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LEGIONELLA AND OTHER GRAM-NEGATIVE BACTERIA IN POTABLE WATER FROM VARIOUS RURAL AND URBAN SOURCES

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Abstract: A total of 107 potable water samples were collected from various rural and urban sources located in the Lublin region (eastern Poland). 54 samples from rural sources comprised 32 samples of untreated well water and 22 samples of treated (chlorinated) tap water from rural dwellings distributed by the municipal water supply system (MWSS). 53 samples of treated water from urban sources were supplied by the city of Lublin MWSS. They comprised: 11 samples of tap water from offices and shops, 8 samples of tap water from dwellings, 19 samples from showerheads in health care units, and 15 samples from the outlets of medical appliances used for hydrotherapy in a rehabilitation centre. 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 family Enterobacteriaceae (GNB-NE), by filtering through cellulose filters and culture on respectively GVPC, CIN, EMB and tryptic soya agar media. *Legionella* was recovered from samples of well water, tap water from rural dwellings, tap water from urban dwellings, and water from medical appliances - with the isolation frequency of 27.8-50.0%, and the low concentrations ranging from 0.7-13.3 × 10^1 cfu/l. No *Legionella* strains were detected in tap water from offices and shops, and in water from showerheads in health care units. Strains of the *Legionella pneumophila* types 2-14 predominated, forming 89.9% of total *Legionella* isolates, while other species of *Legionella* formed 10.1%. Neither *Legionella pneumophila* type 1 strains nor *Yersinia* strains were isolated from the examined water samples. The isolation frequency and mean concentration of GNB-E in water samples from rural sources was significantly greater than in water samples from urban sources (respectively 61.1% vs. 20.8%, 17.1 vs. 3.4 × 10^1 cfu/l, $p<0.001$). Isolation frequency of GNB-NE in water samples from rural sources was significantly greater compared to that from urban sources (77.8% vs. 47.2%, $p<0.01$), but there was no significant difference in the concentration of GNB-NE in both sample sets. A significant correlation was found between concentrations of *Legionella* and GNB-NE for total MWSS water samples ($p<0.001$), but not for the total well water samples. Altogether 34 GNB-E and GNB-NE species and/or genera were identified in the examined samples, out of which 21 were potentially pathogenic. *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., and *Pantoea agglomerans* were most common among GNB-E, while *Acinetobacter* spp. and species of Pseudomonadaceae family predominated among GNB-NE.

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INTRODUCTION

Potable water could be a source of various potentially infectious microorganisms that could be contracted by drinking, by inhaling droplet aerosol or by dermal exposure. The best known potential pathogens are *Escherichia coli* and related Gram-negative species of faecal origin belonging to Enterobacteriaceae family,

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usually referred to as "coliforms", which are commonly used as a sanitary indicator of potable water quality [12, 53, 54]. Important agents of waterborne infections comprise various genera of Gram-negative bacteria (*Legionella*, *Yersinia*, *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*), mycobacteria, enteroviruses and intestinal protozoa (*Giardia*, *Cryptosporidium*) [28, 30]

Legionella pneumophila and related species are fastidious Gram-negative bacteria, developing mostly in water, which may cause in humans atypical pneumonia (legionellosis, legionnaires' disease) or flu-like illness [10, 17, 52, 64, 70, 71, 72]. People become infected with *Legionella* most often by the inhalation of bacteria-laden droplet aerosol when bathing [21, 44], taking showers [11, 45], performing various occupations [18, 72], sprinkling plants [66], and at similar occasions such as exposure to contaminated whirlpool spas [5, 8], humidifiers [17, 27] or greenhouse misting systems [79]. *Legionella* could be also transmitted by the oral route through drinking water [74] and through traumatized skin or mucous membranes [35]. According to Stout *et al.* [68] potable water supplies that harbour *Legionella pneumophila* are an important source of community-acquired legionnaires' disease.

The aim of the present study was to assess the degree of contamination of potable water from various rural and urban sources, comprising wells and different outlets of water supply systems, with *Legionella*, *Yersinia* and non-fastidious Gram-negative bacteria belonging and not belonging to the family Enterobacteriaceae.

MATERIALS AND METHODS

Samples of water from rural sources. 32 samples of well water used as potable water for consumption and 22 water samples from outlets of municipal water supply system (MWSS) were taken in the years 2003-2005 during summer months (June-August) in 8 villages (A-H) located in the Lublin province (eastern Poland). Water samples were taken into sterile glass bottles of the volume of 700 ml at the following places:

• 14 samples of well water were taken immediately from 14 private household wells located on farms in 3 villages (A-C). The wells were shallow (mostly of the depth of 4-5 m) with a surrounding casing of concrete and a manual winch. Wells were in a poor hygienic state, 8 out 14 of them were uncovered. They were situated in close proximity (7-15 m) to animal houses.

• 18 samples of well water were collected from taps of the 18 private water supply systems conducting untreated and unheated water from household wells to outlets within farm buildings located in 6 villages (A, B, D-G). Household wells used as a source of potable water were shallow (mostly of the depth of 6-7 m), in a good hygienic state, all covered. They were situated in a close proximity (7-15 m) to animal houses.

• 22 samples were taken on 22 farms in 5 villages (B-D, G, H) from cold-water taps of the municipal water supply system distributing treated (chlorinated) groundwater, pumped from the depth of 40-100 m. The taps were equipped with aerators or other endings for better outflow of water.

Samples of water from urban sources. Altogether, 53 water samples were collected in the years 2003-2005, during the period from May-November, in the city of Lublin (eastern Poland), from outlets of the municipal water supply system (MWSS) distributing treated (chlorinated) groundwater, pumped from the depth of 40-100 m. Water samples were taken into sterile glass bottles of the volume of 700 ml at the following places:

• 11 samples were taken from hot-water taps in 6 offices and 5 shops.

• 8 samples were taken from hot-water taps, equipped with aerators or other endings, in 8 dwellings.

• 19 samples of hot water were taken from showerheads in 5 following health care units (in parentheses numbers of samples): outpatient health care unit A (6), outpatient health care unit B (3), rehabilitation centre (1), pulmonology department of a clinic (3), internal department of a clinic (6).

• 15 samples of hot water were taken from the outlets of the 4 following medical appliances used for hydrotherapy in a rehabilitation centre (in parentheses numbers of samples): underwater manual massage (4), underwater vibration massage (3), needle-bath (4), Hubard's tank (4). The outlets (nozzles) of investigated medical appliances 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) *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 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 the 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, UK) [4, 40, 70] was used for isolation of *Legionella* (further referred to as GVPC medium). Inoculated agar plates were incubated for 7 days at 37ºC 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, on the basis of microagglutination with latex particles sensitised with specific rabbit antibodies, enables 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*) [40]. Only isolates giving a 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) medium with mannitol [4] (Merck, Darmstadt, Germany) was used for isolation of *Yersinia*. Inoculated agar plates were incubated for 24 hrs at 37ºC. 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 Enterobacteriaceae family. Inoculated agar plates were incubated for 24 hrs at 37ºC. The grown colonies were counted and differentiated, and the isolates identified to the 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 Enterobacteriaceae family. Inoculated agar plates were incubated for 24 hrs at 37ºC. The grown colonies were counted and differentiated, and the isolates 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, Mann-Whitney U-test, Spearman's test for correlation, and Student's t-test 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 rural sources. Strains of *Legionella* were isolated from the samples taken immediately from wells and from the private water supply systems distributing well water (PWSS) with similar frequency amounting to 28.6% and 27.8%, respectively (Tab. 1-2). *Legionella* was recovered with slightly greater frequency (40.9%) from the outlets of the municipal water supply system (MWSS) (Tab. 3). The concentrations of *Legionella* in positive water samples from wells, PWSS water samples, and MWSS water samples were low, ranging from 1.3-3.3, 4.0-13.3, and 0.7-2.0 × 10^1 cfu/l, respectively (Tab. 1-3). Strains of the *Legionella pneumophila* types 2-14 predominated, forming 84.4% of total *Legionella* isolates from rural sources, while other species of *Legionella* (referred to as *Legionella* spp.) formed 15.6% of the total count. Neither *Legionella pneumophila* type 1 strains nor *Yersinia* strains were isolated from the examined water samples from rural sources.

Isolation frequencies of GNB-E from water samples from wells, PWSS water samples, and MWSS water samples were respectively 71.4%, 77.8%, and 40.9% (Tab. 1-3). Concentrations in positive samples were low, ranging from 5.0-68.0, 5.0-97.0, and 3.0-130.0 × 10^1 cfu/l, respectively (Tab. 1-3). Both isolation frequency and concentration of GNB-E in total well water samples (wells + PWSS) were significantly greater compared to rural MWSS samples ($p<0.05$, and $p<0.001$, respectively).

Isolation frequencies of GNB-NE from water samples from wells, PWSS water samples, and MWSS water samples were respectively 85.7%, 77.8%, and 72.7%, while concentrations in positive samples were low, ranging from 10.0-150, 7.0-70.0, and 2.0-120 × 10^1 cfu/l, respectively (Tab. 1-3). There was no statistically significant difference between total well water samples (wells + PWSS) and rural MWSS samples in the frequency of occurrence and concentration of GNB-NE.

Isolation frequency and concentration of GNB in potable water from urban sources. *Legionella* was recovered from 50.0% of water samples taken in dwellings (Tab. 5) and from 33.3% of water samples taken from the outlets of various medical appliances used for hydrotherapy in a rehabilitation centre (Tab. 7), in low concentrations, ranging in positive samples from 3.3-4.0 and 2.3-6.7 × 10^1 cfu/l, respectively (Tables 5, 7). *Legionella* was not found in water samples taken in offices and shops (Tab. 4) and in water samples taken from showerheads in various health care units (Tab. 6). Both frequencies of occurrence and concentrations of *Legionella* in water samples from dwellings or medical appliances were significantly higher compared to offices and shops or showers ($p<0.05$, and $p<0.01$, respectively). No significant differences were found between the samples from dwellings and medical appliances. Similar to the rural environment, strains of the *Legionella pneumophila* types 2-14 predominated in water from urban sources, forming 95.5% of total *Legionella* isolates, while *Legionella* spp. formed 4.5% of the total count. Neither *Legionella pneumophila* type 1 strains nor *Yersinia* strains were isolated from the examined water samples from urban sources.

Table 1. Occurrence of *Legionella* and other Gram-negative bacteria in samples of water taken from wells in 3 villages (14 samples)[a]. Concentration of bacteria is expressed in cfu × 10^1/l.

Village, well	*Legionella pneumophila* type 2-14 (GVPC)	*Citrobacter freundii*	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella oxytoca*	*Salmonella* spp.	Total Enterobacteriaceae (EMB)	*Acinetobacter* spp.	*Aeromonas hydrophila*	*Brevundimonas diminuta*	*Chryseomonas luteola*	*Pseudomonas* spp.	*Stenotrophomonas maltophilia*	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Village A														
Well 1	3.3	0	0	0	0	0	0	0	20.0	0	0	0	0	20.0
Well 2	0	0	0	0	0	0	0	20.0[d]	0	0	15.0	0	19.0	54.0
Village B														
Well 1	0	10.0	4.0	5.0	0	0	19.0	20.0[c]	0	0	0	0	0	20.0
Well 2	0	0	60.0	8.0	0	0	68.0	0	0	0	0	0	0	0
Well 3	0	0	10.0	0	0	0	10.0	0	0	0	0	0	15.0	15.0
Well 4	0	0	0	0	0	0	0	0	8.0	10.0	14.0	0	0	32.0
Village C														
Well 1	0	0	3.0	10.0	14.0	0	27.0	20.0[c]	0	0	0	0	0	20.0
Well 2	1.7	8.0	20.0	0	12.0	0	40.0	10.0[c]	0	0	0	0	0	10.0
Well 3	0	0	5.0	0	0	0	5.0	15.0[c]	0	0	0	30.0[e]	0	45.0
Well 4	2.0	0	0	0	0	0	0	0	0	0	0	50.0[f]	0	50.0
Well 5	0	0	30.0	0	0	0	30.0	65.0[c]	0	0	0	0	0	65.0
Well 6	0	0	0	0	27.0	0	27.0	30.0[c]	0	0	0	0	0	30.0
Well 7	1.3	0	0	0	30.0	0	30.0	150.0[c]	0	0	0	0	0	150.0
Well 8	0	0	10.0	2.0	10.0	5.0	27.0	0	0	0	0	0	0	0
Total positive	4/14 (28.6%)	2/14 (14.3%)	8/14 (57.1%)	4/14 (28.6%)	5/14 (35.7%)	1/14 (7.1%)	10/14 (71.4.%)	8/14 (57.1%)	2/14 (14.3%)	1/14 (7.1%)	2/14 (14.3%)	2/14 (14.3%)	2/14 (14.3%)	12/14 (85.7%)
Median	0	0	3.5	0	0	0	23.0	12.5	0	0	0	0	0	25.0
Mean[b]	0.6±1.1	1.3±3.3	10.1±16.9	1.8±3.4	6.6±10.6	0.4±1.3	20.2±19.6	23.6±40.5	2.0±5.6	0.7±2.7	2.1±5.3	5.7±15.0	2.4±6.2	36.5±38.2

[a]At each site one sample was taken; [b] $\bar{x}$ ± S.D.; [c]*Acinetobacter calcoaceticus*; [d]*Acinetobacter lwoffii*; [e]*Pseudomonas aeruginosa*; [f]*Pseudomonas fluorescens*.

No Enterobacteriaceae (GNB-E) strains were detected in water samples from dwellings (Tab. 5). Isolation frequencies of GNB-E from water samples from offices and shops (Tab. 4), showers (Tab. 6), and medical appliances in a rehabilitation centre (Tab. 7) were respectively 9.1%, 5.3%, and 60.0%, while concentrations in positive samples were low, being 8.0, 30.0 and 5.0-45.0 × 10^1 cfu/l, respectively (Tables 4, 6-7). Both isolation frequencies and concentrations of GNB-E in water samples from medical appliances were significantly higher compared to dwellings, offices and shops, and showers ($p<0.01$). No significant differences were found between the samples from dwellings, offices and shops, and showers.

Isolation frequencies of GNB-NE from water samples from offices and shops, dwellings, showers and medical appliances were respectively 18.2%, 52.6%, 5.3% and 100%, while mean concentrations were low, being within the ranges of 20.0-60.0, 10.0-95.0, 45.0, and 35.0-106.0 × 10^1 cfu/l, respectively (Tab. 4-7). Both isolation frequencies and concentrations of GNB-NE in water samples from dwellings or medical appliances were significantly higher compared to offices and shops or showers ($p<0.01$). The concentration of GNB-NE in water samples from medical appliances was significantly higher compared to those from dwellings ($p<0.05$), but there was no significant difference in isolation frequency of GNB-NE in both sets of samples.

Comparison of isolation frequency and concentration of GNB in potable water from rural and urban sources. No statistically significant differences in the isolation frequency and concentration of *Legionella* were found between water samples from rural and urban sources, between total well water samples (wells + PWSS) and urban MWSS samples, and between total well water samples and total MWSS (rural + urban) samples. The isolation frequency of *Legionella* from rural MWSS water samples was significantly higher compared to those from urban MWSS water samples ($p<0.05$), but there was no significant difference in concentration of *Legionella* in both sets of samples.

Table 2. Occurrence of *Legionella* and other Gram-negative bacteria in samples of water taken from outlets of the private water supply systems distributing well water on farms in 6 villages (18 samples)[a]. Concentration of bacteria is expressed in cfu × 10^1/l.

Village, site	*Legionella pneumophila* type 2-14	*Legionella* spp.	Total *Legionella* (GVPC)	*Citrobacter freundii*	*Enterobacter* spp.	*Escherichia coli*	*Klebsiella* spp.	*Pantoea agglomerans*	*Salmonella* spp.	*Serratia* spp.	Total Enterobacteriaceae (EMB)	*Acinetobacter* spp.	*Chryseomonas luteola*	*Pseudomonas* spp.	*Stenotrophomonas maltophilia*	Other Non Enterobacteriaceae	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Village A																	
Site 1	0	0	0	0	20.0[d]	0	0	0	0	0	20.0	25.0[i]	10.0	0	0	0	35.0
Site 2	0	0	0	0	0	0	20.0[f]	0	0	0	20.0	0	0	0	0	0	0
Village B																	
Site 1	0	0	0	0	0	0	0	0	0	0	0	30.0[j]	0	40.0[l]	0	0	70.0
Site 2	0	0	0	0	0	6.0	0	20.0	0	0	26.0	0	0	0	0	9.0[t]	9.0
Village D																	
Site 1	5.7	0	5.7	0	0	0	0	0	0	0	0	0	10.0	10.0[m]	0	6.0[r]	26.0
Site 2	0	5.0	5.0	0	0	0	0	0	0	0	0	0	30.0	0	10.0	0	40.0
Site 3	0	0	0	0	0	0	0	0	0	0	0	0	9.0	0	10.0	11.0s	30.0
Village E																	
Site 1	0	0	0	15.0	20.0[c]	0	0	0	0	0	35.0	0	0	0	0	0	0
Site 2	0	0	0	0	19.0[c]	0	0	0	0	0	19.0	0	0	0	0	7.0[p]	7.0
Village F																	
Site 1	0	0	0	0	0	0	37.0[h]	10.0	0	10.0[n]	57.0	0	0	14.0[k]	0	0	14.0
Site 2	0	0	0	0	0	0	0	60.0	0	37.0[o]	97.0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	29.0[f]	30.0	0	10.0[o]	69.0	0	0	0	0	18.0[q]	18.0
Site 4	13.3	0	13.3	0	0	0	7.0[g]	17.0	0	10.0[o]	34.0	0	0	0	0	0	0
Site 5	4.0	0	4.0	0	0	0	10.0[f]	0	0	0	10.0	0	0	0	0	11.0[pq]	11.0
Village G																	
Site 1	5.0	0	5.0	0	0	5.0	0	0	0	0	5.0	0	0	0	0	7.0[v]	7.0
Site 2	0	0	0	0	19.0[c]	6.0	0	0	0	0	25.0	0	0	0	0	16.0[p]	16.0
Site 3	0	0	0	0	0	0	7.0[e]	20.0	0	0	27.0	0	0	0	14.0	10.0[u]	24.0
Site 4	0	0	0	0	0	6.0	0	0	8.0	10.0[o]	24.0	0	0	0	0	0	0
Total positive*	4/18 (22.2)	1/18 (5.6)	5/18 (27.8)	1/18 (5.6)	4/18 (22.2)	4/18 (22.2)	6/18 (33.3)	6/18 (33.3)	1/18 (5.6)	5/18 (27.8)	14/18 (77.8)	2/18 (11.1)	4/18 (22.2)	3/18 (16.7)	3/18 (16.7)	9/18 (50.0)	14/18 (77.8)
Median	0	0	0	0	0	0	0	0	0	0	22.0	0	0	0	0	3.0	12.5
Mean[b]	1.5 ±3.5	0.3 ±1.2	1.8 ±3.6	0.9 ±3.5	4.3 ±8.3	1.3 ±2.5	6.1 ±11.2	8.7 ±15.9	0.4 ±1.9	4.3 ±9.2	26.0 ±26.0	3.1 ±8.9	3.3 ±7.6	3.6 ±9.9	1.9 ±4.4	5.2 ±6.1	17.1 ±18.3

* - % in brackets; [a]At each site one sample was taken; [b] $\bar{x}$ ± S.D.; [c]*Enterobacter cloacae*; [d]*Enterobacter intermedius*; [e]*Klebsiella ornithinolytica*; [f]*Klebsiella oxytoca*; [g]*Klebsiella planticola*; [h]*Klebsiella terrigena*; [i]*Acinetobacter calcoaceticus*; [j]*Acinetobacter lwoffii*; [k]*Pseudomonas aeruginosa*; [l]*Pseudomonas alcaligenes*; [m]*Pseudomonas chlororaphis*; [n]*Serratia liquefaciens*; [o]*Serratia marcescens*; [p]*Aeromonas hydrophila*; [q]*Burkholderia cepacia*; [r]*Chromobacterium violaceum*; [s]*Flavimonas oryzihabitans*; [t]*Ochrobactrum anthropi*; [u]*Oligella urethralis*; [v]*Vibrio fluvialis.*

The isolation frequency and mean concentration of GNB-E in water samples from rural sources was significantly greater than in water samples from urban sources (respectively 61.1% vs. 20.8%, 17.1 vs. 3.4 × 10^1 cfu/l, $p<0.001$). The GNB-E isolation frequency and mean concentration were also distinctly greater in total well water samples than in urban MWSS samples (respectively 75.0% vs. 26.7%, 23.5 vs. 3.4 × 10^1 cfu/l, $p<0.00001$) and in total MWSS samples (respectively 75.0% vs. 26.7%, 23.5 vs. 4.6 × 10^1 cfu/l, $p<0.00001$). No significant differences in the isolation frequency and concentration of GNB-E were found between the water samples from rural and urban MWSS.

Isolation frequency of GNB-NE in water samples from rural sources was significantly greater compared to that from urban sources (77.8% vs. 47.2%, $p<0.01$), but there was no significant difference in the concentration of GNB-NE in both sample sets. Similarly, the isolation frequency of GNB-NE in total well water samples was greater compared to that in total (rural + urban) MWSS samples (81.3% vs. 54.7%, $p<0.05$), but there was also no significant difference in the concentration of GNB-NE in both sample sets. The isolation frequency and concentration of GNB-NE in total well water samples were significantly greater compared to samples from offices and shops and showers ($p<0.01$). In contrast, the

Table 3. Occurrence of *Legionella* and other Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system on farms in 5 villages (22 samples)[a]. Concentration of bacteria is expressed in cfu × 10^1/l.

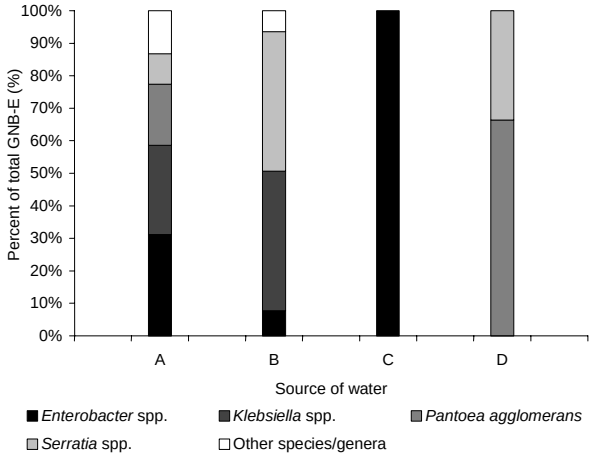
Village, site	*Legionella pneumophila* type 2-14	*Legionella* spp.	Total *Legionella* (GVPC)	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella oxytoca*	*Serratia plymuthica*	*Shigella* spp.	Total Enterobacteriaceae (EMB)	*Acinetobacter* spp.	*Chryseobacterium meningosepticum*	*Chryseomonas luteola*	*Flavimonas oryzihabitans*	*Pseudomonas fluorescens*	*Stenotrophomonas maltophilia*	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Village B																
Site 1	1.3	0	1.3	5.0	0	0	0	0	5.0	30.0[c]	0	0	0	0	7.0	37.0
Site 2	1.7	0	1.7	0	0	0	0	0	0	40.0[d]	0	0	0	0	0	40.0
Site 3	1.3	0	1.3	6.0	0	0	0	0	6.0	13.0[d]	4.0	0	0	0	0	17.0
Site 4	0	0	0	3.0	5.0	0	0	0	8.0	0	0	0	0	0	0	0
Site 5	0	1.3	1.3	0	0	0	0	4.0	4.0	60.0[c]	0	0	0	0	0	60.0
Site 6	0	0	0	0	0	0	0	0	0	120.0[d]	0	0	0	0	0	120.0
Village C																
Site 1	1.0	0	1.0	0	0	3.0	0	0	3.0	2.0[c]	0	0	0	0	0	2.0
Site 2	1.3	0	1.3	0	0	0	0	3.0	3.0	0	0	0	0	0	0	0
Site 3	0	0	0	0	0	0	0	0	0	4.0[c]	0	0	0	0	0	4.0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.0	10.0
Site 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.0	2.0
Site 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.0	20.0
Site 7	1.3	0	1.3	0	0	0	0	0	0	40.0[c]	0	0	0	0	8.0	48.0
Village D																
Site 1	0	2.0	2.0	0	0	0	0	0	0	0	0	30.0	6.0	0	2.0	38.0
Site 2	0	0	0	0	0	0	0	0	0	0	0	9.0	11.0	10.0	0	30.0
Village G																
Site 1	0	0	0	0	0	60.0	70.0	0	130.0	0	0	0	0	0	0	0
Site 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.0	2.0
Site 3	0	0	0	0	0	3.0	2.0	0	5.0	0	0	0	0	0	0	0
Site 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Site 5	0	0	0	0	0	6.0	0	0	6.0	0	0	0	0	0	0	0
Site 6	0.7	0	0.7	0	0	0	0	0	0	0	0	4.0	0	0	0	4.0
Village H																
Site 1	0	0	0	0	0	0	0	0	0	5.0[d]	0	0	7.0	0	0	12.0
Total positive*	7/22 (31.8)	2/22 (9.1)	9/22 (40.9)	3/22 (13.6)	1/22 (4.5)	4/22 (18.2)	2/22 (9.1)	2/22 (9.1)	9/22 (40.9)	9/22 (40.9)	1/22 (4.5)	3/22 (13.6)	3/22 (13.6)	1/22 (4.5)	7/22 (31.8)	16/22 (72.7)
Median	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.0
Mean[b]	0.4 ± 0.6	0.2 ± 0.5	0.6 ± 0.7	0.6 ± 1.7	0.2 ± 1.1	3.3 ± 12.8	3.3 ± 14.9	0.3 ± 1.0	7.7 ± 27.4	14.3 ± 29.1	0.2 ± 0.9	2.0 ± 6.6	1.1 ± 2.9	0.5 ± 2.1	2.3 ± 4.9	20.4 ± 28.9

* - % in brackets; [a]At each site one sample was taken; [b] $\bar{x}$ ± S.D.; [c]*Acinetobacter calcoaceticus*; [d]*Acinetobacter lwoffii*.

concentration of GNB-NE in water samples from medical appliances was significantly greater compared to all other sets of water samples, including total well water samples ($p<0.001$). Altogether, the isolation frequencies of GNB-NE in total well water samples and in rural MWSS water samples were significantly greater compared to that in urban MWSS water samples ($p<0.05$), but there were no significant differences in concentration of GNB-NE between those sets of samples.

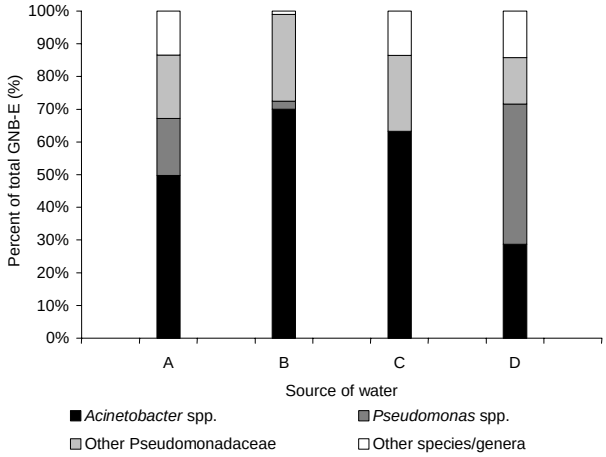
Relationships between particular groups of GNB in potable water. Concentrations of *Legionella* in the water samples from rural sources, urban sources, and in the total examined samples (both from rural and urban sources) were significantly smaller compared to GNB-NE concentrations ($p<0.0001$). Concentrations of *Legionella* in the water samples from rural sources and in total examined samples, but not in water samples from urban sources, were significantly smaller compared to GNB-E concentrations ($p<0.001$). A significant correlation was found between concentrations of *Legionella* and GNB-NE for rural MWSS water samples ($p<0.05$), urban MWSS water samples ($p<0.001$), total MWSS water samples ($p<0.001$), and total examined water samples ($p<0.001$), but not for the total well water samples. No significant correlation was found between concentrations of *Legionella* and GNB-E for either set of water samples.

GNB-NE concentrations were significantly greater than GNB-E concentrations in the rural MWSS water samples ($p<0.05$), urban MWSS water samples ($p<0.001$), and total examined water samples ($p<0.001$), but not in the total well water samples. A significant correlation was found between concentrations of GNB-E and GNB-NE for the urban MWSS water samples ($p<0.001$), but not for



A – well water sampled directly from wells and from private systems supplying well water; B – water from rural MWSS; C – water from urban MWSS sampled in offices, shops and dwellings; D – water from urban MWSS sampled in health care units.

Figure 1. Gram-negative bacteria belonging to family Enterobacteriaceae (GNB-E) isolated from various sources of water.



A – well water sampled directly from wells and from private systems supplying well water; B – water from rural MWSS; C – water from urban MWSS sampled in offices, shops and dwellings; D – water from urban MWSS sampled in health care units.

Figure 2. Gram-negative bacteria not belonging to family Enterobacteriaceae (GNB-NE) isolated from various sources of water.

the total well water samples, for rural MWSS water samples, and for the total examined water samples.

Species composition of GNB-E and GNB-NE in potable water. In the total well water samples the most common were GNB-E species of the genera *Enterobacter* (*E. cloaceae*, *E. intermedius*) and *Klebsiella* (*K. ornithinolytica*, *K. oxytoca*, *K. planticola*, *K. terrigena*) which formed respectively 31.2% and 27.5% of the total count, while in the rural MWSS water samples predominated species *Klebsiella oxytoca* and *Serratia plymuthica*, each of them forming 42.9% of the total count (Fig. 1, Tab. 1-3). In the urban MWSS water samples from shops, offices and dwellings the only detected GNB-E species was *Enterobacter intermedius*, occurring in low concentration, whereas in the urban MWSS water samples from health care units predominated *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) and *Serratia liquefaciens* which formed respectively 66.4% and 33.6% of the total count (Fig. 1, Tab. 4-7). Altogether, the following 14 GNB-E species and/or genera were identified in the examined samples of potable water: *Citrobacter freundii*, *Enterobacter cloaceae*, *Enterobacter intermedius*, *Escherichia coli*, *Klebsiella ornithinolytica*, *Klebsiella oxytoca*, *Klebsiella planticola*, *Klebsiella terrigena*, *Pantoea agglomerans*, *Salmonella* spp., *Serratia liquefaciens*, *Serratia marcescens*, *Serratia plymuthica*, *Shigella* spp. (Tab. 1-7).

Among GNB-NE strains, isolated from the examined potable water samples, distinctly predominated 2 groups of bacteria: • *Acinetobacter* strains; • strains of *Pseudomonas* and related genera, belonging to family Pseudomonadaceae (Fig. 2). In the total well water samples, the most common were *Acinetobacter* species (*A. calcoaceticus*, *A. lwoffii*), *Pseudomonas* species (*Ps. aeruginosa*, *Ps. alcaligenes*, *Ps. chlororaphis*, *Ps. fluorescens*), and other species of family Pseudomonadaceae (*Brevundimonas diminuta*, *Burkholderia cepacia*, *Chryseomonas luteola*, *Flavimonas oryzihabitans*, *Stenotrophomonas maltophilia*) which formed respectively 49.8%, 17.4%, and 19.4% of the total count (Fig. 2, Tab. 1-2).

Table 4. Occurrence of *Legionella* and other Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system of the city of Lublin in offices and shops (11 samples)[a]. Concentration of bacteria cfu × 10^1/l.

	Legionella (GVPC)	*Enterobacter intermedius*	Total Enterobacteriaceae (EMB)	*Acinetobacter calcoaceticus*	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Offices					
Site 1	0	0	0	20.0	20.0
Site 2	0	0	0	0	0
Site 3	0	0	0	60.0	60.0
Site 4	0	0	0	0	0
Site 5	0	0	0	0	0
Site 6	0	0	0	0	0
Shops					
Site 1	0	0	0	0	0
Site 2	0	0	0	0	0
Site 3	0	8.0	8.0	0	0
Site 4	0	0	0	0	0
Site 5	0	0	0	0	0
Total positive	0/11 (0)	1/11 (9.1%)	1/11 (9.1%)	2/11 (18.2%)	2/11 (18.2%)
Median	0	0	0	0	0
Mean[b]	0	0.7±2.4	0.7±2.4	7.3±18.5	7.3±18.5

[a]At each site one sample was taken; [b] $\bar{x}$ ± S.D.

Table 5. Occurrence of *Legionella* and other Gram-negative bacteria in samples of tap water taken from the outlets of the municipal water supply system in dwellings of the city of Lublin (8 samples)[a]. Concentration of bacteria is expressed in cfu × 10^1/l.

Site	*Legionella pneumophila* type 2-14	*Legionella* spp.	Total *Legionella* (GVPC)	Total Enterobacteriaceae (EMB)	*Acinetobacter* spp.	*Chryseomonas luteola*	*Empedobacter brevis*	*Flavimonas oryzihabitans*	*Stenotrophomonas maltophilia*	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Site 1	0	0	0	0	0	0	0	10.0	0	10.0
Site 2	0	0	0	0	20.0[c]	0	0	0	0	20.0
Site 3	0	0	0	0	10.0[c]	0	0	0	0	10.0
Site 4	0	0	0	0	0	0	0	0	0	0
Site 5	3.3	0	3.3	0	60.0c	0	0	0	0	60.0
Site 6	1.7	1.7	3.4	0	0	35.0	20.0	0	40.0	95.0
Site 7	3.3	0	3.3	0	60.0[d]	0	0	0	0	60.0
Site 8	4.0	0	4.0	0	25.0[c]	0	30.0	0	0	55.0
Total positive	4/8 (50.0%)	1/8 (12.5%)	4/8 (50.0%)	0/8 (0)	5/8 (62.5%)	1/8 (12.5%)	2/8 (25.0%)	1/8 (12.5%)	1/8 (12.5%)	7/8 (87.5%)
Median	0.9	0	1.7	0	15.0	0	0	0	0	37.5
Mean[b]	1.5 ± 1.8	0.2 ± 0.6	1.7 ± 1.9	0	21.9 ± 25.3	4.4 ± 12.4	6.2 ± 11.9	1.3 ± 3.5	5.0 ± 14.1	38.8 ± 33.5

[a]At each site one sample was taken; [b] $\bar{x}$ ± S.D.; [c]*Acinetobacter calcoaceticus*; [d]*Acinetobacter lwoffii*.

Table 6. Occurrence of *Legionella* and other Gram-negative bacteria in samples of tap water taken from showerheads in various health care units located in the city of Lublin (19 samples). Concentration of bacteria is expressed in cfu × 10^1/l.

Health care unit, sampling sites	Number of samples	*Legionella* (GVPC)	*Pantoea agglomerans*	Total Enterobacteriaceae (EMB)	*Pseudomonas putida*	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Outpatient health care unit A, sites 1-6	6	0	0	0	0	0
Outpatient health care unit B, sites 1-3	3	0	0	0	0	0
Rehabilitation centre, site 1	1	0	30.0	30.0	45.0	45.0
Clinic, pulmonology department, sites 1-3	3	0	0	0	0	0
Clinic, internal department, sites 1-6	6	0	0	0	0	0
Total positive		0/19(0)	1/19(5.3%)	1/19 (5.3%)	1/19 (5.3%)	1/19 (5.3%)
Median concentration		0	0	0	0	0
Mean concentration		0	1.6 ± 6.9	1.6 ± 6.9	2.4 ± 10.3	2.4 ± 10.3

In the rural MWSS water samples predominated *Acinetobacter* species (*A. calcoaceticus*, *A. lwoffii*), and other species of the family Pseudomonadaceae (*Ch. luteola*, *Flavimonas oryzihabitans*, *St. maltophilia*) which formed respectively 70.1% and 26.5% of the total count (Fig. 2, Tab. 3). *Acinetobacter* species (*A. calcoaceticus*, *A. lwoffii*), and other species of family Pseudomonadaceae (*Ch. luteola*, *Fl. oryzihabitans*, *St. maltophilia*) predominated also in the urban MWSS water samples from shops, offices and dwellings, forming respectively 63.3% and 23.2% of the total count (Fig. 2, tab. 4-5).

In the urban MWSS water samples from health care units predominated *Pseudomonas* species (*Ps. aeruginosa*, *Ps. alcaligenes*, *Ps. putida*), and *Acinetobacter lwoffii* which formed respectively 42.8% and 28.8% of the total count (Fig. 2, Tab. 6-7). Altogether, the following 20 GNB-NE species were identified in the examined samples of potable water: *Acinetobacter calcoaceticus*, *Acinetobacter lwoffii*, *Aeromonas hydrophila*, *Brevundimonas diminuta*, *Burkholderia cepacia*, *Chromobacterium violaceum*, *Chryseobacterium meningosepticum*, *Chryseomonas luteola*, *Empedobacter brevis*, *Flavimonas oryzihabitans*, *Ochrobactrum anthropi*, *Oligella urethralis*, *Photobacterium damsela*, *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, *Vibrio fluvialis*. In the total examined water samples, the numbers of *A. calcoaceticus* and *A. lwoffii*

Table 7. Occurrence of *Legionella* and other Gram-negative bacteria in samples of tap water taken from the outlets of various medical appliances used for hydrotherapy in a rehabilitation centre located in the city of Lublin (15 samples)[a]. Concentration of bacteria is expressed in cfu × 10^1/l.

Appliance, site	*Legionella pneumophila* type 2-14 (GVPC)	*Pantoea agglomerans*	*Serratia liquefaciens*	Total Enterobacteriaceae (EMB)	*Acinetobacter lwoffii*	*Burkholderia cepacia*	*Chryseomonas luteola*	*Photobacterium damsela*	*Pseudomonas aeruginosa*	*Pseudomonas alcaligenes*	Total Non Enterobacteriaceae (Tryptic Soya Agar)
Underwater manual massage											
Site 1	0	0	6.0	6.0	0	25.0	0	16.0	0	0	41.0
Site 2	0	0	6.0	6.0	0	25.0	0	35.0	0	0	60.0
Site 3	0	0	6.0	6.0	0	0	0	26.0	10.0	0	36.0
Site 4	0	0	6.0	6.0	35.0	0	0	36.0	0	35.0	106.0
Underwater vibration massage											
Site 1	2.3	20.0	6.0	26.0	10.0	0	0	0	0	50.0	60.0
Site 2	0	10.0	0	10.0	15.0	0	0	20.0	0	60.0	95.0
Site 3	0	10.0	10.0	20.0	20.0	0	0	0	0	60.0	80.0
Needle-bath											
Site 1	5.0	0	10.0	10.0	25.0	0	10.0	0	0	60.0	95.0
Site 2	6.7	0	0	0	35.0	0	16.0	0	0	30.0	81.0
Site 3	0	0	0	0	30.0	0	15.0	0	0	55.0	100.0
Site 4	0	0	5.0	5.0	10.0	0	17.0	0	0	35.0	62.0
Hubard's tank											
Site 1	3.3	45.0	0	45.0	30.0	0	0	0	0	6.0	36.0
Site 2	6.7	0	0	0	25.0	0	0	0	0	10.0	35.0
Site 3	0	0	0	0	35.0	30.0	0	0	0	0	65.0
Site 4	0	0	0	0	30.0	10.0	0	15.0	0	0	55.0
Total positive	5/15 (33.3%)	4/15 (26.7%)	8/15 (53.3%)	9/15 (60.0%)	12/15 (80.0%)	4/15 (26.7%)	4/15 (26.7%)	6/15 (40.0%)	1/15 (6.7%)	10/15 (66.7%)	15 (100%)
Median concentration	0	0	5.0	6.0	25.0	0	0	0	0	30.0	62.0
Mean concentration[b]	1.6±2.6	5.7±12.4	3.7±3.8	9.4±12.4	20.0±13.2	6.0±11.0	3.9±6.8	9.9±13.7	0.6±2.6	26.7±25.5	67.1±24.5

[a]At each site one sample was taken; [b] $\overline{x}$ ± S.D.

were similar, forming respectively 53.3% and 46.7% of the total *Acinetobacter* isolates. However, it is noteworthy that *A. calcoaceticus* distinctly predominated in the well water samples forming 87.0% of total *Acinetobacter* strains while *A. lwoffii* prevailed in the MWSS water samples, forming 61.7% of total *Acinetobacter* strains. Among *Pseudomonas* strains isolated from the MWSS water samples, the predominant species was *Pseudomonas alcaligenes*, which occurred abundantly in water samples from medical appliances and formed 86.1% of the total MWSS isolates. By contrast, in well water samples, the species *Ps. fluorescens*, *Ps. aeruginosa*, and *Ps. alcaligenes* occurred in similar numbers, forming respectively 34.7%%, 30.6%, and 27.8% of the total *Pseudomonas* isolates.

DISCUSSION

Bacteria of the genus *Legionella* colonize a wide range of natural and artificial water biotopes, mostly those with high temperature (over 20ºC) and high oxygen content, containing biofilms and amoebae [20, 49, 72]. The presence of legionellae in hot springs used as a source of potable water was reported [1, 74], but only a little is known about their occurrence in cold water springs and wells. Leoni *et al.* [33] have not isolated *Legionella* from wells providing spa water for aerosol hydrotherapy, but found them in the aerosol generating devices.

The present study demonstrates that *Legionella* occurs in 27.8-28.6% samples of untreated, cold well water used for consumption by rural population in eastern Poland in the concentration of 1.3-13.3 × 10^1 cfu/l. Incidence of *Legionella* in the well water and MWSS water samples from villages of eastern Poland may explain, at least in part, a high (34.3%) prevalence of seropositive reactions to *Legionella* antigen among rural inhabitants of this region found by us in an earlier work [65]. The results of the present study are in accordance with those reported by Straus *et al.* [69] who found by epidemiological interview that the non-municipal water supply increases signifi-

cantly the risk of community-acquired legionellosis in the state of Ohio, USA.

Nevertheless, the results of the present study demonstrate also the presence of *Legionella* in the outlets of the municipal water supplying system distributing chlorinated water, mostly in those where the presence of aerators or other endings in taps or other outlets favoured proliferation of legionellae. Such conditions occurred in rural and urban dwellings and medical appliances used for hydrotherapy in a rehabilitation centre, where *Legionella* was found in respectively 40.9%, 50.0% and 33.3% of samples in the concentrations of 0.7-1.7, 1.7-4.0, and 2.3-6.7 × 10^1 cfu/l, respectively. No *Legionella* strains were isolated from water samples taken from taps in offices and shops, which were mostly not equipped with aerators, and from showerheads in health care centres. According to criteria quoted by Arnow *et al.* [2] and Stypułkowska-Misiurewicz and Pancer [72], the concentrations of Legionella found in the present study in the MWSS water samples are low, as they do not exceed the levels of 1.0 × 10^4 cfu/l and 1.0 × 10^3 cfu/l, respectively. However, according to Kool *et al.* [25], the numbers of cases of nosocomial legionellosis in Texas, USA correlated better with the isolation frequency than with the concentration of *Legionella*, and thus the results of the present study indicate the presence of a moderate health risk in examined settings.

The frequency of *Legionella* isolation in dwellings, recorded in the present study, is similar to the values hitherto reported for: Italian dwellings [7, 34], German dwellings [36], Polish dwellings (Warsaw) [72], US dwellings [2], and Finnish apartment buildings [77]; it is lower compared to data reported for Italian hotels [34]. The stated concentrations of legionellae are usually lower compared to the above-cited authors [2, 36, 77].

The common occurrence of *Legionella* in the water distribution systems of hospitals and other health care units has been reported by many authors [11, 19, 25, 28, 31, 34, 55], and this bacterium is regarded as a common cause of nosocomial infections which may be transmitted by potable water through contaminated taps, showers, inhalators, dental unit waterlines, eyewash stations, or bathing appliances [11, 19, 20, 21, 25, 28, 30, 35, 44, 48, 49, 51, 55, 71, 72, 73]. These infections could be evoked by the strains belonging to *Legionella pneumophila* serogroups 2-14 - such as serogroup 5 [44, 51] or serogroup 6 [11, 21] - which were predominant among the *Legionella* strains isolated in this study.

Our results show that the medical appliances used for hydrotherapy, such as needle-bath, Hubard's tank or appliance for underwater vibration massage should be considered as a potential source of infection, either via the pulmonary route by the inhalation of droplet aerosol generated during treatment [72], or via an extrapulmonary route by direct exposure of traumatized skin to contaminated tap water [35]. For the possibility of infection during hydrotherapy remedies indicate the described in Japan cases of legionellosis acquired by taking a bath in a hospital [44], by taking a private whirlpool bath [21], or by exposure to communal bath water [47].

In contrast to some earlier papers [11, 20, 27, 28, 32], this study does not confirm the role of showers in the spreading of *Legionella* infection. Altogether, isolation frequency and/or concentration of *Legionella* found in the present work in water samples from health care centres is similar to those reported for a hospital in Canada [39], and lower compared to those reported for hospitals in Italy [31, 34], Germany [22, 36, 55], Denmark [3], the Netherlands [20], Poland (Warsaw) [48], and the USA [19]. They are also lower compared to those reported for balneotherapy facilities in Poland [27], and eyewash stations in the USA [49]. Until recently, no cases of nosocomial legionellosis were diagnosed in the health care units examined in the present study.

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 isolation of *Yersinia* strains from well water in rural environment [38, 63].

Gram-negative bacteria belonging to the family Enterobacteriaceae (GNB-E) occurred in well water samples in numbers similar to those of Gram-negative bacteria not belonging to family Enterobacteriaceae (GNB-NE). By contrast, in water samples from the outlets of municipal water supply systems (MWSS), the GNB-E numbers were significantly smaller compared to those of GNB-NE. Most probably this was due to greater vulnerability to chlorination, and imperfect adaptation to persist and proliferate in specific ecological niches of MWSS, such as aerated taps.

Most of GNB-E isolates from well water belonged to lactose-fermenting strains (*Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*) described as "coliforms", the presence of which in quantities equal to or exceeding 1 cfu per 100 ml of potable water (1.0 × 10^1 cfu/l) is not permitted by Polish [54] and international [12] sanitary regulations. According to this criterion, 71.9% of well water samples examined in this study did not meet sanitary requirements. These results correspond with those reported by Tymczyna and Gołuszka [75] who found excessive numbers of coliforms in 80-90% of examined wells in southern Poland. The frequent presence of GNB-E in well water may be due, at least in part, to contamination with manure and sewage from neighbouring animal houses, and/or with decaying plant and animal matter. The water samples from rural and urban MWSS were contaminated with coliforms to a much lesser extent than well water, in 31.8% and 1.9%, respectively.

Isolation frequency of GNB-NE in water samples from rural sources was significantly greater compared to that from urban sources, but relatively large concentrations of GNB-NE were noted in water samples from medical appliances used for hydrotherapy and from tap water in dwellings. Most probably this was due to the fact that the

nozzles of medical appliances and taps in dwellings are equipped with aerators or other endings for better outflow of water. These gadgets enable the formation of biofilms and create optimal, oxygen-rich micro-ecological conditions for persistence and proliferation of aerobic bacteria, such as GNB-NE and legionellae. Accordingly, a highly significant correlation was found between the numbers of GNB-NE and *Legionella* in the MWSS water samples, but not in well water samples where micro-ecological conditions were different. This finding is in accordance with that of Zietz *et al.* [79] who described parallel occurrence of *Legionella* and *Pseudomonas* in greenhouse watering systems, and with the observation made by Koide *et al.* [24] that the multiplication of *Legionella* was supported by a strain of *Pseudomonas vesicularis*. By contrast, it does not confirm the lack of correlation between *Legionella* and Pseudomonadaceae stated in MWSS water samples in Italy [34], similar to the inverse correlation between *Legionella* and other Gram-negative bacteria or between *Legionella* and *Pseudomonas aeruginosa* in shower water samples that was reported by Leoni *et al.* [32]. On the contrary, in our MWSS samples, Pseudomonadaceae were one of the main GNB-NE components and contributed to the overall positive correlation of GNB-NE with *Legionella*. Moreover, a separate positive correlation could be found between Pseudomonadaceae and *Legionella* ($p<0.05$). These results could well be explained by the fact that both Pseudomonadaceae and *Legionella* grow well in similar aerobic niches constituted by water aerators. In an earlier work, Stojek [67] found 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%). In this context, it is noteworthy that Weber *et al.* [76] described cases of nosocomial infections caused by *Stenotrophomonas maltophilia* which colonized tap aerators in the patients' rooms, and contaminated potable water. Our results support the recommendations of these authors that consideration should be given to routine disinfection or removal of the aerators.

Numbers of GNB-NE in the examined samples of potable water were relatively low, distinctly below the permissible values for total heterotroph (Gram-negative and Gram-positive) bacteria (2.0×10^4-5.0×10^5 cfu/l) recommended by Polish [54] and international [50] regulations. They were much lower compared to the numbers of *Pseudomonas* spp. detected by Zietz *et al.* [79] in greenhouse watering systems and by Paszko-Kolva *et al.* [49] in eyewash stations, compared to the numbers of Pseudomonadaceae found by Leoni *et al.* [33] in water used for aerosol hydrotherapy, and compared to the numbers of GNB-NE found by Botzenhart *et al.* [9] in MWSS water samples from hospitals.

Out of 34 GNB-E and GNB-NE species and/or genera isolated from the samples of potable water examined in the present study, at least 21 were reported as obligatory or opportunistic agents of infectious diseases. These are the following species and/or genera: *Acinetobacter calcoaceticus*, *Acinetobacter lwoffii*, *Aeromonas hydrophila*, *Burkholderia cepacia*, *Chromobacterium violaceum*, *Chryseobacterium meningosepticum*, *Chryseomonas luteola*, *Citrobacter freundii*, *Enterobacter cloaceae*, *Escherichia coli*, *Klebsiella oxytoca*, *Ochrobactrum anthropi*, *Pantoea agglomerans*, *Photobacterium damsela*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Serratia liquefaciens*, *Serratia marcescens*, *Shigella* spp., *Stenotrophomonas maltophilia*, *Vibrio fluvialis* [78]. Some of these pathogens, such as *Acinetobacter calcoaceticus*, *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 [30, 50, 56, 67]. *Acinetobacter calcoaceticus*, a predominant species among GNB-NE recovered in this study, may cause skin and wounds infections, pneumonia, endocarditis, meningitis, urethritis, and other ailments [67]. *Aeromonas hydrophila*, isolated in this study from well water, is a known fish pathogen which may cause in humans bacteremia, gastroenteritis and wound infections after contact with contaminated water [56, 67, 78]. Rylander *et al.* [58] described cases of gastroenteritis in sewage workers after inhaling a droplet aerosol contaminated with *A. hydrophila*.

Inhaling of droplet aerosol from water contaminated with various species of Gram-negative bacteria at bathing, taking hydrotherapeutic remedies, exposure to polluted humidifiers, misting fountains, or similar sources of bioaerosol could also be a cause of allergic and/or immunotoxic diseases [26, 46]. These diseases, such as allergic alveolitis or toxic alveolitis [26], are less known compared to infectious ones and are often underestimated. They are evoked, among other factors, by various species of Gram-negative bacteria that produce biologically potent allergens, endotoxin and other toxins [16, 42, 43, 46]. Some of these species were isolated in relatively high numbers from potable water in the course of this study. Thus, the frequently isolated species *Acinetobacter calcoaceticus* was experimentally identified by Skórska as a producer of potent allergens and endotoxin [60, 61]. *Klebsiella oxytoca*, a coliform pathogen isolated in this study from well water and rural MWSS water samples, was identified as a cause of allergic alveolitis after repeated exposure to contaminated aerosol from a home humidifier [23]. *Pseudomonas fluorescens*, a bacterium isolated in this study from well water and rural MWSS, was implicated as a causative agent of allergic alveolitis in workers exposed to oil mist in metallurgic industry facilities [6].

Another potentially pathogenic species is *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*), isolated in the present study commonly from the samples of water from wells and medical appliances. This epiphytic bacterium occurs abundantly in airborne organic dusts [14, 16, 62], possesses strong

endotoxic and allergenic properties [13, 15, 41, 42, 57, 59] and was identified as a cause of allergic alveolitis and other diseases due to exposure to the dust [29, 37, 43].

CONCLUSIONS

1. Potable water from household wells in the rural environment of eastern Poland is contaminated by various Gram-negative bacteria which represent, in spite of relatively low numbers, a health risk for consumers because of the frequent presence of *Legionella*, coliform bacteria and other potentially pathogenic species.

2. Treated groundwater distributed by the rural and urban municipal water supply systems, on average, is less contaminated with *Legionella* and other Gram-negative bacteria compared to well water. A relatively high degree of water contamination with potentially pathogenic Gram-negative bacteria was revealed by medical appliances used for hydrotherapy. This poses for the users a potential risk of exposure to these bacteria by skin contact and inhaling of droplet aerosol.

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