWSS because of the high prevalence and concentration of *Legionella*, and with exposure to well water from PWSS because of the correlation of occurrence of *Legionella* and potentially pathogenic Enterobacteriaceae strains and the possibility of synergistic effects. The adverse effects could be also due to the significant correlation of *Legionella* and *Pseudomonas aeruginosa* that occurs in water from various sources.

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