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Escherichia coli and *Serratia fonticola* ESBLs as a potential source of antibiotics resistance dissemination in the Tricity water reservoirs*

Bartosz Rybak, Natalia Wawrzyniak, Lidia Wolska and Marta Potrykus✉

Department of Environmental Toxicology, Faculty of Health Sciences with Institute of Maritime and Tropical Medicine, Medical University of Gdansk, Gdańsk, Poland

Despite the fact that cephalosporins are rarely used in medical or veterinary treatment, the presence of Enterobacterales strains resistant to this group of anti-bacterial drugs (ESBL) is an important issue that requires attention. Between 2019 and 2021, 14 retention reservoirs, 12 streams, 3 rivers and 1 lake situated in the Tricity area (in northern Poland) were sampled for the presence of ESBL strains. Out of 40 water samples, characteristic growth (*Escherichia coli* and the KESC group) on Chromagar ESBL plates was observed for 33 samples. The average number of ESBL *E. coli* was 42±132 CFU/100 ml, while the KESC group was 73±147 CFU/100 ml. Out of 33 positive samples, 57 ESBL Enterobacterales strains were isolated, of which the most abundant species were *E. coli* (13 isolates) and *Serratia fonticola* (23 isolates). The *E. coli* ESBL isolates not only showed resistance to third generation cephalosporins but also to antibiotics from other groups, such as fluoroquinolones, aminoglycosides and sulfonamides. The *S. fonticola* ESBL isolates were also found to be mainly resistant to the third generation cephalosporins, with the exception of 5 imipenem and 2 ertapenem-resistant strains. These strains presented highly diverse fingerprinting profiles, as well as significant differences in phenotypic traits helpful for survival in the environment, such as biofilm formation and motility. Moreover, biofilm formation and the swimming ability were species and temperature dependent. We confirmed the presence of highly diverse ESBL strains with multiple drug resistance patterns in the Tricity water reservoirs. This could possibly pose a threat to human health and create a suitable ground for acquiring antibiotics resistance in the natural environment.

Keywords: Multi-drug resistant strains, ESBL-beta-lactamase, freshwater microbial contamination

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✉e-mail: marta.potrykus@gumed.edu.pl

***This paper is dedicated to Professor Wacław Tadeusz Szybalski on the 100th anniversary of his birth**

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Abbreviations: AMR, antimicrobial resistance; CFU, colony forming units; ESBL, Extended-Spectrum Beta-Lactamases; DID, defined daily doses; DDS, double disk synergy test; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization with Time Of Flight Mass Spectrometry; MICs, minimal inhibitory concentrations; OD, optical density; UPGMA, unweighted pair group method with arithmetic mean; PCA, principal component analysis

INTRODUCTION

Antibiotics have existed in the world since bacteria and fungi appeared on Earth, but people have noticed their effects relatively recently. The first potentially health-promoting use of tetracycline-containing beer was found in ancient Nubia around 350-550 BC (Kozińska *et al.,* 2017). In 1897, Ernest Duchesne concluded that some moulds inhibit the growth of pathogenic bacteria (Duckett, 1999). The discovery of antibiotics became a turning point in the history of mankind. The "age of antibiotics" with antibiotics considered as "miracle drugs" that could fight almost any bacterial infection, began in 1928 after the discovery of penicillin by Alexander Fleming (Tan *et al.,* 2015). Antibiotics have not only revolutionized medicine, but also had a huge impact on the socioeconomic well-being of people worldwide. Life expectancy has increased significantly, and mortality from many infectious diseases has decreased. People's productivity has increased by reducing absenteeism related to infectious diseases (Hutchings *et al.,* 2019). The production of antibiotics and chemotherapeutic agents is growing every year (Kümmerer *et al.,* 2009a; Kümmerer *et al.,* 2009b). The amount of antibiotics produced in the world is measured in millions of tons per year (ECDC, 2015). Antibiotics, when administered to humans or animals, are partially metabolized and then excreted via faeces and/or urine, both in metabolically active and inactivated forms. Furthermore, waste from the pharmaceutical industry and agri-food processing is of great importance with regards to the amount of metabolites and active forms of antibiotics released into the environment. They are released to municipal and industrial wastewater, and ultimately end up in surface waters. Waste and manure from animal husbandry is used as fertilizer on arable lands. This leads to the accumulation of antibiotics in the soil of farmlands and bottom sediments of water reservoirs, where antibiotics are being washed out from the soil, run off with rainwater and penetrate into the groundwater (Kümmerer *et al.,* 2003). Fish farms likewise can be a source of antibiotics and their residues for other water reservoirs, such as rivers, lakes or ponds. However, municipal sewage, including sewage from hospitals, is considered to be the main source of contamination with antibacterial drugs. During biological waste water treatment, bioreactors contain various active and inactive forms of antimicrobial agents and a high content of microorganisms. The concentrations of drugs at this stage of treatment can vary, but even if low, they can affect microorganisms and promote horizontal gene exchange of resistance genes (Thanner *et al.,* 2016). Next, the an-

tibiotic residues can be rinsed from the sewage sludge and move further, reaching rivers and the seas. While the majority of microorganisms resistant to antibiotics remains in the sludge of the sewage treatment plant, some of the drug-resistant bacteria may enter the natural environment (Galvin *et al.,* 2010; Zhi *et al.,* 2019). In fact, many antibiotics have been already found at detectable levels in the Polish water reservoirs and elsewhere (Koniuszewska *et al.,* 2020; Rodríguez-Pérez & Bajorath, 2020; Szymańska *et al.*, 2019; Rodriguez-Mozaz *et al.,* 2015; Zhang *et al.,* 2020).

The presence of antimicrobial contaminants in water, even at low concentrations, at first may not seem dangerous, but in the long run, a local accumulation of individual antimicrobial substances may occur, e.g. in the bottom sediments of water reservoirs, or in aquatic plants. At low concentrations, antibiotics are being detected both, in the surface and drinking waters. Despite low levels, these drugs are constantly released into the environment, which additionally increases their hazardous potential. Furthermore, their constant presence in a given area affects many generations of microorganisms.

Effluents from municipal wastewater plants contain a mixture of various groups of drugs, including antibiotics. Such mixtures may have a synergistic effect on individual drugs, enhancing their impact on the environment. The presence of antibiotics in waters influences the composition of the microbiome of a given ecological niche: pond, lake or river (Koniuszewska *et al.,* 2020). This may lead to selection of new drug resistance mechanisms in the environment or to enhanced spread of multi-drug resistant strains in water reservoirs. Antibiotics present in municipal wastewater pose a selective pressure, which not only promotes the growth of drug-resistant strains, but also causes a decrease in the number of drug-sensitive strains (Kümmerer *et al.,* 2003).

Surface waters mostly contain drug-sensitive bacteria, however drug-resistant bacteria are also detected in growing amounts. Antibiotic-resistant bacteria have been detected virtually in all water bodies in the world (Manji *et al.,* 2012; Mishra *et al.,* 2018; Kurekci *et al.,* 2017; El-Zanfaly, 2015; Azzam *et al.,* 2017; Danner *et al.,* 2019). People who use water bodies for recreational purposes constantly expose their skin and mucous membranes to bacteria which may be potentially antibiotic resistant. These bacteria can be either commensal, environmental or pathogenic strains which may cause infections, including *Pseudomonas aeruginosa*. On the other hand, drinking water contaminated with potentially pathogenic bacteria may be associated with food poisoning (Ling *et al.,* 2018). The risk of contamination becomes even greater when the bacteria that cause the infections are not susceptible to antibiotics. Worldwide, 700,000 people a year die from bacterial diseases that would be largely curable if the bacteria had not acquired resistance genes (De Oliveira *et al.,* 2020). The number of resistant bacteria is constantly increasing, and according to the World Health Organisation, by 2050 as many as 10 million people a year will have died from infectious diseases caused by drug-resistant microorganisms. The increasing number of people suffering from infectious diseases in areas where sewage treatment plants are unable to disinfect wastewater sufficiently causes in turn large-scale contamination of the environment with drug-resistant microorganisms (WHO Antimicrobial resistance: global report on surveillance 2014).

Despite the fact that cephalosporins are rarely used in hospital and veterinary treatment, the presence of strains resistant to this group of anti-bacterial drugs is an important fact that requires attention (Berendonk *et al.,* 2015). Bacilli of the Enterobacterales family, producing ESBL beta-lactamases (Extended-Spectrum Beta-Lactamases), have been and are still associated with nosocomial infections. Thus, during the 2019–2021 period we sampled 14 retention reservoirs, 12 streams, 3 rivers and 1 lake situated in the Tricity area, Poland, looking for the presence of ESBL strains, which could possibly pose a threat to human health and create a suitable ground for acquiring antibiotics resistance in the natural environment. We have analysed not only their antibiotic resistance patterns, but also phenotypic traits important for bacterial survival in the environment, as well as for their pathogenesis.

MATERIALS AND METHODS

Water sample collection

40 water samples were collected in the Pomorskie voivodship (Poland) on 17.06.2019, 30.05.2019, 06.12.2019, 20.03.2020, 21.03.2020, 13.03.2021 and 17.03.2021, from different water reservoirs, such as the retention reservoirs, streams and rivers passing towns (Fig. 1, Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). The water samples were collected into sterile 500-ml glass bottles, 1 meter away (if possible) from the shore of the water reservoirs, about 20 cm below the surface. Then, the samples were immediately transported to the laboratory in an isothermal box, kept at 4°C and processed within 24h. There are no intensive animal husbandry, slaughterhouses, fish farms or pharmaceutical plants in the vicinity of the studied water reservoirs. However, there are several large hospital centres in the area, although they are not in close vicinity of any of the sampled water reservoirs. In Gdansk, there are 57 retention reservoirs localized at 7 streams. All of the streams flow into the Gulf of Gdansk. Samples were collected from 14 randomly selected retention reservoirs, 3 rivers, 1 lake and 12 streams localized in Gdansk, Gdynia, Sopot, Reda and Puck (Fig. 1, Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp).

Isolation of ESBL strains

Membrane filtration method (Baird *et al.,* 2017) was used to examine the water samples. 50 mL samples were passed through sterile filters (0.22 μm pore diameter, Merck, Germany) with the use of a Merck Millipore membrane filtration set. Then, the filters were placed on the Chromagar ESBL medium (Graso Biotech, Poland) and incubated for 17–24 h at 37°C. Positive colonies were then counted (pink colonies for the presence of *E. coli*, blue colonies for the presence of Enterobacterales from the KESC group (*Klebsiella, Enterobacter, Serratia, Citrobacter*), and expressed as CFU/ 100 ml (Colony Forming Units) for each tested water reservoir. For positive plates, at least one colony was transferred onto a fresh medium and grown until pure culture was obtained. Isolated colonies were identified using MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization with Time Of Flight Mass Spectrometry) (MALDI Biotyper; Bruker Daltonics, USA) according to the manufacturer's procedure. Isolated colonies were also preserved by growing them in the LB medium (Graso Biotech, Poland) at 37°C for 24 h (150 rpm) and transferring 1 ml of bacterial cultures into 1.5 ml Eppendorf tubes and freezing them at –60°C with 20% glycerol (Epoch, Poland).

Antimicrobial Susceptibility Testing

The double-disk synergy (DDS) assay was carried out to identify ESBL-producing strains (Jarlier *et al.,* 1988). Subsequently, *E. coli* and KESC isolates were tested using a Kirby-Bauer disk diffusion assay for susceptibility to the following 16 antimicrobial agents according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) v.10.0 (2020) guidelines (European Committee on Antimicrobial Susceptibility Testing. Breakpoints tables for interpretation of MICs and zones diameters. Version 10.0, 2020, 2020): ampicillin (10 μg), amoxicillin/clavulanic acid (10/30 μg), piperacillin/tazobactam (30/6 μg), cefuroxime (30 μg), ceftazidime (10 μg), cefepime (30 μg), cefotaxime (5 μg), ciprofloxacin (5 μg), gentamycin (10 μg), amikacin (10 μg), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), tigecycline (15 μg), sulfamethoxazole/trimethoprim (25/25 μg), cefoxitin (30 μg) (Oxoid). *E. coli* ATCC 25922 (C49) was used as a reference strain (Supplementary Table 2 at https://ojs.ptbioch.edu.pl/index.php/abp).

Enzyme production, motility and biofilm formation assays

For all enzyme production assays, as well as motility and biofilm formation, the bacterial cultures were freshly streaked on LA medium (Graso Biotech, Poland) and grown at 37°C for 24 h. Then, bacterial suspensions were prepared from these fresh cultures in sterile 0.85% NaCl and adjusted to 0.5 MacFarland units (DensiCheK plus, Biomeriux). For enzyme and motility assays, 10 μl of each bacterial suspension was placed on the medium surface. Protease production was measured on a medium containing skim milk (2 g/l) after incubation for 48 h at 37°C and at 20°C (Ji *et al.,* 1987). The ability to produce DNases was assessed on DNase agar plates (Graso Biotech, Poland) after 48 h of incubation at 37°C and flooding the plates with 1N HCl (Sigma Aldrich, Germany). For both tests, the diameter of clear halo around the colonies was measured. All experiments were performed twice, with 4 replicates. To determine the swarming and swimming motility, bacterial strains were inoculated onto 0.8% and 0.3% semisolid LA agar plates, respectively, and incubated at 37°C and at 20°C for 24 h (Harshey *et al.,* 2003). The diameter of each halo was measured. In some cases, for swimming ability assays, the bacteria had overgrown the plates. In such cases, a 40 mm diameter was recorded. These tests were performed twice, with 4 replicates for swimming and 6 replicates for swarming motility assessment.

For biofilm formation, bacterial suspensions prepared as described above were 10x diluted in ½ LB medium (decreased amount of yeast extract and peptone). The biofilm formation assay was performed as in Nykyri *et al.* (2013), with some modifications. 1 ml of prepared bacterial culture was inoculated into 24-well Nunclon Delta Surface (Thermo Scientific) plates and kept for 48h at 37°C and at 20°C without agitation. After the incubation period, OD_{600} of bacterial cultures was measured and the bacterial cultures were removed from the wells. Then, 70 μl of 1% crystal violet solution was added into each well and left for 20 min without agitation. After incubation, the wells were washed 3 times with 900 μl of distilled water. Then, 900 μl of ethanol (96%, Sigma Aldrich, Germany) was added into each well and OD_{540} of each well was measured. For calculations, the OD_{540} value of the negative control was subtracted from the values obtained for the strains. Negative values obtained after subtraction were set to 0. The experiment was performed three times with two replicates.

As the reference for the above mentioned assays, *E. coli* ATCC25922 (C49) and *Serratia marcescens* KPD102-BA (C19) were used (Supplementary Table 2 at https://ojs.ptbioch.edu.pl/index.php/abp).

DNA fingerprinting

The genomic DNA from all chosen strains was isolated with the use of Genomic Mini AX Bacteria Kit (A&A Biotechnology, Poland). The genomic DNA concentration was measured with the use of Epoch Microplate Spectrophotometer (BioTek Instruments) and the chosen strains were analysed using a repetitive-sequence-based rep-PCR with ERIC primers, as described by Versalovic and others (Versalovic *et al.,* 1998). After PCR, 5 μl of the products were resolved in 0.8% agarose gels (0.5×TBE) at 50V for 2.5 h. After electrophoresis, the band patterns obtained for different strains were compared with the use of GelJ (Heras *et al.,* 2015). Similarity trees were composed with the unweighted pair group method with arithmetic mean (UPGMA) and band difference was set to 1.0. As a reference, *E. coli* strains C38, C44, C47, C48, C49 and *S. marcescens* C19 were used for comparative purposes (Supplementary Table 2 at https://ojs.ptbioch.edu.pl/index.php/abp).

Statistical analysis

The phenotypic assays (biofilm formation, motility) were tested for statistical significance with ANOVA followed by Tuckey post hoc test ($p<0.05$), with Statistica 13.3 (StatSoft Inc.). The principal component analysis was performed with the use of the Past software to enable global comparisons between the tested groups of strains (Hammer & Harper, 2001). The heatmap of phenotypic data was created in Python 3.8 (Van Rossum, 2007) with the Seaborn 0.11.1 package (Waskom, 2021) and Pandas 1.2.4 for visualization (McKinney, 2010) with Euclidean distance matrix. The map (Figure 1) was prepared with the Python 3.8.10 Folium package (Python wrapper to leaflet.js maps 1.7.1, https://python-visualization.github.io/folium/).

RESULTS

ESBLs are present in the Tricity waters

There are 57 retention reservoirs in Gdansk, and more are being designed and built. Due to the 3 geological elevation zones in the city, from depressed areas to the upland in the city, there is a high risk of flooding in case of heavy rainfall, when huge amounts of water flow from the upper terraces of the city towards the Gdansk Bay. Retention reservoirs in Gdansk have a total capacity of approx. 700,000 m^3, and are located in cascades on 7 streams. It creates an exceptional system for draining rainwater into the sea, not implemented in other Polish cities (Gdanskie Wody, 2021). During the 2019-2021 period we sampled 14 retention reservoirs, 12 streams, 3 rivers and 1 lake situated in the Tricity area (Poland) searching for the presence of ESBL strains (Fig. 1).

Of these, 19 samples were collected in Gdansk, 10 in Gdynia, 9 in Sopot and 1 each in Puck and Reda (the Plutnica and Reda rivers). Out of the 19 samples collected in Gdansk, 14 came from retention reservoirs, 1 from the Jasien Lake and 4 from streams. In Sopot and Gdynia, the samples were collected from 9 different

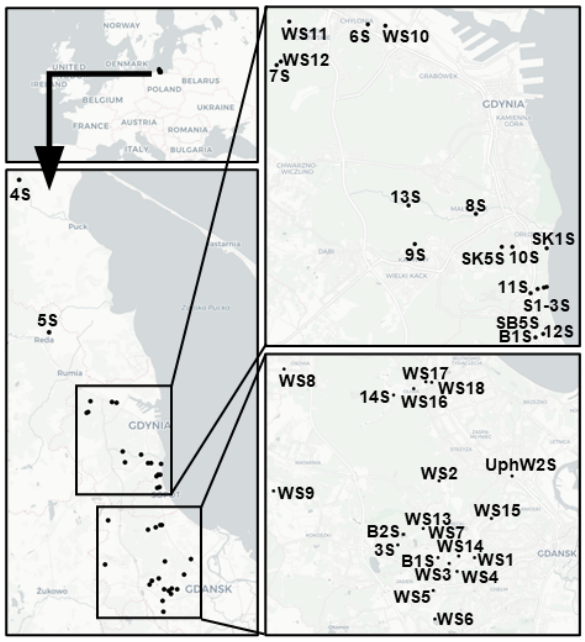

Figure 1. Geographical distribution of the water sampling points (rivers, lakes, streams, retention reservoirs) in the Tricity area (Gdansk, Gdynia, Puck, Reda, Sopot).
For details see Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp.

streams and 1 river (the Kacza River in Gdynia) (Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). In total, among the 40 water samples that were collected, characteristic growth (*E. coli* and KESC) on the Chromagar ESBL plates was observed for 33 samples (Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). The growth of the colonies characteristic for *E. coli* was observed in 20 samples, while for the KESC group in 32 samples. Average number of *E. coli* CFU/100 ml in water samples plated on Chromagar ESBL was 42±132 CFU/100 ml, while for the KESC group it was 73±147 CFU/100 ml. At two sampling points, WS16 (the Grunwaldzka retention reservoir) and UPH_W2S (Potok Krolewski stream), located in close proximity to each other, high amounts of both – *E. coli* and KESC group were found (Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). From the perspective of the whole set of data obtained from different sites, there are large differences between the studied cities. For example, for Gdansk, the number of *E. coli* and KESC group was the highest (81±185 and 130±200 CFU/100 ml, respectively), while in Sopot the lowest (1±1.4 and 4±4 CFU/100 ml, respectively) (Fig. 2, Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). Intermediary values were observed for Gdynia, Reda and Puck (Fig. 2). On average, the water samples coming from the streams were two times less contaminated with *E. coli* ESBL and 3 times less contaminated with KESC than the samples from retention reservoirs (Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). The rivers (Kacza, Plutnica, Reda) had very low levels of *E. coli* ESBL contamination (0.7±1.2 CFU/100 ml), while the load of KESC bacteria was similar to that observed for the streams (rivers – 50.0±14.0 CFU/100 ml, streams – 42.3±124.1 CFU/100 ml).

Strains with confirmed resistance to cephalosporins were isolated from 33 samples (82.5%). From a total of 33 positive samples, 56 strains belonging either to *E. coli* or the KESC group were isolated. The species of the isolated strains were identified with MALDI-TOF MS analysis (Table 1, Table 2). The most frequently isolated species were *Serratia fonticola* (41%) and *Escherichia coli* (21%). Moreover, 3 (5%) strains of *Enterobacter cloacae* were isolated, followed by 2 (4%) strains of *Citrobacter braakii* and *Citrobacter freundii* each. Only one strain belonging to *Enterobacter xiangfangensis* was identified, and as many as 11 (20%) strains from the *Aeromonas* genus were isolated that belonged to 5 different *Aeromonas* species (Table 1, Table 2).

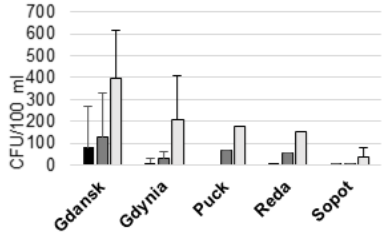

Figure 2. Average amount of ESBL strains detected with the ESBL Chromagar plates in different cities of the Tricity area (Gdansk, Gdynia, Puck, Reda, Sopot), expressed as CFU/100 ml of water sample +/- standard deviation.
Black bars represent ESBL *E. coli* strains, dark grey bars strains belonging to the KESC group, while light grey bars all other microorganisms able to grow on the medium. For details see Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp..

ESBLs isolated from the Tricity waters present diverse resistance to antibiotics

For further studies, strains belonging to two of the most representative species, namely *S. fonticola* and *E. coli,* were selected (35 isolates, Table 2). All analysed strains were tested for the presence of ESBL beta-lactamases using the DDS phenotypic test. Among the 35 strains tested, 34 were ESBL positive. One negative DDS-test strain (155) showed resistance to all analysed third generation cephalosporins, which may result from the AmpC

Table 1. Number of ESBL strains isolated from the Tricity waters (n=56) belonging to different species, as identified with MALDI-TOF MS.

Species	Number of isolates	% of isolates
Aeromonas bestiarum	3	5%
Aeromonas eucrenophila	2	4%
Aeromonas hydrophila	2	4%
Aeromonas salmonicida	1	2%
Aeromonas veronii	2	4%
Aeromonas spp.	1	2%
Citrobacter braakii	2	4%
Citrobacter freundii	2	4%
Enterobacter cloacae	3	5%
Enterobacter xiangfangensis	1	2%
Escherichia coli	12	21%
Rahnella aquatica	1	2%
Serratia fonticola	23	41%
Yersinia mollaretii	1	2%

Table 2. The ESBL Enterobacterales strains isolated from the Tricity water reservoirs in 2019–2021, identified with MALDI-TOF MS analysis. The strains taken for phenotypic and genotypic analysis are marked in bold.

Collection ID	Date of collection	City	Water reservoir	Species
127	06.12.2019	Gdansk	Zabornia retention reservoir	***Escherichia coli***
128	06.12.2019		Potokowa retention reservoir	***Escherichia coli***
129	06.12.2019		Potokowa retention reservoir	*Aeromonas hydrophila*
130	06.12.2019		Potokowa retention reservoir	*Aeromonas veronii*
131	06.12.2019		Potokowa retention reservoir	*Aeromonas hydrophila*
150	12.03.2020		Oliwa Park retention reservoir	***Escherichia coli***
151	12.03.2020		Grunwaldzka retention reservoir	***Escherichia coli***
152	12.03.2020		Grunwaldzka retention reservoir	***Serratia fonticola***
153	12.03.2020		Wilenska retention reservoir	***Serratia fonticola***
154	12.03.2020		Wilenska retention reservoir	***Serratia fonticola***
155	12.03.2020		Mysliwska retention reservoir	***Serratia fonticola***
163	12.03.2020		Osowa retention reservoir	*Enterobacter cloacae*
164	12.03.2020		Jasien lake	*Enterobacter cloacae*
165	12.03.2020		Jasien lake	*Enterobacter xiangfangensis*
166	12.03.2020		Swietokrzyska retention reservoir	***Serratia fonticola***
167	12.03.2020		Jabloniowa retention reservoir	*Aeromonas veronii*
168	12.03.2020		Jabloniowa retention reservoir	***Serratia fonticola***
169	12.03.2020		Cedrowa retention reservoir	***Escherichia coli***
170	12.03.2020		Labedzia retention reservoir	***Escherichia coli***
172	12.03.2020		Potokowa retention reservoir	***Serratia fonticola***
173	12.03.2020		Zabornia retention reservoir	***Escherichia coli***
174	12.03.2020		Potokowa retention reservoir	***Serratia fonticola***
182	12.03.2020		Oliwa Park retention reservoir	***Escherichia coli***
876	18.03.2021		Siedlicki stream	***Serratia fonticola***
890	18.03.2021		Nowiec stream	***Serratia fonticola***
156	12.03.2020	Gdynia	Cisowska stream	***Serratia fonticola***
157	12.03.2020		Cisowska stream	*Enterobacter cloacae*
158	12.03.2020		Cisowska stream	***Serratia fonticola***
159	12.03.2020		Cisowska stream	***Serratia fonticola***
160	12.03.2020		Chylońska stream	***Serratia fonticola***
199	12.03.2020		Chylonska stream	***Serratia fonticola***
802	18.03.2021		Cisowska stream	*Rahnella aquatica*
810	18.03.2021		Kacza river	***Serratia fonticola***
845	18.03.2021		Zrodlo Marii stream	***Serratia fonticola***
874	18.03.2021		Zrodlo Marii stream	*Citrobacter braakii*
877	18.03.2021		Chylonska stream	*Yersinia mollaretii*
886	18.03.2021		Chylonska stream	*Citrobacter braakii*
88	30.05.2019		Kolibkowski stream	*Aeromonass almonicida*
89	30.05.2019		Kolibkowski stream	*Aeromona seucrenophila*
90	30.05.2019		Kolibkowski stream	*Aeromonas bestiarum*
811	18.03.2021		Kolibkowski stream	***Serratia fonticola***
805	18.03.2021	Puck	Plutnica river	***Serratia fonticola***
809	18.03.2021		Plutnica river	***Serratia fonticola***

807	18.03.2021	Reda	Reda river	*Citrobacter freundii*
842	18.03.2021		Reda river	*Aeromonas spp.*
853	18.03.2021		Reda river	*Citrobacter freundii*
863	18.03.2021		Reda river	***Escherichia coli***
86	30.05.2019	Sopot	Babidolski stream	*Aeromonas eucrenophila*
87	30.05.2019		Babidolski stream	*Aeromonas bestiarum*
91	30.05.2019		Babidolski stream	*Aeromonas bestiarum*
102	17.06.2019		Swelina stream	***Escherichia coli***
103	17.06.2019		Swelina stream	***Escherichia coli***
104	17.06.2019		Swelina stream	***Escherichia coli***
866	18.03.2021		Elizy stream	***Serratia fonticola***
878	18.03.2021		Babidolski stream	***Serratia fonticola***
882	18.03.2021		Swelina stream	***Serratia fonticola***

beta-lactamase mechanism, however further confirmation is necessary.

All tested *E. coli* and *S. fonticola* strains were resistant to ampicillin (Supplementary Table 3 at https://ojs.ptbioch.edu.pl/index.php/abp). The analysed *E. coli* strains were more frequently resistant to more than one tested antibiotic than *S. fonticola* strains (Supplementary Table 3 at https://ojs.ptbioch.edu.pl/index.php/abp). However, one *S. fonticola* strain (155) was resistant to all tested antibiotics apart from tigecycline. This particular strain is of special interest since it was also negative in the DDS test for the presence of ESBL enzymes. Regarding groups of antibiotics, most strains (19/35) were resistant to at least one cephalosporin, while 10 strains were also resistant to fluoroquinolones. On the other hand, the most active antibiotic groups against bacteria were tetracycline and aminoglycosides (only 1 and 4 resistant strains, respectively). Similarly, only 5 strains were resistant to carbapenems and betalactams/betalactam inhibitors. Resistance to different third generation cephalosporins was different among the tested *E. coli* and *S. fonticola* strains. Noteworthy, all *E. coli* and *S. fonticola* strains were resistant to cefuroxime. Among the third generation cephalosporins, the most active against the tested strains was ceftazidime, to which 2 (9.1%) *S. fonticola* strains, and as many as 7 (63.6%) *E. coli* strains were sensitive (Fig. 3). The number of *E. coli* strains sensitive to third generation cephalosporins was much higher than that of *S. fonticola* strains. All *E. coli* strains were resistant to at least one of third generation cephalosporins, while among *S. fonticola* strains only 8 out of 22 showed a similar resistance.

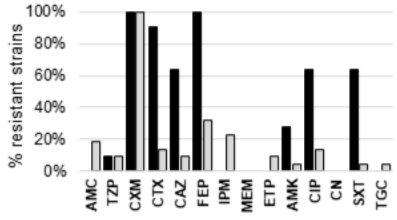

Figure 3. Percentage of *E. coli* (black bars) and *S. fonticola* (grey bars) strains resistant to different antibiotics.
AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; CXM, cefuroxime; CAZ, ceftazidime; FEP, cefepime; CTX, cefotaxime; CIP, ciprofloxacin; CN, gentamycin; AMK, amikacin; IPM, imipenem; MEM, meropenem; ETP, ertapenem; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole; FOX, cefoxitin

Similarly, the number of *E. coli* strains resistant to ciprofloxacin and trimethoprim/sulfamethoxazole was much higher (7 strains, 63.6%) than the number of *S. fonticola* resistant strains (3 strains, 13.6% and 1 strain, 4.5%, respectively) (Fig. 3).

The *E. coli* and *S. fonticola* isolates were most sensitive to carbapenems and especially to meropenem, to which no resistant strains were found (Fig. 3, Supplementary Table 3 at https://ojs.ptbioch.edu.pl/index.php/abp). All *E. coli* strains were sensitive to imipenem and ertapenem, contrary to *S. fonticola* isolates, where 5 strains (160, 199, 155, 153, 166) were not sensitive to imipenem and 2 (155, 199) were resistant to ertapenem. In this study, the mechanism of lower sensitivity to carbapenems of *S. fonticola* strains was not examined.

Another group of tested antibiotics with high antimicrobial activity were aminoglycosides. Almost all tested strains were sensitive to gentamycin, except for 3 *E. coli* strains (127, 128, 151) isolated from two streams and one reservoir in Gdansk, and 1 *S. fonticola* strain (155) isolated from a reservoir in Gdansk (Supplementary Table 3 at https://ojs.ptbioch.edu.pl/index.php/abp). Similarly, only 1 *E. coli* strain (170) and 2 *S. fonticola* strains (158, 155) were resistant to piperacillin/tazobactam, along with 4 other *S. fonticola* strains (809, 199, 158, 155) that were resistant to amoxicillin/clavulanic acid. Among the tested strains, only *S. fonticola* 155 was resistant to all tested groups of antibiotics, while 3 *E. coli* strains (127, 128, 151) were resistant to four groups (cephalosporins, aminoglycosides, fluoroquinolones and sulphonamides) of antibiotics.

Comparing isolates from both species, *E. coli* strains showed a higher incidence of multidrug resistance pattern, as 7 strains (104, 102, 127, 182, 150, 128, 151) were resistant to more than one antibiotic (Supplementary Table 3 at https://ojs.ptbioch.edu.pl/index.php/abp).

E. coli and *S. fonticola* ESBL strains show large diversity in phenotypic traits

Not only do the *E. coli* and *S. fonticola* strains show differences in antibiotics resistance, but they also present high diversity when looking at phenotypic traits important for pathogenesis and survival in harsh environments, such as motility, biofilm formation and production of extracellular enzymes. For 12 *E. coli* strains and 23 *S. fonticola* strains diverse phenotypic tests were performed at two different temperatures (20°C and 37°C) to

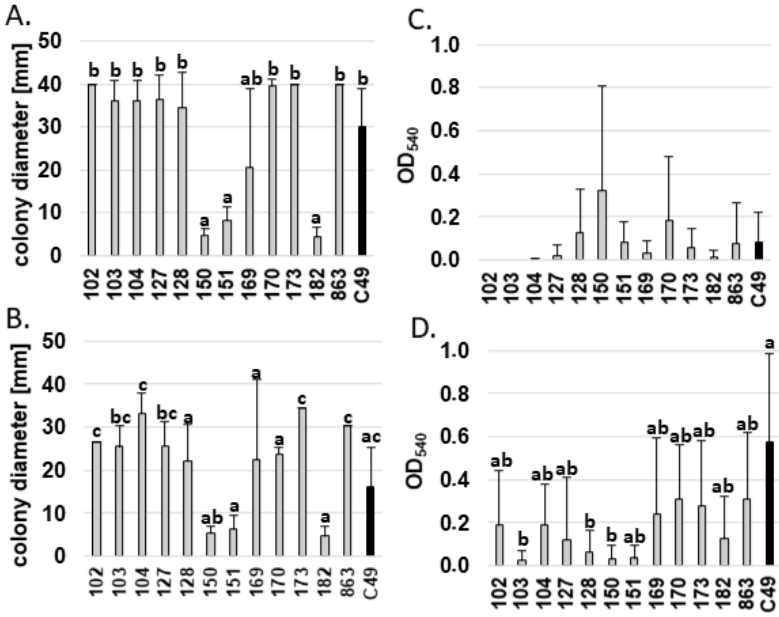

Figure 4. Swimming motility (A; B) and biofilm formation (C; D) of *E. coli* ESBL strains isolated from the Tricity water reservoirs tested at 37°C (A; C) and 20°C (B; D).
Swimming motility was expressed as an average colony diameter (mm) with standard deviation (n=4), while biofilm formation was expressed as an average OD_{540} value of crystal violet ethanol extractions after background subtraction, with standard deviation (n=2). Swimming motility was repeated twice, while biofilm formation three times. The results marked with different letters are statistically different as assayed with ANOVA and the Tuckey post hoc test, $p<0.05$.

highlight any differences in the strains' behaviour. None of the strains were able to produce DNases, nor proteases under tested conditions, except for the *S. marcescens* C19 reference strain (not shown). The swarming motility of the strains was similar for both *E. coli* and *S. fonticola* environmental isolates, and was about 3 times lower than the one observed for the *S. marcescens* C19 reference strain (not shown).

The swimming motility of the *E. coli* strains was approximately 30% higher at 37°C than at 20°C (Fig. 4AB). Only 3 strains presented statistically lower swimming motility (150, 151 and 182) at 37°C than other strains. Strains 150 and 182 where isolated from the same retention reservoir (Oliwa Park), while *E. coli* 151 was isolated from the Grunwaldzka retention reservoir, in close vicinity to the previous one. At 20°C, the swimming motility of most *E. coli* environmental isolates was higher than the one observed for *E. coli* C49, although this was not statistically significant. The ability to form biofilm was higher at 20°C than at 37°C for most strains except for *E. coli* 151. Interestingly, at 20°C the strains showing low swimming motility also showed low biofilm formation capability (150, 151, 182) under tested conditions (Fig. 4BD).

When looking at *S. fonticola* strains, they were compared to the *S. marcescens* C19 reference strain. *S. fonticola* environmental isolates presented higher swimming motility at 20°C than at 37°C. At 20°C, the swimming motility for all the *S. fonticola* strains (except for 882) was significantly lower than the one found for *S. marcescens* C19, while at 37°C only a few isolates were less motile than the reference strain (155, 172, 805, 876, 890) (Fig. 5AB). When looking at biofilm formation capability of *S. fonticola*, it was generally higher at 37°C than at 20°C, while swimming motility was generally higher at 20°C than at 37°C. However, at 37°C the strains with low biofilm formation capability also expressed low swimming motility (152, 166, 809, 810, 811, 845, 866, 876, 878) (Fig. 5AC). Interestingly, *S. fonticola* 155, which had low swimming ability at 20°C, showed high ability to form biofilm under the same conditions.

We also performed a combined principal component analysis (PCA) taking into account all phenotypic tests (swimming, swarming, biofilm formation) and antibiotics resistance for the tested *E. coli* and *S. fonticola* strains (Fig. 6). By combining all of these data, we could explain 40% and 27% of the variance among the strains with component PC1 and PC2, respectively. The *E. coli* strains seem to form clusters of strains which are more similar to each other, like for example strains 151, 150 and 182 and another cluster of *E. coli* 102 and 104 which were isolated either from the same retention reservoir or in close vicinity (Fig. 6, Table 2). However, there were also strains clustered with each other but isolated from different water reservoirs, such as *E. coli* 173 and 863, and *E. coli* 127 and 128. Contrary to *E. coli* isolates, *S. fonticola* isolates did not seem to form any clusters. Most of the strains were grouped together regardless of the place of isolation. Interestingly, there was one *S. fonticola* strain (155), which was highly different from other *S. fonticola* isolates and turned out to be more similar to *E. coli* isolates when looking at phenotypic traits (Fig. 6). Detailed analysis of phenotypic traits, together with antibiotics resistance, showed that biofilm formation at 37°C, swarming at both temperatures and resistance to cefuroxime are showing similar trends in the tested group of *S. fonticola* and *E. coli* strains (Supplementary Fig. 1 at https://ojs.ptbioch.edu.pl/index.php/abp). Other traits, such as swimming, biofilm formation at 20°C and resistance to other antibiotics, form another cluster of features. In a global analysis based on Euclidean distance, some of the *E. coli* and *S. fonticola* isolates are clustered together. In this case, *S. fonticola* 155 again stands out from the group of strains, showing even more differences from other strains than *S. marcescens* C19 (Supplementary Fig. 1 at https://ojs.ptbioch.edu.pl/index.php/abp).

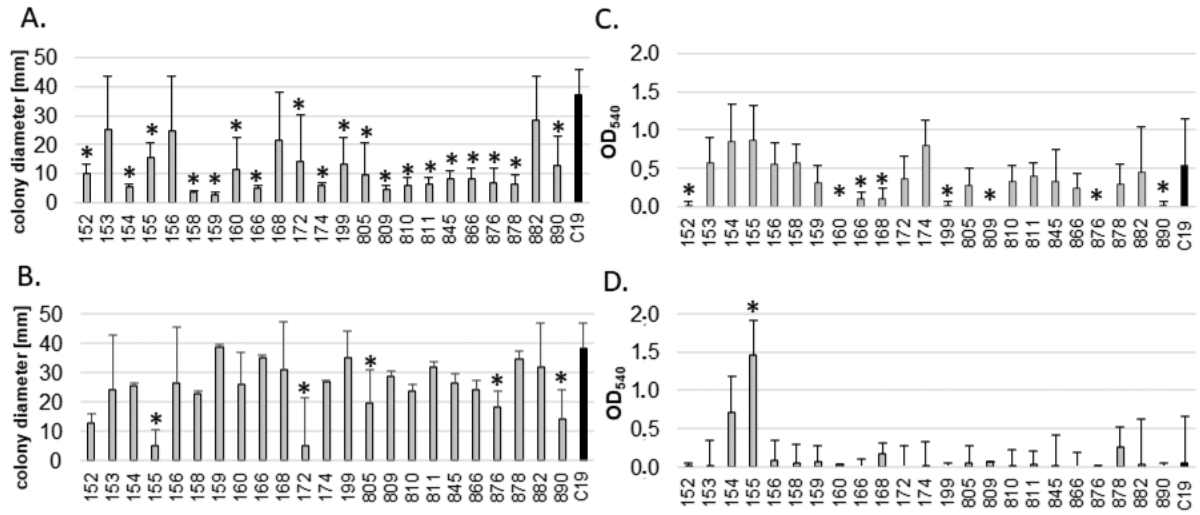

Figure 5. Swimming motility (A; B) and biofilm formation (C; D) of *S. fonticola* ESBL strains isolated from the Tricity water reservoirs tested at 37°C (A; C) and 22°C (B; D).
Swimming motility was expressed as an average colony diameter (mm) with standard deviation (n=4), while biofilm formation was expressed as an average OD_{540} value of crystal violet ethanol extractions after background subtraction, with standard deviation (n=2). Swimming motility was repeated twice, while biofilm formation three times. The results marked with an asterisk are statistically different from the reference strain *S. marcescens* C19 as assayed with ANOVA and the Tuckey post hoc test, $p<0.05$.

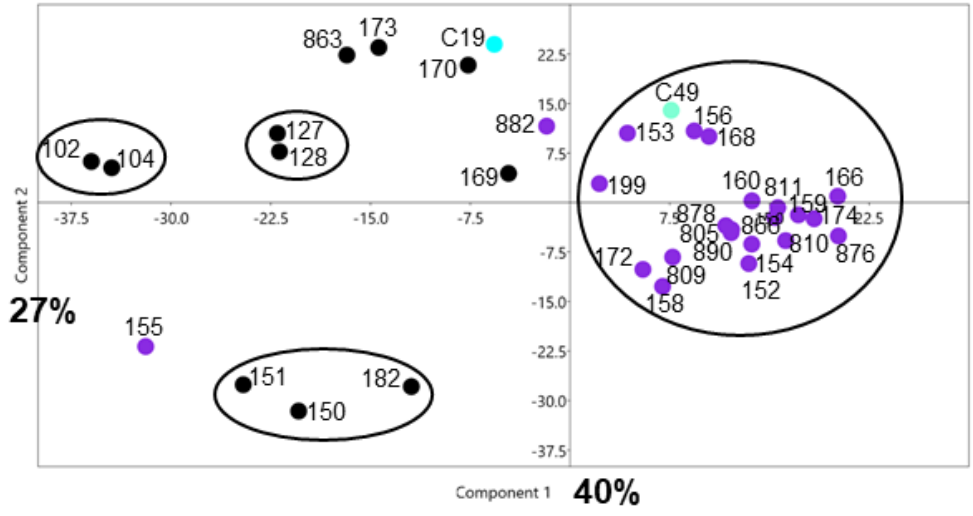

Figure 6. Principal component analysis (PCA) of biofilm formation, swimming and swarming motility, and antibiotics resistance of *S. fonticola* and *E. coli* ESBL strains. *E. coli* C49 and *S. marcescens* C19 were used as a reference.
The biofilm formation and motility assays were performed at 37°C and 22°C. *E. coli* strains are marked with black dots, while *S. fonticola* with violet dots.

ESBLs from *S. fonticola* and *E. coli* genera isolated from the Tricity waters present diverse fingerprinting profiles

Given a large phenotypic diversity among the isolated *E. coli* and *S. fonticola* ESBL strains, it was decided to verify their genotypic relationships. With the use of rep-PCR fingerprinting profiles obtained with ERIC primers, it was possible to assess the genetic variability among the isolated strains (Fig. 7). Two strains (*E. coli* 103 and *S. fonticola* 845) were excluded from the analysis as their isolated genomic DNA was unstable, preventing appropriate execution of the experiments. When taking into account profiles obtained for *E. coli* strains, 5 *E. coli* reference strains were used in the analysis that possessed similar or identical profiles forming a separate clade. Another clade was formed by *E. coli* environmental isolates, which in turn presented unique profiles for each strain (Fig. 7A).

For *S. fonticola* isolates, similar to *E. coli*, diversity among obtained rep-PCR profiles could be observed (Fig. 7B). Two *S. fonticola* strains (153 and 154) isolated from the Wilenska retention reservoir (Gdansk) showed almost the same band patterns, while other strains presented diverse profiles. Still, the strains could be divided into a few clades. However, these clades do not reflect geographical origin nor phenotypic analysis, apart from a few cases. For example, differently from all other strains in the phenotypic analysis, *S. fonticola* 155 formed a clade with several other strains (152, 153, 154, 156, 159, 805, 890) in the fingerprinting analysis. *E. coli* strains (150, 151, 182) that prove to be similar in the PCA analysis of the

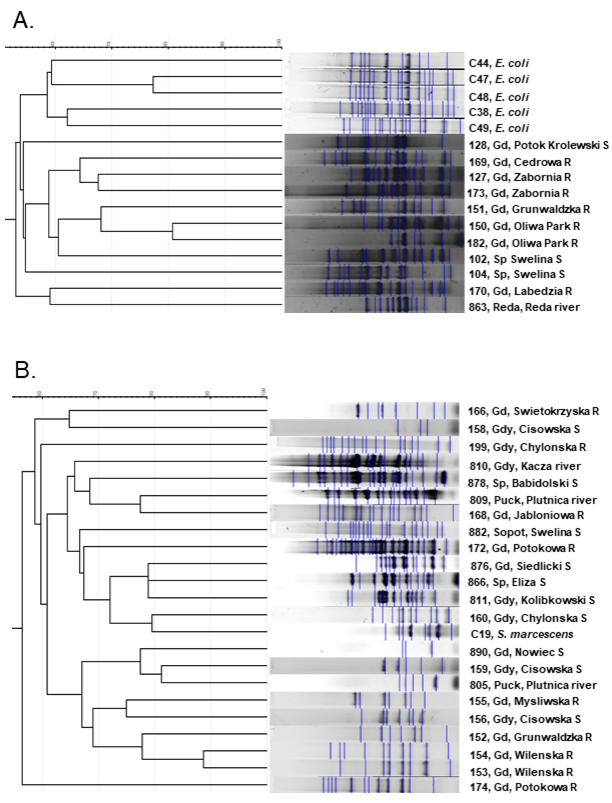

Figure 7. Rep-PCR profiles similarity of *E. coli* (A) and *S. fonticola* (B) ESBL strains isolated in the Tricity water reservoirs in 2019–2021.
The profiles were obtained with ERIC rep-PCR primers and analysed with GelJ software using UPGMA and band similarity index set to 1.0. *S. marcescens* C19 and *E. coli* C38, C44, C47, C48, C49, were used as a reference. Gd, Gdansk; Gdy, Gdynia; Sp, Sopot; 'S', stream; 'R', retention reservoir.

phenotypic traits, also fall in the same clade in the rep-PCR profile analysis. In contrast, *E. coli* 102 and 104 which are similar in PCA, tend to have very different rep-PCR profiles (Fig. 7A, Fig. 6).

DISCUSSION

Faecal microorganisms can enter water bodies in diverse ways, including runoff, sewage discharge, and direct faecal deposition (Korajkic *et al.,* 2019). Many investigations have shown the presence of multi-drug resistant coliforms in water (Manji *et al.,* 2012; Mishra *et al.,* 2018; Kurekci *et al.,* 2017; El-Zanfaly, 2015; Azzam *et al.,* 2017). In water reservoirs, such as rivers, streams, lakes and retention reservoirs, both pathogenic and commensal Enterobacterales may be present (Muraleedharan *et al.,* 2019). Pathogenic bacteria present in the water reservoirs may in turn cause infections in humans, which often have to be treated with antibiotics (Manji *et al.,* 2012). The antibiotic therapy is effective when the causative agent is drug-sensitive, otherwise the treatment is much more challenging. Thus, there is a growing concern regarding the occurrence of multi-drug resistance in coliforms which can render antibiotic therapy ineffective (Mishra *et al.,* 2018). Presently, antimicrobial pharmaceuticals are widely used in patient treatment, as well as in treatment of animals. Poland is one of the European Union countries with the highest usage of antibiotics, reaching as much as 25.87 DID (Defined Daily Doses per 1000 inhabitants per day) in 2015 (Olczak-Pieńkowska *et al.,* 2017). When regional usage is considered, the Pomorskie Voivodeship was the fourth largest antibiotic user in Poland (26.57 DID). Pharmaceutical residues excreted by treated humans and animals tend to end up in the water reservoirs where they can be a potential selection factor for multi-drug resistance arising in pathogenic, commensal and environmental microorganisms. In this phenomenon, horizontal and lateral gene transfers could be potent mechanisms (Emamalipour *et al.,* 2020).

The inland water catchment area of the Gulf of Gdansk, together with the Vistula River mouth, are important sources of bacterial contamination, including multi-drug resistant strains feeding into the Baltic Sea. In this study, we verified the presence of multiple ESBL species carrying resistance to different groups of antibiotics. Already in 2014, Bartoszewicz and others (Bartoszewicz *et al.,* 2014) reported the presence of antibiotics resistant *E. coli* strains in the Sopot streams, while Łuczkiewicz and others (Łuczkiewicz *et al.,* 2010) reported only one *E. coli* ESBL out of 155 strains isolated from effluent of the water treatment plant in Gdansk. Here, growth of ESBLs strains could be observed in 80% of samples, with greater load of strains observed in larger water reservoirs, such as the retention reservoirs (Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp). In fact, this is a larger number than the one observed in other rivers, streams and retention reservoirs in the territory of Poland. For example, Lenart-Boroń *et al.* (Lenart-Boroń *et al.,* 2017) reported that 14% of the isolated *E. coli* strains were ESBL *E. coli* in the Białka and Zakopianka rivers (Southern Poland), while at a later date this percentage rose to 20.6% of ESBL *E. coli* strains in the Białka river (Lenart-Boroń *et al.*, 2020a). On the other hand, no ESBL strains were detected in the Szreniawa river (Lenart-Boroń *et al.*, 2020b). Contrary, as many as 37.1% of *E. coli* isolates from effluents from a hospital situated in Olsztyn (Poland) belonged to ESBL, while from the city wastewater treatment plant effluents there was only 17.7% of such strains present (Korzeniewska *et al.,* 2013). In rivers, the load of ESBL bacteria was usually lower than in streams, but the water flow rate of the river is usually less turbulent, which enables deposition of the microorganisms in the sediments with the speed of 0.066 m/h (Garcia-Armisen *et al.,* 2009). In contrast, streams with more ESBL *E. coli* than in the rivers, usually have a high flow rate, which can prevent microorganisms from depositing themselves on the way. It is not surprising that in the retention reservoirs much more ESBL strains were found, as these reservoirs are fed by both, the streams and in case of heavy rain also with rain waters flowing from the urban areas. Moreover, the retention reservoirs in Gdansk are rich with birds which can also deposit their faeces contaminated with ESBL strains (B. Rybak, data not published). In fact, already in 2009, faeces of birds caught in the Tricity area were in 27% contaminated with ESBL *E. coli* (Literak *et al.,* 2010). We managed to isolate 56 ESBLs strains out of all of the water reservoirs, the majority of which belonged to *E. coli* (12) and *S. fonticola* (23) species.

Within Enterobacterales, strains of the *Serratia* genus are frequently encountered in the human nosocomial infections. Apart from *Serratia marcescens* and *Serratia liquefaciens* complex (*S. liquefaciens*, *Serratia proteamaculans*, *Serratia grimesii*), which are regarded as a major agent causing human *Serratia* infections, there is little information

about the remaining species of *Serratia* ('unusual' *serratiae*), including their susceptibility patterns to antimicrobial agents or underlying mechanisms of resistance (Grimont *et al.,* 1992). Strains of *S. fonticola* are widespread in the environment (drinking water, sewage and soil), with birds being reported as possible natural hosts (Stock *et al.,* 2003). As a human pathogen, *S. fonticola* has been associated with multiple diseases, such as diarrhoea, septic arthritis, wound, respiratory, urinary tract, bloodstream, or skin and soft tissue infections (Stock *et al.,* 2003). Resistance to beta-lactams in *S. fonticola* has been mediated by chromosomal class A extended-spectrum b-lactamases ESBLs belonging to the FONA family (Philippon *et al.,* 2016; Fuentes-Castillo *et al.,* 2020).

Among the tested *E. coli* and *S. fonticola* strains, large differences in resistance to groups of antibiotics could be observed. Strains that belonged to the S. *fonticola* (except 155) species were rarely resistant to other antibiotic groups except for third generation cephalosporins, contrary to *E. coli* isolates. Stock and others (Stock *et al.,* 2003) have also pointed out the natural susceptibility of several *Serratia* species (*S. ficaria, S. fonticola, S. odorifera, S. plymuthica*and *S. rubidaea*) to a wide range of antimicrobial agents (Stock *et al.,* 2003). *S. fonticola* strains are naturally sensitive to tetracycline and naturally resistant to ticarcillin and amoxicillin. At the same time, this species is sensitive or intermediately sensitive to aminopenicillins in the presence of β-lactamase inhibitors. Stock *et al.* (2003) have shown that their *Serratia* spp. isolates were able to express their own naturally occurring AmpC β-lactamase, which might be inducible (*S. ficaria, S. fonticola, S. odorifera*) or not inducible (*S. rubidaea*). Interestingly, *S. fonticola* 155 isolated from the Mysliwska retention reservoir (Gdansk) has shown a multi-drug resistant pattern for third generation cephalosporins and at the same time was negative in the DDS-test. Taking into consideration Stockand others (Stock *et al.*, 2003), it seems possible that *S. fonticola* 155 may be facilitated with inducible AmpC β-lactamase. The unique β-lactam susceptibility pattern of *S. fonticola* 155, showing resistance to amoxicillin and several cephalosporins, may be due to expression of a chromosomally encoded class A β-lactamase with an enhanced cephalosporinase activity, which in case of *S. fonticola* 155 needs further investigation (Stock *et al.,* 2003). Contrary to *S. fonticola*, *E. coli* ESBL isolates were generally more resistant to the tested antibiotics. However, a few *S. fonticola* strains were not sensitive to imipenem, a representative of carbapenems. Carbapenems are considered as antibiotics of the 'last resort', used for treatment of patients infected with drug-resistant pathogens. *S. fonticola* can harbour its drug resistance to imipenem on a plasmid, which in turn can be disseminated to other microorganisms present in the environment, such as the isolated ESBL *E. coli* strains. Similarly to *S. fonticola* 155, it was found that environmental *S. fonticola* UTAD54 was resistant to multiple antibiotics and possessed genes coding for multiple enzymes (A β-lactamase with carbapenemase activity, carbepenemases SFC-1 and Sfh-I), which are not common among *S. fonticola* strains (Henriques *et al.,* 2004; Saavedra *et al.,* 2003).

Apart from the antibiotics resistance of the tested *E. coli* and *S. fonticola* strains, closer consideration was taken for other phenotypic features of these bacteria which may facilitate their survival in the water reservoirs, as well as be potential factors enabling efficient pathogenesis. Enzyme production, biofilm formation and motility were investigated at two different temperatures, reflecting both, the host environment (37°C) and the water environment (20°C). In general, we noticed differences in biofilm formation ability and swimming motility, which were both species and temperature dependent. Higher swimming motility and lower ability to form biofilm was observed for *S. fonticola* grown at 20°C. The *E. coli* isolates were more motile and produced less biofilm at 37°C, which may suggest that they are more suited to invade the host than to persist in the environment. Interestingly, 7 *E. coli* isolates, which were more motile at 37°C, simultaneously produced more biofilm (102, 104, 127, 169, 170, 173, 863), while 2 *E. coli* strains (103 and 128) were not efficient in biofilm production. This finding is in agreement with Wood and others (Wood *et al.,* 2006) who showed that in some cases *E. coli* requires motility for biofilm formation in its early stage. Wood and others (Wood *et al.,* 2006) compared motility and biofilm formation of multiple *E. coli* strains which proved to be correlated. The strains capable of producing the largest biofilms under tested conditions were also the most motile (Wood *et al.,* 2006). In the case of *S. fonticola* species, 4 *S. fonticola* strains (153, 155, 156 and 882) also showed a similar pattern of being more motile and a better biofilm producer at 37°C. This ability is particularly important because production of biofilms often complicates chronic and difficult-to-treat infections by protecting bacteria from the immune system, decreasing antibiotic efficacy, and dispersing planktonic cells to distant body sites (Jackson *et al.,* 2002).

Due to large diversity among the tested strains regarding their phenotypic traits, such as antibiotics resistance, motility and biofilm formation, their genotypic variability with the use of rep-PCR fingerprinting profiles was analysed. The tested *E. coli* and *S. fonticola* ESBL strains presented large diversity of profiles, which are dissimilar from the reference strains used in this study. High variability of rep-PCR profiles of bacteria isolated from water reservoirs is widely documented (Moreira *et al.,* 2012; Potrykus *et al.,* 2016; Khare *et al.,* 2020; Chandrasekaran *et al.,* 2015; Kotlarska *et al.,* 2015; Lenart-Boroń *et al.* 2020a). Similar results for aquatic *E. coli* were observed in the Yamuna River (India) (Khare *et al.,* 2020). The isolates were highly diverse at all sampling sites of the river except for the entry site (Delhi at Palla). However, in this site the influence of anthropogenic activities and the pollution was the lowest when compared to other sampling sites. Similar situation was observed in Ontario (Canada), where fingerprinting profiles of periphytic *E. coli* were variable (Moreira *et al.,* 2012). Moreover, unlike Kon *et al.* (2007), we have observed diverse rep-PCR profiles even for the isolates coming from the same water reservoir, except for *S. fonticola* 153 and 154. In the case of *S. fonticola* no fingerprinting analysis in the literature had been found for comparison purposes. However, the profiles are as diverse as the *E. coli* profiles obtained in this study (Chandrasekaran *et al.*, 2015).

To sum up, the water reservoirs situated in the urban Tricity area are a habitat for ESBL Enterobacterales strains and thus can be a source of growing antibiotics resistance of the microorganisms in this area. The antimicrobial resistance (AMR), supported by a vertical and/or horizontal transfer of antibiotic resistance genes, is a serious public health challenge globally. AMR has been widely associated with pathogens in clinical settings, and it is becoming increasingly recognized that nonclinical environments and nonhuman hosts may also be reservoirs of AMR genes. As a result, constant monitoring of these bacteria (present not only in the water reservoirs but also in drinking water and at bathing areas localized by the Baltic Sea) should be taken under consideration,

since all of the freshwater reservoirs feed into the Baltic Sea with their waters containing ESBL strains.

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