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Original Research

Identification and Antimicrobial Susceptibility of Fecal Coliforms Isolated from Surface Water

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Abstract

Of 274 fecal coliforms isolated from two watercourses influencing the costal water quality of the Gdańsk and Puck bays, 265 were identified as *Escherichia coli*. The remained strains belonged to: *Acinetobacter* spp. (n=1), *Enterobacter* spp. (n=3), *Klebsiella* spp. (n=4), and *Shigella* spp. (n=1). The susceptibility of 222 *E. coli* was tested against 19 antimicrobial agents: aminoglycosides, carbapenems, cephalosporines, folate antagonists, fluoroquinolones, monobactam, penicillins, penicillins/β-lactamase inhibitors, and tetracycline. The highest number of isolates was resistant to penicillins (ampicillin 21% and piperacillin 14%), as well as to tetracycline (16%). Up to 19% of *E. coli* isolates were resistant to 3 or more of the analyzed antimicrobial agents, and 9% were regarded as multiple-antibiotic-resistant (MAR) strains. Two of the analyzed isolates were regarded as extended-spectrum β-lactamase – producing strains.

Keywords: surface water, fecal coliforms, identification, antimicrobial resistance

Introduction

Bacterial resistance to antimicrobial agents has been recognized as crucial for public health by the European Commission, the U.S. Centers for Disease Control and Prevention, and the World Health Organization. The antibacterial agents used in hospitals and at homes, as well as their residues, are continuously discharged with wastewater to municipal sewage systems [1, 2]. Thus, municipal wastewater is regarded as a second, following clinical material, reservoir of antimicrobial agents and bacteria with antimicrobial resistance patterns [1, 2]. Since the positive selection of resistance patterns has been observed in wastewater processes [3-7], the treated wastewater is suggested to contribute in the dissemination of resistance genes in the receiving water [8, 9]. In surface and groundwater the resistance genes can be spread also by fecal contamination originated from leaking septic tanks, agricultural activities, grazing fields, and fish farming.

In this study the taxonomic diversity of fecal coliforms and antimicrobial resistance among *E. coli* isolated from Oliwski Stream and the Reda River was determined. These two watercourses are two main direct tributaries of the Gdańsk Bay and the Bay of Puck, respectively, which significantly influence the sanitary quality of coastal water and beach areas [10-12]. Since no effluents of wastewater treatment plants impacted the quality of studied rivers, it was suggested that fecal pollutants are brought mainly through the surface runoff and soil leaching processes, representing non-point sources [11]. For identification and susceptibility testing of Gram-negative fecal bacteria isolated from the two watercourses, a Phoenix Automated Microbiology

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System (BD Biosciences, USA) dedicated to clinical microbiology laboratory practice was used.

Materials and Methods

The Study Area

Two watercourses that drain the edge of the Gdańsk Plateau and influence the costal water quality of the Gdańsk and Puck bays were examined in this study. Oliwski Stream, with its average flow rate (0.52 $m^3 \cdot s^{-1}$), length (10.9 km) and catchment area (26 km^2), is regarded as a typical watercourse of this area, while the Reda River (4.58 $m^3 \cdot s^{-1}$, 50.6 km, 485 km^2, respectively) is the main tributary of Puck Bay.

The upper part of Oliwski Stream (sampling points: O1) is located in a forested area within the protective zone of the Tri-City Landscape Park, where human activity is connected mostly with recreation. The fish farm is, however, located just above sampling point O2. In the central part of the catchments, the major tributary (Rynarzewski Stream), which drains the area of municipal Zoological Garden, flows into Oliwski Stream (sampling point O3). Then the watercourse runs through Oliwa and Jelitkowo, the two districts of Gdańsk, with very diverse landscape including: a municipal park, semi-intense, and intense built-up area (sampling point O4), as well as allotments (small vegetable and fruit gardens), a seaside park, a beach resort, and hotels in the mouth area (sampling point O5).

The basin of the Reda River lies in three geographical mesoregions: the proglacial valley of the Reda and Łeba rivers, the Kaszubian Lake District, and the Kaszubian coast. The rural landscape with small villages or single dispersed buildings dominates in the upper part of the catchments (sampling points R1 and R2). Intense human activity occurs in the central part of the river, in the area of Wejherowo and Reda (sampling points R3 and R4, respectively). In the marshland area of the Reda River's mouth is one of the most important stop-over sites for waders during autumn migration, Beka Nature Reserve (sampling point R5). The river discharges water into Puck Lagoon, an internal part of the Puck Bay.

Detailed characteristics of Oliwski Stream and the Reda River are given by Łuczkiewicz et al. [13].

Sample Collection

The samples of riverine water were collected into sterile 250 cm^3 bottles from May 2007 to April 2008. Immediately refrigerated, the samples were transported to a laboratory and analyzed within 4 h of sampling.

Determination of Environmental Parameters

The physical and chemical characteristics of surface water: pH, temperature, suspended matter (SM), chemical oxygen demand (COD), biological oxygen demand over 5 days (BOD_5), total nitrogen (N_{tot}), ammonium nitrogen (N_{NH4}), nitrate nitrogen (N_{NO3}), and total phosphorus (P_{tot}), were determined according to the Standard Methods for Examination the Water and Wastewater, APHA [14].

Enumeration and Isolation of Fecal Bacteria

The presence of fecal coliforms was determined using mFC agar [15]. Water samples (vol. 10 and 1 cm^3) were filtered through 0.45 μm cellulose-acetate filters (Merck). The filters were placed on mFC agar (Merck Cat. No. 1.11278) and incubated at 44.5ºC for 24 h. Blue colonies, regarded as fecal coliforms, were selected and subcultured onto the nutrient agar, then kept at 4ºC for further investigation.

Additionally in surface water samples, fecal enterococci were determined via membrane filtration [16]. The cellulose-acetate filters (Sartorius 0.45 μm) were incubated on Enterococcus selective agar according to Slanetz-Bartley (Merck Cat. No. 1.05262) at 37ºC for 48 h. Then, the Bile Esculin Azide Agar (Merck Cat. No 1.00072) at 44ºC for 2 h was used for selective cultivation of intestinal enterococci.

Species Identification and Antimicrobial Susceptibility

In this study the identification and drug susceptibility of bacterial strains, isolated on mFC medium and regarded as fecal coliforms, were tested by the Phoenix Automated Microbiology System. Commercially available panels (BD Phoenix) were applied for bacterial identification (ID) and antimicrobial susceptibility (AST) tests. The ID side of the panel contains 2 fluorescent control wells and 45 wells with dried biochemical substrates (enzymatic, carbon source, and utilization substrates).

The AST side contains wells with dried antimicrobial agents, with two-fold doubling dilution concentrations and a growth control well. In this paper the resistance profiles are presented only for riverine strains identified as *E. coli.* The investigated antimicrobial agents are listed in Table 1.

According to the aim of our study an appropriate Phoenix panel was selected (NMIC/ID 50). Panel inoculation was performed according to the manufacturer's recommendations. Pure cultures of bacterial strains (18-24 hours) were Gram stained and then used for inoculation of ID broth. Inoculum's concentration was adjusted to a 0.5 McFarland standard using BBL™ Crystal Spec™ Nephelometer (Becton Dickinson Diagnostics). Next, AST broth was inoculated with 25 μl of ID suspension. Then the filled panels were placed into the instrument, and incubated at 35ºC. Quality control was performed according to the manufacturer's instruction.

Data Analyses

Interpretation of the AST results was carried out using the standards for antimicrobial susceptibility testing of the Clinical and Laboratory Standards Institute (CLSI) [17]. A confidence level of 90% was required as the lowest limit of acceptability for the Phoenix ID/AST system.

Table 1. Antimicrobial agents used in the BD Phoenix panel.

Antimicrobial agents			
Class	Code	Name	Range µg/cm^3
Aminoglycoside	AN	Amikacin	4-32
	GM	Gentamicin	1-8
	NN	Tobramycin	1-4
Carbapenem	IPM	Imipenem	1-4
	MEM	Meropenem	1-8
Cephalosporin	CZ	Cefazolin	4-16
	CXM	Cefuroxime	4-16
	CAZ	Ceftazidime	4-16
	CTX	Cefotaxime	1-32
	FEP	Cefepime	2-16
Monobactam	ATM	Aztreonam	2-16
Penicillins	AM	Ampicillin	2-16
	PIP	Piperacillin	4-64
Penicillins/β-lactamase inhibitors	AMC	Amoxicillin/Clavulanate	4/2-16/8
	TZP	Piperacillin/Tazobactam	4/4-64/4
Folate antagonist	SXT	Trimethoprim/Sulfamethoxazole	0.5/9.5-2/32
Fluoroquinolone	CIP	Ciprofloxacin	0.5-2
	LVX	Levofloxacin	1-4
Tetracyclines	TE	Tetracycline	1-8
Others	ESR	ESBL	yes

Under the "resistant" category, all strains showing "resistant" or "intermediate resistant" behaviour were subsumed. As cross resistance, the resistance to two or more antimicrobial agents belonging to the same chemical family, due to the common resistance mechanisms, was defined. The resistance, due to the unrelated mechanisms, to two or more antimicrobial agents belonging to the different chemical family was defined as associated resistance. The isolates resistant to 3 or more chemical classes of antibiotic were taken as the multiple antibiotic resistant (MAR). Data concerning *E. coli* strains resistance against tested antimicrobial agents were analyzed using exact Fisher's of independence (eFt).

Results and Discussion

Environmental Parameters

According to the obtained results, the Reda River and Oliwski Stream can be regarded as a cold watercourses. During the studied period the average water temperature did not exceed 11ºC, but in July 2007 reached even 18ºC. The obtained pH values of water samples were within the range from 7 to 8. In both watercourses chemical parameters such as BOD_5, COD, N_{tot}, N_{NH4}, N_{NO3}, and P_{tot}, were detected at the level commonly found in the river water. The relationship between bacteriological and meteorological parameters was, however, observed throughout the sampling year [13]. Fecal coliforms were detected in the majority of the analyzed water samples. In the analyzed sampling points (O4 and O5, as well as R3 and R4) the number of fecal coliforms periodically exceeded the proposed levels of the European Union bathing water standards for the inland water [18].

The fecal coliform noted in the upper (agricultural) part of the Reda River (R1, R2), was pretty steady (80% of bacterial counts vary from 10 to 200 CFU/100 cm^3), and probably connected with continuous non-point sources of fecal contamination originating from leaking septic tanks, onsite wastewater treatment systems, or wildlife. A similar situation was found at the mouth of the Reda River (R5) at the marshland area of a bird nature reserve.

Generally, the fluctuations of bacterial counts are more distinguished at Oliwski Stream. Wildlife, especially birds, can contribute to fecal contamination mainly in the middle

part of the stream, running through the municipal park (O3). In sampling points O4 and O5, the decrease of water quality was noted mainly in July to September (summer), probably due to the cultivation of vegetables and fruits in the allotments located near point O5. It should be noted that at that time, from July to September, because of the warmest water and air temperature, the number of people visiting the sea coast is the largest.

Identification of Selected Bacterial Strains

Thermotolerant *E. coli,* the most common fecal coliform found in surface water samples [19], was also identified as a dominated species in this study (97%). Eight of the remaining isolates were identified as fermentative rods of *Klebsiella pneumoniae* ssp *pneumoniae* (n=4), *Enterobacter cloacae* (n=3), and *Shigella sonnei* (n=1), and one belonging to the non-fermentative rods of *Acinetobacter baumannii/calcoaceticus* complex.

From the species listed above, only *K. pneumoniae* (although much less frequent in environmental samples than *E. coli*) is also regarded as fecal coliform [20, 21]. Others, like *E. cloacae*, commonly present among the total coliforms, and *S. sonnei*, the bacterial pathogen implicated in outbreaks caused by water contaminated with human waste, were found to be able to grow under conditions defined for thermotolerant coliforms [22, 23]. In the case of bacterial strains identified as *A. baumannii/calcoaceticus,* a nosocomial pathogen, their role in natural reservoirs remains unknown.

Susceptibility Tests of *Escherichia coli*

In the present study, together with identification, the susceptibility of the isolates identified as *E. coli* was determined against the antimicrobial agents listed in Table 1. The growth limitation in control on the AST Phoenix panel was observed for 16% of 265 isolates and can be explained by environmental stresses [24]. The loss of culturability occurs especially in specific laboratory media, like AST broth used in this study. In consequence, the susceptibility results were obtained for 222 *E. coli* isolates.

Resistance to ampicillin, the most prevalent among *E. coli* isolated from clinical material [25] and from wastewater [1, 3, 4], was also the most common in this study (21% of isolates) (Fig. 1). In Poland the resistance rate for clinical strains varied from year to year (2000-08) and reached up to 58% [25], while the isolates originating from treated municipal wastewater reached 34% [4].

Resistance to tetracycline followed resistance to ampicillin, and reached 16% of isolates. Since the tetracycline itself is not used to treat *E. coli* infections in humans, the resistance rate was not tested among clinical isolates. However, resistance to TE has been commonly observed among *E. coli* isolates, the bystander effect is suggested to induce resistance among the commensal *E. coli*. For *E. coli* of wastewater origin, the resistance rate to TE was about 23% [1, 4, 26].

In the case of fluoroquinolones, 13% of studied *E. coli* isolates were CIP-resistant and 11% were LVX-resistant. In Poland, a similar resistance rate was observed among the wastewater strains, about 10% [4]. For the clinical strains resistance varied from 7% to 11% in 2001-04, and then increased up to 20% in 2008 [25]. The considerable increase of fluoroquinolone resistance was reported by many European countries, with the important role of gastrointestinal tract colonization with fluoroquinolones-resistant *E. coli* [27]. The mechanism of quinolone resistance among Gram-negative bacteria is mostly connected with chromosomal mutations, but plasmid – mediated resistance was also observed [28, 29].

Besides the fluoroquinolones, the combination trimethoprim with sulphamethoxazole (STX), which shows synergistic activity against many microbes, is frequently used to treat uncomplicated urinary tract infections. In consequence, the resistance to STX among urinary isolates of *E. coli* reached 20% [30]. On the basis of the obtained results, the resistance rate among riverine isolates reached 8% and was similar to the rate detected for wastewater isolates 11% [4]. For studied carbapenems (IPM and MEM), no resistance was detected, while for aminoglycosides (AN, GN, NN) we found only resistance to gentamicin (6%). To tested cephalosporins, 3% of *E. coli* isolates (n=7) were resistant to CZ, CXM, CAZ, CTX, and 1% (n=3) was resistant to all of them, including cefepime. It should be stressed that cefepime is fourth-generation cephalosporines, used only in clinical (hospital) practice, thus FEP-resistant *E. coli* were reported only among clinical isolates [31-33]. The presence of FEP-

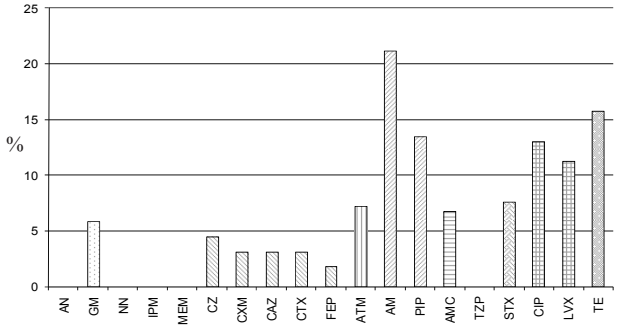

Fig. 1. Antimicrobial susceptibility of riverine strains identified as *Escherichia coli*.

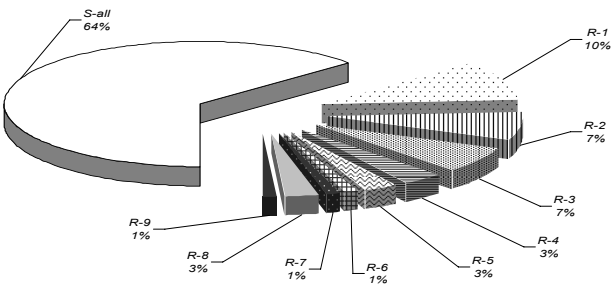

Fig. 2. Distribution of antimicrobial patterns among *E. coli* isolates sensitive to all (S-all) and resistant to X of analyzed antimicrobial agents (R-X).

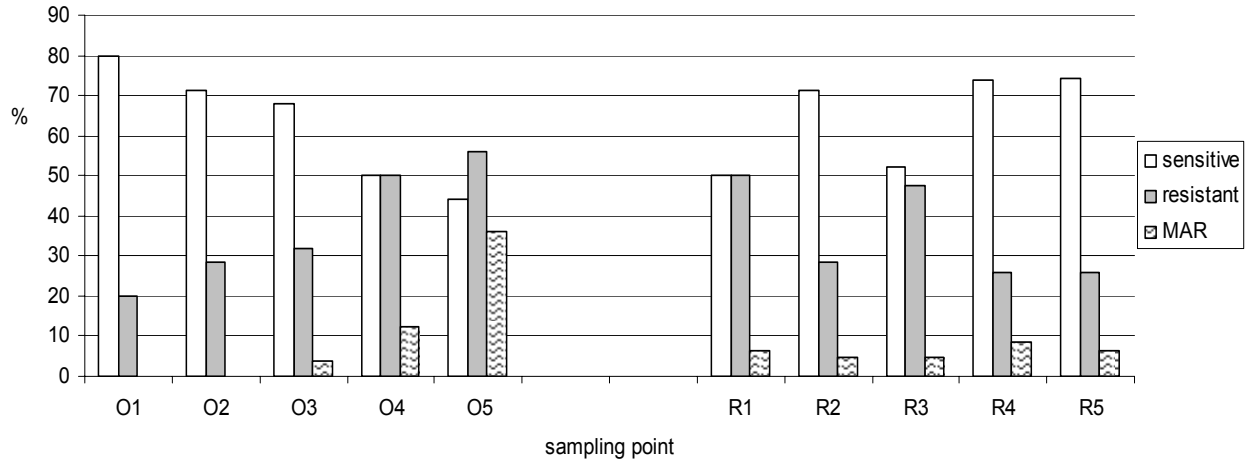

Fig. 3. Antimicrobial susceptibility of *Escherichia coli* strains isolated from the different sampling points.

resistant *E. coli* in water samples of Oliwski Stream (points O2 and O5) needs future study.

Of analyzed *E. coli* isolates, 36% were resistant to at least one and 9% to five or more antibiotic agents (Fig. 2). The most common associated resistance ($p<0.01$) involved ampicillin with piperacillin (AM-PIP), ampicillin with tetracycline (AM-TE), ampicillin with trimetoprim-sulfamethoxazole (AM-STX), and ampicillin with ciprofloxacin (AM-CIP) (64%, 43%, 34%, and 32% of ampicillin resistant strains, respectively) (Table 2). Similar resistance patterns were observed by Jakobsen et al. [34]. Since mentioned antimicrobial agents, except tetracycline, are used to treat urinary tract infections caused by *E. coli,* the spread of resistance is undesirable. In the case of tetracycline, it is suggested that the resistance patterns in bacterial population may be stored over time, regardless of selection pressure [35], and that TE-resistant *E. coli* may also become resistant to additional antimicrobial agents, probably by co-selection [36]. Multiple-antibiotic-resistance patterns (MAR) were observed among 9% of *E. coli* (13 strains were isolated from Oliwski Stream and 7 from the Reda River). Among MAR-isolates, *E. coli* exhibited resistance to the combination of antimicrobial agents, mainly ampicillin (n=15), tetracycline (n=15), and fluoroquinolones (n=15) (Table 3). The multiple-resistant phenotype involving fluoroquinolnes is suggested to be strongly associated with resistance to other antimicrobial agents [37] and considered as an increasing problem among clinical *E. coli* [25].

Since horizontal gene transfer is regarded as the main mechanism of resistance dissemination [38] the presence of antimicrobial-resistance genes on mobile genetic elements such as plasmids, transposable elements or integron-specific gene cassettes was wildly studied and reported among the environmental isolates [9, 39]. The presence of multiresistance plasmids in the studied riverine *E. coli* donors, as well as the possibility of horizontal gene transfer, was also indirectly confirmed by a preliminary study of Łuczkiewicz et al. [40].

E. coli isolates resistant to three or more chemical classes of antimicrobial agents (MAR) were isolated from the Reda River, mainly during long-lasting rainfall in March 2008 (Table 3), suggesting the contribution of surface runoff and soil leaching processes in the fecal contamination of the river body. In the case of Oliwski Stream, MAR patterns were detected in the central and lower parts of the catchments (sampling points O3-O5), although the highest number of multiple-resistant isolates were derived in the bathing season (May and August 2007) from the in-mouth area (sampling point O5). Also, the ratio of *E. coli* with resistance patterns to all tested *E. coli* isolates against the sampling points suggested positive correlation downstream (Fig. 3) [41]. At the Reda River such association was not observed.

The identification of fecal contamination sources seems to be crucial in order to effectively estimate the inherent risk. The preliminary analyses employing a BOX-PCR fingerprinting method have indicated that the phylogenetic structure of riverine isolates is influenced by catchment characteristics [42].

Conclusions

The fecal pollution of the studied watercourses were mainly related to runoff and soil leaching. Among 265 isolates identified as *Escherichia coli*, 36% of strains were resistant to at least one of the analyzed antimicrobial agents, and resistance patterns were detected in all studied sampling points. At the Reda River the resistance rate was correlated with agricultural activity and no centralized sewage system. At Oliwski Stream, the highest resistance rate was observed at the urbanized watershed and the multiple-resistant isolates of *E. coli* were mainly detected in the mouth area. Thus, in coastal water used for recreational purposes, the presence of bacterial strains resistant to clinically applied antimicrobial agents should be considered.

Table 2. Associated and cross resistance among *Escherichia coli* isolates (n=222).
a) concerning resistant-resistant E. coli isolates.

No of isolates resistant to antimicrobial agent	No (%) of *E. coli* resistant strains among the isolates resistant other antimicrobial agents																		
	AM	PIP	AMC	TZP	CZ	CXM	CAZ	CTX	FEP	ATM	STX	CIP	LVX	TE	GM	AN	NN	IPM	MEM
AM (n=47)	**47 (100)**	30 (64)	15 (32)	0 (0)	9 (19)	6 (13)	6 (13)	6 (13)	2 (4)	8 (17)	16 (34)	15 (32)	10 (21)	20 (43)	8 (17)	0 (0)	0 (0)	0 (0)	0 (0)
PIP (n=30)	30 (100)	**30 (100)**	6 (20)	0 (0)	3 (10)	2 (7)	2 (7)	2 (7)	2 (7)	2 (7)	11 (37)	13 (43)	8 (27)	18 (60)	5 (17)	0 (0)	0 (0)	0 (0)	0 (0)
AMC (n=15)	15 (100)	6 (40)	**15 (100)**	0 (0)	8 (53)	6 (40)	6 (40)	6 (40)	2 (13)	6 (40)	8 (53)	2 (13)	3 (20)	4 (27)	2 (13)	0 (0)	0 (0)	0 (0)	0 (0)
TZP (nd)	nd	nd	nd	**nd**	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CZ (n=10)	9 (90)	3 (30)	8 (80)	0 (0)	**10 (100)**	7 (70)	7 (70)	7 (70)	3 (30)	7 (70)	5 (50)	0 (0)	2 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CXM (n=7)	6 (86)	2 (29)	6 (86)	0 (0)	7 (100)	**7 (100)**	7 (100)	7 (100)	3 (43)	7 (100)	5 (71)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CAZ (n=7)	6 (86)	2 (29)	6 (86)	0 (0)	7 (100)	7 (100)	**7 (100)**	7 (100)	43	7 (100)	5 (71)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CTX (n=7)	6 (86)	2 (29)	6 (86)	0 (0)	7 (100)	7 (100)	7 (100)	**7 (100)**	43	7 (100)	5 (71)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
FEP (n=4)	2 (50)	2 (50)	2 (50)	0 (0)	3 (75)	3 (75)	3 (75)	3 (75)	**4 (100)**	3 (75)	3 (75)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ATM (n=16)	8 (50)	2 (13)	6 (38)	0 (0)	7 (44)	7 (44)	7 (44)	7 (44)	3 (19)	**16 (100)**	5 (31)	2 (13)	1 (6)	1 (6)	2 (13)	0 (0)	0 (0)	0 (0)	0 (0)
STX (n=17)	16 (94)	11 (65)	8 (47)	0 (0)	5 (29)	5 (29)	5 (29)	5 (29)	1 (6)	5 (29)	**17 (100)**	8 (47)	8 (47)	12 (71)	4 (24)	0 (0)	0 (0)	0 (0)	0 (0)
CIP (n=29)	15 (52)	13 (45)	2 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (7)	8 (28)	**29 (100)**	18 (62)	19 (66)	10 (34)	0 (0)	0 (0)	0 (0)	0 (0)
LVX (n=25)	10 (40)	8 (32)	3 (12)	0 (0)	2 (8)	1 (4)	1 (4)	1 (4)	1 (4)	1 (4)	8 (32)	18 (72)	**25 (100)**	13 (52)	6 (24)	0 (0)	0 (0)	0 (0)	0 (0)
TE (n=35)	20 (57)	18 (51)	4 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	12 (34)	19 (54)	13 (37)	**35 (100)**	7 (20)	0 (0)	0 (0)	0 (0)	0 (0)
GM (n=13)	8 (62)	5 (38)	2 (15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15)	4 (31)	10 (77)	6 (46)	7 (54)	**13 (100)**	0 (0)	0 (0)	0 (0)	0 (0)
AN (nd)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**nd**	nd	nd	nd
NN (nd)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**nd**	nd	nd
IPM (nd)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**nd**	nd
MEM (nd)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**nd**

nd – resistance patterns to TZP, AN, NN, IPM, MEM were not detected

Table 2. Continued.
b) concerning sensitive-resistant *E. coli* isolates.

No of isolates sensitive to antimicrobial agent	No (%) of *E. coli* resistant isolates among the isolates sensitive other antimicrobial agents																		
	AM	PIP	AMC	TZP	CZ	CXM	CAZ	CTX	FEP	ATM	STX	CIP	LVX	TE	GM	AN	NN	IPM	MEM
AM (n=175)	**0 (0)**	0 (0)	0 (0)	0 (0)	1 (1)	2 (1)	1 (1)	1 (1)	1 (1)	8 (5)	1 (1)	14 (8)	15 (9)	15 (9)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)
PIP (n=192)	17 (9)	**0 (0)**	9 (5)	0 (0)	7 (4)	6 (3)	5 (3)	5 (3)	1 (1)	14 (7)	6 (3)	16 (8)	17 (9)	17 (9)	8 (4)	0 (0)	0 (0)	0 (0)	0 (0)
AMC (n=207)	32 (15)	24 (12)	**0 (0)**	0 (0)	2 (1)	2 (1)	1 (0,5)	1 (0,5)	1 (0,5)	10 (5)	9 (4)	27 (13)	22 (11)	31 (15)	11 (5)	0 (0)	0 (0)	0 (0)	0 (0)
TZP (n=222)	47 (21)	30 (14)	15 (7)	**0 (0)**	9 (4)	7 (3)	7 (3)	7 (3)	3 (1)	16 (7)	17 (8)	29 (13)	25 (11)	35 (16)	13 (6)	0 (0)	0 (0)	0 (0)	0 (0)
CZ (n=212)	38 (18)	27 (13)	7 (3)	0 (0)	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)	9 (4)	12 (6)	29 (14)	23 (11)	35 (17)	13 (6)	0 (0)	0 (0)	0 (0)	0 (0)
CXM (n=215)	41 (19)	28 (13)	9 (4)	0 (0)	3 (1)	**0 (0)**	0 (0)	0 (0)	0 (0)	9 (4)	12 (6)	29 (14)	24 (11)	35 (17)	13 (6)	0 (0)	0 (0)	0 (0)	0 (0)
CAZ (n=215)	41 (19)	28 (13)	9 (4)	0 (0)	3 (1)	0 (0)	**0 (0)**	0 (0)	0 (0)	9 (4)	12 (6)	29 (14)	24 (11)	35 (17)	13 (6)	0 (0)	0 (0)	0 (0)	0 (0)
CTX (n=215)	41 (19)	28 (13)	9 (4)	0 (0)	3 (1)	0 (0)	0 (0)	**0 (0)**	0 (0)	9 (4)	12 (6)	29 (14)	24 (11)	35 (17)	13 (6)	0 (0)	0 (0)	0 (0)	0 (0)
FEP (n=218)	45 (21)	28 (13)	13 (6)	0 (0)	7 (3)	5 (2)	4 (2)	4 (2)	**0 (0)**	13 (6)	16 (7)	29 (13)	24 (11)	35 (16)	13 (6)	0 (0)	0 (0)	0 (0)	0 (0)
ATM (n=206)	39 (19)	28 (14)	9 (4)	0 (0)	3 (1)	1 (0,5)	0 (0)	0 (0)	0 (0)	**0 (0)**	12 (6)	27 (13)	24 (12)	34 (17)	11 (5)	0 (0)	0 (0)	0 (0)	0 (0)
STX (n=205)	31 (15)	19 (9)	7 (3)	0 (0)	5 (2)	3 (1)	2 (1)	2 (1)	2 (1)	11 (5)	**0 (0)**	21 (10)	17 (8)	23 (11)	9 (4)	0 (0)	0 (0)	0 (0)	0 (0)
CIP (n=193)	32 (17)	17 (9)	13 (7)	0 (0)	10 (5)	8 (4)	7 (4)	7 (4)	3 (2)	14 (7)	9 (5)	**0 (0)**	7 (4)	16 (8)	3 (2)	0 (0)	0 (0)	0 (0)	0 (0)
LVX (n=197)	37 (19)	22 (11)	12 (6)	0 (0)	8 (4)	7 (4)	6 (3)	6 (3)	2 (1)	15 (8)	9 (5)	11 (6)	**0 (0)**	22 (11)	7 (4)	0 (0)	0 (0)	0 (0)	0 (0)
TE (n=187)	27 (14)	12 (6)	11 (6)	0 (0)	10 (5)	8 (4)	7 (4)	7 (4)	3 (2)	15 (8)	5 (3)	10 (5)	12 (6)	**0 (0)**	6 (3)	0 (0)	0 (0)	0 (0)	0 (0)
GM (n=209)	41 (20)	27 (13)	13 (6)	0 (0)	10 (5)	8 (4)	7 (4)	7 (4)	3 (1)	14 (7)	15 (7)	22 (11)	24 (11)	31 (15)	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)
AN (n=222)	47 (21)	30 (14)	15 (7)	0 (0)	9 (4)	7 (3)	7 (3)	7 (3)	3 (1)	16 (7)	17 (8)	29 (13)	25 (11)	35 (16)	13 (6)	**0 (0)**	0 (0)	0 (0)	0 (0)
NN (n=222)	47 (21)	30 (14)	15 (7)	0 (0)	9 (4)	7 (3)	7 (3)	7 (3)	3 (1)	16 (7)	17 (8)	29 (13)	25 (11)	35 (16)	13 (6)	0 (0)	**0 (0)**	0 (0)	0 (0)
IPM (n=222)	47 (21)	30 (14)	15 (7)	0 (0)	9 (4)	7 (3)	7 (3)	7 (3)	3 (1)	16 (7)	17 (8)	29 (13)	25 (11)	35 (16)	13 (6)	0 (0)	0 (0)	**0 (0)**	0 (0)
MEM (n=222)	47 (21)	30 (14)	15 (7)	0 (0)	9 (4)	7 (3)	7 (3)	7 (3)	3 (1)	16 (7)	17 (8)	29 (13)	25 (11)	35 (16)	13 (6)	0 (0)	0 (0)	0 (0)	**0 (0)**

Table 3. Antimicrobial patterns among multiple-antibiotic-resistant (MAR) isolates of *E. coli*.

Sampling point	Date	*Escherichia coli*	
		No. of isolates	MAR pattern
The Oliwski Stream			
Point O3	March 2008	1	[AM, PIP, AMC]*, [STX], [TE]
	June 2007	1	[GM], [ATM], [CIP]
Point O4	November 2007	1	[GM], [ATM, AM], [CIP], [TE]
	December 2007	1	[GM], [AM, PIP], [LVX]
Point O5	May 2007	2	[AM, PIP], [CIP], [TE]
	August 2007	1	[GM], [AM, PIP, AMC], [STX], [CIP, LVX], [TE]
		2	[GM], [AM, PIP], [STX], [CIP, LVX], [TE]
	November 2007	1	[CZ, CXM, CAZ, CTX, FEP, ATM], [STX], [LVX]
	December 2007	1	[AM, PIP], [STX], [LVX], [TE]
	March 2008	1	[AM, PIP], [STX], [TE]
	April 2008	1	[AM, PIP] [STX], [LVX], [TE]
The Reda River			
Point R1	March 2008	1	[AM, PIP, AMC], [STX], [TE]
Point R2	March 2008	1	[GM], [AM, AMC], [STX], [CIP, LVX], [TE]
Point R3	March 2008	1	[AM, PIP], [STX], [CIP, LVX], [TE]
Point R4	December 2007	1	[AM, PIP], [STX], [CIP, LVX], [TE]
	March 2008	1	[AM, PIP], [CIP], [TE]
Point R5	December 2007	1	[AM, PIP], [STX], [CIP], [TE]
	March 2008	1	[GM], [CIP], [TE]

*each bracket represent different class of antimicrobial agents.

Abbreviations

Abbreviations of Antimicrobial Agents

AM – Ampicillin
ATM – Aztreonam
CTX – Cefotaxime
FEP – Cefepime
LVX – Levofloxacin
PIP – Piperacillin
TE – Tetracycline
AMC – Amoxicillin/Clavulanate
CAZ – Ceftazidime
CXM – Cefuroxime
GM – Gentamicin
MEM – Meropenem
SXT – Trimethoprim/Sulfamethoxazole
TZP – Piperacillin/Tazobactam
AN – Amikacin
CIP – Ciprofloxacin
CZ – Cefazolin
IPM – Imipenem
NN – Tobramycin

Other Abbreviations

AMS – Automated Microbiology System
BOD_5 – Biological oxygen demand over 5 days
DDD – Defined daily dose
ESBL – Extended spectrum of β-lactam antibiotics
ID – Bacterial identification
MAR – Multiple antibiotic resistance
N_{NO3} – Nitrate nitrogen
P_{tot} – Total phosphorus
AST – Antimicrobial susceptibility test
COD – Chemical oxygen demand
DID – DDD/1000 inhabitants per day
FC – Fecal coliforms
IE – Intestinal enterococci
N_{NH4} – Ammonium nitrogen
N_{tot} – Total nitrogen
SM – Suspended matter

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