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GRAM-NEGATIVE BACTERIA IN WATER DISTRIBUTION SYSTEMS OF HOSPITALS

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Abstract: A total of 67 samples of tap water were collected from faucets and showerheads in 6 hospitals located in the Lublin province (eastern Poland). The samples were examined for the presence and species composition of *Legionella*, Gram-negative bacteria belonging to family Enterobacteriaceae (GNB-E) and Gram-negative bacteria not belonging to family Enterobacteriaceae (GNB-NE), by filtering through cellulose filters and culture on respectively GVPC, EMB and tryptic soya agar media. On average, *Legionella* was isolated from 65.7% of the water samples taken in hospitals. Strains of the *Legionella pneumophila* types 2–14 predominated, forming 74.6% of total *Legionella* isolates. *Legionella pneumophila* type 1 strains constituted 13.5% of the total count, while other species of *Legionella* (referred to as *Legionella* spp.) formed 11.9% of the total. The concentrations of *Legionella* in positive water samples ranged from 3–350 cfu/100 ml. GNB-E were not found in the examined water samples. GNB-NE were isolated from 79.1% of the water samples taken in hospitals in the concentrations 11–300 cfu/100 ml. Species of the family Pseudomonadaceae predominated among GNB-NE strains isolated from the examined water samples, forming on average 71.5% of the total count. Altogether, 20 GNB-NE species were identified in the examined samples, out of which 12 were potentially pathogenic. In conclusion, Gram-negative flora of water samples taken in the examined hospitals complies with potable water sanitary standards by the lack of Enterobacteriaceae species, but creates a moderate health risk because of mediocre concentrations of *Legionella* and the presence of potentially pathogenic non-enterobacterial species.

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INTRODUCTION

It has been demonstrated that potable water supplied to hospitals could be contaminated with various potentially infectious Gram-negative bacteria, including coliforms (members of Enterobacteriaceae family), *Legionella*, and other species not belonging to Enterobacteriaceae [4, 5, 7, 11, 13, 14, 23, 28, 35]. These organisms may be a cause of nosocomial infections, mainly in immunocompromised patients, that could be contracted by drinking, by inhaling droplet aerosol, or by dermal exposure [5, 7, 9, 12, 15, 19, 22, 23, 25, 30, 31, 32, 35].

The aim of the present study was to assess the degree of contamination of potable water from different outlets of water supply systems in 6 hospitals, with *Legionella* and non-fastidious Gram-negative bacteria belonging and not belonging to the family Enterobacteriaceae.

MATERIALS AND METHODS

Samples of water. In the years 2007-2008, a total of 67 samples of tap water were collected from different outlets (faucets or showerheads) of the hospital water supply systems distributing treated (chlorinated) groundwater,

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pumped from the depth of 40-100 m. The samples were collected in the following 6 hospitals located in the Lublin province (eastern Poland): clinic for lung diseases (city of Lublin), gynecology clinic (city of Lublin), clinic for infectious diseases (city of Lublin), clinic for hemato-oncology and bone marrow transplantation (city of Lublin), dental clinic (city of Lublin), and regional hospital (Biała Podlaska). At all places the samples of hot water were collected except for the dental clinic, where samples of cold water were taken. Water samples of the volume of 300 ml were taken into sterile plastic bottles of the volume of 500 ml at following sites (sampling points):

• Clinic for lung diseases (18 sites): 1) Ward A, showerhead; 2) Ward A, faucet; 3) Ward B, showerhead; 4) Ward C, faucet; 5) Ward C, showerhead; 6) Ward D, showerhead; 7) Bathroom for personnel A, faucet; 8) Bathroom for personnel A, showerhead; 9) Kitchen, faucet; 10) Bathroom for personnel B, faucet; 11) Bathroom for personnel B, showerhead; 12) Ward E, faucet; 13) Ward E, showerhead; 14) Ward F, faucet; 15) Ward F, showerhead; 16) Bathroom for personnel C, faucet; 17) Bathroom for personnel C, showerhead; 18) Bathroom for personnel D, showerhead.

• Gynecology clinic (17 sites): 1) Bathroom for patients, faucet A; 2) Bathroom for patients, faucet B; 3) Bathroom for patients, showerhead A; 4) Bathroom for patients, showerhead B; 5) Bathroom for patients, showerhead C; 6) Bathroom for personnel, faucet; 7) Bathroom for personnel, showerhead; 8) Ward A, faucet; 9) Ward B, faucet; 10) Ward C, faucet; 11) Ward D, faucet; 12) Ward E, faucet; 13) Ward F, faucet; 14) Nurses' room, faucet; 15) Treatment room, faucet A; 16) Treatment room, faucet B; 17) Kitchen, faucet.

• Clinic for infectious diseases (18 sites): 1) Physicians' room, faucet; 2) Treatment room, faucet A; 3) Treatment room, faucet B; 4) Ward A, faucet; 5) Bathroom for personnel, showerhead; 6) Bathroom for personnel, faucet; 7) Ward B, showerhead; 8) Ward B, faucet; 9) Passage to Ward B, faucet; 10) Kitchen, faucet; 11) Passage to Ward C, faucet; 12) Ward C, showerhead; 13) Ward C, faucet; 14) Ward D, faucet; 15) Ward D, showerhead; 16) Passage to Ward D, faucet; 17) Passage to Ward E, faucet; 18) Ward E, showerhead.

• Clinic for hemato-oncology and bone marrow transplantation (6 sites): 1) Tissue bank, faucet; 2) Transplantation room, showerhead; 3) Transplantation room, faucet; 4) Passage to transplantation room, faucet; 5) Passage to clinic, faucet; 6) Nurse's station, faucet.

• Dental clinic (5 sites): 1) Dental unit A, waterline outlet; 2) Dental unit B, waterline outlet; 3) Dental unit C, waterline outlet; 4) Dental unit D, waterline outlet; 5) Dental unit E, waterline outlet.

• Regional hospital (3 sites): 1) Bathroom for patients A, faucet; 2) Bathroom for patients B, faucet; 3) Bathroom for patients C, faucet.

The faucets in examined hospitals were equipped with aerators or other endings for better outflow of water.

Processing of samples. Water samples were examined for the presence of following Gram-negative bacteria (GNB): (a) *Legionella*; (b) non-fastidious Gram-negative bacteria belonging to the Enterobacteriaceae family (GNB-E); (c) non-fastidious Gram-negative bacteria not belonging to the Enterobacteriaceae family (GNB-NE). For recovery of *Legionella*, water samples of 100 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 the isolation agar medium. For recovery of 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, UK) [3, 18, 29] was used for isolation of *Legionella* (further referred to as GVPC medium). Inoculated agar plates were incubated for 7 days at 37°C with 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, UK) 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*) [18]. Only isolates giving positive reaction in the latex test were considered as strains of *Legionella*.

Isolation and identification of GNB-E. The eosin methylene blue (EMB) agar (Merck, Darmstadt, Germany) was used for isolation of bacteria of Enterobacteriaceae family. Inoculated agar plates were incubated for 24 hrs at 37°C.

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

Statistical analysis. The data were analysed by Shapiro-Wilk W-test for distribution and Spearman's test for

correlation with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

Isolation frequency and concentration of GNB in potable water from hospital distribution systems. In water samples from hemato-oncology and dental clinics no strains of *Legionella* were found. In the samples taken in the remaining 4 hospitals the prevalence of *Legionella* was within a range of 55.6-100% (Tab. 1–4). On average, *Legionella* was isolated from 65.7% of the water samples taken in hospitals. Strains of the *Legionella pneumophila* types 2-14 predominated, forming 74.6% of total *Legionella* isolates from hospital water systems. *Legionella pneumophila* type 1 strains constituted 13.5% of the total count, while other species of *Legionella* (referred to as *Legionella* spp.) formed 11.9% of the total.

The concentrations of *Legionella* in 44 positive water samples ranged from 3-350 cfu/100 ml (Tab. 1–4). In 28 samples (63.6% of the positive and 41.8% of the total samples) it was equal to or exceeded 100 cfu/100 ml which is the Polish threshold limit value for mediocre pollution of potable water with *Legionella* [24]. In no case was the

Table 1. Occurrence of Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system in the clinic for lung diseases (18 samples).

Site	*Legionella* (GVPC)		Entero-bacteriaceae (EMB)	Non-Enterobacteriaceae (NE) (Tryptic Soya Agar)				
				Concentration cfu/100 ml				
	Concentration cfu/100 ml	Species	Concentration cfu/100 ml	*Chryseomonas luteola*	*Empedobacter brevis*	*Pseudomonas* spp.	Other NE	Total NE
1	0		0	30	30	0	0	60
2	50	*L. p.* 2–14	0	30	0	50[b]	0	80
3	10	*L. p.* 2–14 *L.* spp.	0	0	30	0	50[e]	80
4	60	*L. p.* 2–14	0	0	10	0	70[dj]	80
5	30	*L. p.* 2–14	0	0	80	0	20[h]	100
6	6	*L. p.* 2–14	0	0	10	0	10[g]	20
7	4	*L. p.* 2–14	0	0	0	300[b]	0	300
8	50	*L. p.* 2–14 *L.* spp.	0	0	0	250[b]	0	250
9	3	*L. p.* 2–14	0	0	0	0	100[d]	100
10	150	*L. p.* 1 *L. p.* 2–14 *L.* spp.	0	0	0	20[b]	0	20
11	100	*L. p.* 2–14	0	100	0	0	0	100
12	40	*L. p.* 1 *L. p.* 2–14	0	0	100	0	100[i]	200
13	200	*L. p.* 2–14 *L.* spp.	0	0	0	100[a c]	0	100
14	80	*L. p.* 2–14	0	100	100	15[b]	0	215
15	100	*L. p.* 2–14 *L.* spp.	0	0	100	0	50[f]	150
16	80	*L. p.* 2–14 *L.* spp.	0	0	50	0	50[f]	100
17	30	*L. p.* 1 *L. p.* 2–14	0	0	80	0	50[f]	130
18	0		0	0	20	100[b]	0	120
Total positive	16/18 (88.9%)		0/18 (0)	4/18 (22.2%)	11/18 (61.1%)	7/18 (38.9%)	9/18 (50.0%)	18/18 (100%)
Median	45.0		0	0	15.0	0	5.0	100.0
Mean*	55.2 ± 55.7		0	14.4 ± 32.6	33.9 ± 39.9	46.4 ± 89.7	27.8 ± 35.6	122.5 ± 75.2

L. p. 1=*Legionella pneumophila* serogroup 1; *L. p.* 2–14=*Legionella pneumophila* serogroups 2–14; *L.* spp.=*Legionella* spp. (other than *L. pneumophila*); [a]*Pseudomonas aeruginosa*; [b]*Pseudomonas alcaligenes*; [c]*Pseudomonas stutzeri*; [d]*Acinetobacter haemolyticus*; [e]*Aeromonas salmonicida*; [f]*Brevundimonas vesicularis*; [g]*Chryseobacterium indologenes*; [h]*Photobacterium damsela*; [i]*Ralstonia pickettii*; [j]*Stenotrophomonas maltophilia*; *$\bar{x}$ ± S.D.

value of 1000 cfu/100 ml exceeded, which is defined as high pollution of potable water with *Legionella* [24].

Gram-negative bacteria belonging to the Enterobacteriaceae family were not found in the examined water samples.

Gram-negative bacteria not belonging to Enterobacteriaceae family (GNB-NE) were not found in the water samples taken in the dental clinic (Tab. 4). In the samples taken in the clinic for infectious diseases the prevalence of GNB-NE was 50% (Tab. 3), while in the remaining 4 hospitals GNB-NE were recovered from all samples examined (100%) (Tab. 1, 2, 4). On average, GNB-NE were isolated from 79.1% of the water samples taken in hospitals. The concentrations of GNB-NE in positive samples ranged from 11-300 cfu/100 ml and in no case exceeded the concentration of 50 cfu/ml recommended as a Polish threshold value for total microorganisms grown at 36°C [24].

No significant correlation could be found between the concentrations of *Legionella* and GNB-NE in the examined water samples taken in hospitals ($p>0.2$).

Species composition of GNB-NE in water samples taken in hospitals. Altogether, the following 20 GNB-NE species were identified in the examined samples of potable water collected in hospitals: *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Brevundimonas diminuta*, *Brevundimonas vesicularis*, *Burkholderia cepacia*, *Chryseobacterium indologenes*, *Chryseomonas luteola*, *Empedobacter brevis*, *Flavimonas oryzihabitans*, *Photobacterium damsela*, *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas mesophilica*, *Pseudomonas stutzeri*, *Ralstonia pickettii*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*, *Vibrio metschnikovii*.

Among GNB-NE strains isolated from the examined water samples, predominated species of the family Pseudomonadaceae belonging to genera *Brevundimonas*, *Burkholderia*, *Chryseomonas*, *Flavimonas*, *Pseudomonas*, *Ralstonia*, *Sphingomonas*, and *Stenotrophomonas*. They formed 25.7–95.1%, on the average 71.5% of the total GNB-NE count.

Table 2. Occurrence of Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system in the gynecology clinic (17 samples).

Site	*Legionella* (GVPC)		Entero-bacteriaceae (EMB)	Non Enterobacteriaceae (NE) (Tryptic Soya Agar)				
				Concentration cfu/100 ml				
	Concentration cfu/100 ml	Species	Concentration cfu/100 ml	*Aeromonas hydrophila*	*Burkholderia cepacia*	*Pseudomonas* spp.	Other NE	Total NE
1	50	*L. p.* 2-14	0	80	0	0	0	80
2	320	*L. p.* 2-14	0	30	0	0	80[f]	110
3	50	*L. p.* 2-14	0	0	150	6[a]	0	156
4	100	*L. p.* 2-14	0	0	150	0	0	150
5	200	*L. p.* 2-14	0	0	0	54[a b]	0	54
6	0		0	0	0	200[a]	0	200
7	200	*L. p.* 1 *L. p.* 2-14	0	0	200	0	0	200
8	250	*L. p.* 2-14	0	0	200	0	0	200
9	150	*L. p.* 2-14	0	0	0	4[a]	200[d]	204
10	150	*L. p.* 2-14	0	0	200	0	0	200
11	200	*L. p.* 2-14	0	0	0	200[b]	0	200
12	200	*L. p.* 2-14	0	0	20	0	0	20
13	0		0	0	100	0	50[e]	150
14	200	*L. p.* 2-14	0	0	80	0	20[c]	100
15	150	*L. p.* 2-14	0	0	50	0	50[c]	100
16	100	*L. p.* 2-14	0	0	200	0	0	200
17	300	*L. p.* 1 *L. p.* 2-14	0	0	25	0	25[c]	50
Total positive	15/17 (88.2%)		0/17 (0)	2/17 (11.8%)	11/17 (64.7%)	5/17 (29.4%)	6/17 (35.3%)	17/17 (100%)
Median	150.0		0	0	50.0	0	0	150.0
Mean*	154.2 ± 94.7		0	6.5 ± 20.3	80.9 ± 84.3	27.3 ± 66.3	25.0 ± 51.1	139.6 ± 63.1

L. p. 1=*Legionella pneumophila* serogroup 1; *L. p.* 2–14=*Legionella pneumophila* serogroups 2–14; [a]*Pseudomonas aeruginosa*; [b]*Pseudomonas stutzeri*; [c]*Flavimonas oryzihabitans*; [d]*Ralstonia pickettii*; [e]*Sphingomonas paucimobilis*; [f]*Stenotrophomonas maltophilia*; *$\bar{x}$ ± S.D.

Table 3. Occurrence of Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system in the clinic for infectious diseases (18 samples).

Site	*Legionella* (GVPC)		Enterobacteriaceae (EMB)	Non Enterobacteriaceae (NE) (Tryptic Soya Agar)				
				Concentration cfu/100 ml				
	Concentration cfu/100 ml	Species	Concentration cfu/100 ml	*Acinetobacter lwoffii*	*Chryseomonas luteola*	*Pseudomonas* spp.	Other NE	Total NE
1	0		0	100	0	0	0	100
2	0		0	0	0	0	0	0
3	80	*L. p.* 1 *L. p.* 2–14	0	0	30	0	0	30
4	0		0	0	0	0	0	0
5	100	*L. p.* 2–14 *L.* spp.	0	0	0	0	0	0
6	350	*L. p.* 2–14	0	0	0	0	0	0
7	0		0	0	0	0	50[b]	50
8	200	*L. p.* 2–14	0	0	0	0	0	0
9	40	*L. p.* 2–14	0	0	0	0	0	0
10	0		0	0	0	0	0	0
11	100	*L. p.* 2–14	0	0	0	0	0	0
12	280	*L. p.* 1 *L. p.* 2–14	0	100	0	0	0	100
13	0		0	200	0	0	0	200
14	0		0	50	0	0	20[c]	70
15	300	*L. p.* 2–14	0	0	0	0	50[c]	50
16	200	*L. p.* 2–14	0	0	30	50[a]	0	80
17	0		0	0	0	0	0	0
18	250	*L. p.* 2–14	0	20	0	0	0	20
Total positive	10/18 (55.6%)		0/18 (0)	5/18 (27.8%)	2/18 (11.1%)	1/18 (5.6%)	3/18 (16.7%)	9/18 (50.0%)
Median	60.0		0	0	0	0	0	10.0
Mean*	105.6 ± 123.9		0	26.1 ± 54.6	3.3 ± 9.7	2.8 ± 11.8	6.7 ± 16.4	38.9 ± 54.4

L. p. 1=*Legionella pneumophila* serogroup 1; *L. p.* 2–14=*Legionella pneumophila* serogroups 2–14; *L.* spp.=*Legionella* spp. (other than *L. pneumophila*). [a]*Pseudomonas mesophilica*; [b]*Empedobacter brevis*; [c]*Stenotrophomonas maltophilia*; *$\bar{x}$ ± S.D.

Frequency of isolation of particular species varied depending on the kind of hospital. Thus, in the clinic for lung diseases the most common species was *Empedobacter brevis* isolated from 61.1% of examined water samples (Tab. 1), while in the gynecology clinic it was *Burkholderia cepacia* isolated from 64.7% of water samples (Tab. 2). In the clinic for hemato-oncology relatively common were species *Brevundimonas vesicularis* and *Pseudomonas stutzeri* isolated each from 50% of water samples (Tab. 4).

DISCUSSION

The mean prevalence of *Legionella* in the water samples taken in 6 different hospitals (65.7%) was smaller compared to analogical data reported from Italy [13, 14], Denmark [2], USA [7], and Warsaw (Poland) [19], similar or greater compared to those reported from Germany [4, 8, 16], and greater compared to those reported from Canada [17], and the UK [20]. It was also greater compared to the values obtained previously by us in various health care units in Lublin, Poland [28]. It is noteworthy that in the present work were isolated potentially pathogenic *Legionella pneumophila* type 1 strains which had not been found previously [28]. Nevertheless, nosocomial infections could be evoked by the strains belonging to *Legionella pneumophila* serogroups 2–14 – such as serogroup 5 [22] or serogroup 6 [5] – which were predominant among *Legionella* strains isolated in this study. Most probably, the presence of aerators or other endings on faucets or showerheads favoured proliferation of legionellae. In contrast to our previous work [28], *Legionella* was recovered commonly from the showerheads in examined hospitals.

The concentration of *Legionella* in the water distribution systems of examined hospitals nowhere exceeded the

Table 4. Occurrence of Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system in various hospitals (14 samples).

Site	*Legionella* (GVPC)		Enterobacteriaceae (EMB)	Non Enterobacteriaceae (NE) (Tryptic Soya Agar)				
				Concentration cfu/100 ml				
	Concentration cfu/100 ml	Species	Concentration cfu/100 ml	*Brevundimonas vesicularis*	*Empedobacter brevis*	*Pseudomonas* spp.	Other NE	Total NE
Clinic for hemato-oncology								
1	0		0	100	0	100[b]	0	200
2	0		0	0	0	13[b]	0	13
3	0		0	100	0	100[b]	0	200
4	0		0	11	0	0	0	11
5	0		0	0	200	0	0	200
6	0		0	0	0	0	200[d]	200
Total positive	0/6 (0)		0/6 (0)	3/6 (50.0%)	1/6 (16.7%)	3/6 (50.0%)	1/6 (16.7%)	6/6 (100%)
Median	0		0	5.5	0	6.5	0	200.0
Mean*	0		0	35.2 ± 50.4	33.3 ± 81.6	35.5 ± 50.2	33.3 ± 81.6	137.3 ± 97.1
Dental clinic								
1	0		0	0	0	0	0	0
2	0		0	0	0	0	0	0
3	0		0	0	0	0	0	0
4	0		0	0	0	0	0	0
5	0		0	0	0	0	0	0
Total positive	0/5 (0)		0/5 (0)	100	0	0	0	0/5 (0)
Median	0		0	0	0	0	0	0
Mean*	0		0	0	0	0	0	0
Regional hospital								
1	200	*L. p.* 2–14	0	0	0	0	20[c]	20
2	300	*L. p.* 1 *L. p.* 2–14	0	0	0	0	200[e]	200
3	350	*L. p.* 2–14	0	0	0	200[a]	0	200
Total positive	3/3 (100%)		0/3 (0)	0/3 (0)	0/3 (0)	1/3 (33.3%)	2/3 (66.7%)	3/3 (100%)
Median	300.0		0	0	0	0	20.0	200.0
Mean*	283.3 ± 76.4		0	0	0	66.7 ± 115.5	73.3 ± 110.2	140.0 ± 103.9

L. p. 1=*Legionella pneumophila* serogroup 1; *L. p.* 2–14=*Legionella pneumophila* serogroups 2–14; [a]*Pseudomonas aeruginos*a; [b]*Pseudomonas stutzeri*; [c]*Aeromonas salmonicida*; [d]*Brevundimonas diminuta*; [e]*Vibrio metschnikovii*; *$\bar{x}$ ± S.D.

threshold limit value of 1,000 (10^3) cfu/100 ml, regarded as a high pollution of potable water with *Legionella* [1, 24, 31]. In 41.8% of the samples it was equal to or exceeded the value of 100 (10^2) cfu/100 ml considered as a mediocre pollution of water with *Legionella* [24, 31]. Overall pollution of hospital water systems with *Legionella* found in the present work was smaller compared to earlier studies made in Germany and Italy [4, 8, 13, 16] where in part of the samples levels up to 10^3-10^6 cfu/100 ml were found, as well as compared to a recent study of water distribution system in a children's hospital in Lublin where in 40% of samples the level of 10^3 cfu/100 ml or greater was noted [10]. Until recently, no cases of nosocomial legionellosis were diagnosed in the health care units examined in the present study.

In contrast to the earlier examined rehabilitation centre [28], the water distribution systems of examined hospitals were totally free of Gram-negative bacteria belonging to family Enterobacteriaceae, meeting the Polish [24] and international [6] sanitary regulations. As much as 79.1% of water samples contained the non-fastidious Gram-negative bacteria not belonging to family Enterobacteriaceae (GNB-NE), which, however, never exceeded threshold limit values proposed for total heterotrophic bacteria [21,

24]. A potential risk was associated rather with the species composition of these bacteria and the presence of potentially pathogenic species.

Out of 20 GNB-NE species isolated from the samples of potable water examined in the present study, at least 12 were reported as obligatory or opportunistic agents of infectious diseases. These are following species: *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Aeromonas hydrophila*, *Brevundimonas vesicularis*, *Burkholderia cepacia*, *Chryseobacterium indologenes*, *Chryseomonas luteola*, *Photobacterium damsela*, *Pseudomonas aeruginosa*, *Ralstonia pickettii*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia* [33, 34, 36]. Some of these pathogens, as *Acinetobacter* spp., *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* are often isolated from water and have been identified as a cause of waterborne infections by drinking water as well as by contact with skin or inhaling of droplet aerosol [4, 12, 21, 26, 27]. The greatest hazard is posed by *Pseudomonas aeruginosa,* a major cause of hospital-acquired infections with a high mortality rate [26]. The presence of this species in quantities equal to or exceeding 1 cfu per 100 ml of potable water is not permitted by Polish [24] and international [6] sanitary regulations.

In conclusion, Gram-negative flora of water samples taken in the examined hospitals complies with potable water sanitary standards by the lack of Enterobacteriaceae species ("coliforms"), but creates a moderate health risk because of mediocre concentrations of *Legionella* and the presence of potentially pathogenic non-enterobacterial species.

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