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Antibiotic resistance and antibiotic-resistance genes of *Pseudomonas* spp. and *Escherichia coli* isolated from untreated hospital wastewater

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ABSTRACT

Hospitals are considered an important factor in the spread of antibiotic-resistant bacteria (ARBs) and antibiotic-resistance genes (ARGs). The purpose of this research was to characterize the microbial populations in hospital wastewater and investigated the prevalence of β-lactamase, *SulI* and *QnrS* resistance genes. In the first step, culture method was used to isolate *Pseudomonas aeruginosa* and *Escherichia coli*. In the next step, accurate identification of isolated bacteria was carried out using the polymerase chain reaction (PCR) method, then the resistance of the bacteria at different concentrations of antibiotics (8–128 μg/mL) was examined. Finally the ARGs were detected using the PCR method. The averages of heterotrophic plate count (HPC) and ARB concentration in wastewater samples were 1.8×10^8 and 4.3×10^6 CFU/100 mL, respectively. The highest resistance rates were found for sulfamethoxazole and the highest resistance rates in the β-lactamase group were for ceftazidime, while highest sensitivity was for gentamicin and there was no isolate that was sensitive to the studied antibiotics. SulI and *QnrS* were the highest and lowest abundance of all ARGs in samples respectively and blaSHV was the highest β-lactam resistance gene. Our results indicated an increase in the resistance of identified bacteria to several antibiotics. So it can be concluded that numerous antibiotic-resistant pathogens and vast numbers of ARGs exist in the human body so that their release from hospitals without effective treatment can cause many dangers to the environment and human health.

Key words: antibiotic resistant, *Escherichia coli*, hospital wastewater, multiplex PCR, *Pseudomonas aeruginosa*

HIGHLIGHTS

- In this study, five types of bacteria were extracted from EMB agar, two types of these bacteria were *Escherichia coli*.
- The identified *Escherichia coli* both had the *SUL*, *SHV*, *TEM* genes and only one type of *Escherichia coli* CTX had the *QnrS* gene
- The highest abundance of ARG, among identified *Pseudomonas* grown on cetrimide agar was sulfadimidine-resistance gene.

INTRODUCTION

Hospital and health care centers discharge considerable amounts of microbial agents and pharmaceutical residues into sewage and municipal wastewaters. Antibiotics are an important group of pharmaceuticals that are widely used in hospitals to treat infectious diseases that finally enter the aquatic environment from various sources (Paulshus *et al.* 2019; Khan *et al.* 2020a). Also, the increasing resistance of pathogenic bacteria to multiple common antibiotics is an emergent global public health problem (Elhariri *et al.* 2017; Peymani *et al.* 2017; Khan *et al.* 2020b). Irrational use of antimicrobials in the last few decades has been converted the clinically important bacteria from susceptible to single and or multiple antibiotics resistant which has led to a threat in public health (Akther *et al.* 2018; Debnath *et al.* 2018). The occurrence and spread of antibiotic-resistant bacteria or antibiotic-resistance genes (ARB, ARGs) are pressing public health problems worldwide and aquatic ecosystems are becoming a recognized reservoir for these micropollutants (Hocquet *et al.* 2016; Hassoun-Kheir *et al.* 2020).

Hospitals are ecological niches for ARB and play a major role in the emergence and spread of these bacteria and contain higher levels of antibiotic-resistant enteric bacteria in wastewater effluents than derived from other sources (Lamba *et al.* 2017; Paulus *et al.* 2019). Bacteria resistance to antibiotics could advance different infectious disease threats to humans,

since they can act as a reservoir for the maintenance and spread of ARGs (Devarajan *et al.* 2017). Also bacterial resistance to antibiotics may be caused through horizontal gene transfer or mutations in the bacterial population and increases the threats presented by antibiotic resistance (Joseph *et al.* 2019). β-Lactam antibiotics are the most commonly used drugs against infections because of their broad spectrum activity and low toxicity and are the largest type of antibiotic used by hospitals to treat infections caused by Gram-negative bacteria (Carlier *et al.* 2015; Ogbolu *et al.* 2018). The presence of β-lactam genes encoding bla*SHV*, bla_{TEM}, bla_{CTX} groups and VIM enzymes has been frequently reported in rivers in different regions over the world (Pérez-Etayo *et al.* 2020). Iran is one of the countries with the highest consumption of antibiotics and antibiotic resistance has been reported in almost all clinical, agricultural, livestock and environmental studies. Use of various antibiotics in different fields eventually causes these substances to enter human-related environments and causes ARB and ARG problems. For example, most antibiotics used in animal husbandry are consistently detected in livestock gastrointestinal environments that slow down the growth of susceptible bacterial populations. When ARBs in livestock gastrointestinal environments are excreted, ARGs are disseminated into receiving environments (e.g., soil, water) (Ogura *et al.* 2020). Subsequent ARG replication and propagation would increase the likelihood of human exposure. Therefore the consumption of antibiotics in any field provides the basis for the study of antibiotic resistance and, due to the nature of hospital wastewaters that are an important source of resistant bacteria, can be one of these fields. Considering that *Escherichia coli* and *Pseudomonas aeruginosa* due to their marked ability to survive on hospital surfaces are among the causative agents of nosocomial infections and given that increasing antimicrobial resistance in these bacteria has reduced the therapeutic potential against these pathogens and posed a challenge to human health, and as the World Health Organization and the Centers for Disease Control and Prevention in the United States have deemed these bacteria to be a serious threat worldwide (Willyard 2017), these bacteria were selected for this study. Due to the widespread use of different groups of antibiotics in medical environments, as well as the informal use of antibiotics by people and according to the results of previous studies on high-consumption antibiotics in the study area, ARGs were selected based on resistance to β-lactam (bla_{TEM}, bla_{SHV}, bla_{CTX}, bla_{VIM}), *QnrS* (reduced susceptibility to fluoroquinolones) and *SulI* (resistance to sulfonamides) genes. Including innovations of this study is that in addition to examining the resistance of bacteria to different groups of antibiotics, the resistance of bacteria to different concentrations of antibiotics was also examined separately. Regarding the study of ARGs, it should be noted that the presence of resistant genes was examined both before exposure to antibiotics and after exposure of identified bacteria to different concentrations of antibiotics.

MATERIALS AND METHODS

Wastewater samples

Untreated wastewater samples, were obtained from the last manhole of sewerage collecting wastewater from Tabriz university-affiliated hospital in Iran. The hospital has 998 general beds, 120 emergency beds, 125 dialysis beds and 26 operating beds with an area of 72,500 m^2.

Samples were taken twice during the year 2019 (warm and cold seasons) and to get a more representative sample 24 h composite samples were taken each period. Samples were collected in three periods in morning, noon and evening times. Samples were collected in sterilized glass bottles and then these bottles were refrigerated at 4 °C until processing in laboratory.

Isolation of bacteria and identification of resistant bacteria species

Cultivation of bacteria

For isolation of *Pseudomonas aeruginosa* cetrimide agar was used and eosin methylene blue (EMB) agar was used to isolate *Escherichia coli* from hospital wastewater samples. Samples were serially diluted with phosphate buffer to achieve an approximate range of 30 to 300 colonies on the plates. A total of 0.1 mL of diluted sample was placed on the agar plates in triplicate and then incubated aerobically at 37 °C for *Escherichia coli* 24 h and for *Pseudomonas* spp. 24–48 h. The different colonies grown on the culture medium were determined based on shape and morphology. Also heterotrophic plate count (HPC) were determined using R_2A agar (Merck, Merck KGaA, Darmstadt, Germany) and incubated at 35 °C for 48–72 h.

Determination of bacteria resistance spectrum

Antibiotics belong to different antimicrobial classes with involving different mechanisms for resistance by inhibition of protein synthesis, folic-acid cycle, DNA gyrase (involved in DNA replication) and synthesis of cell walls.

In this research, the identified bacteria were dosed with four different antibiotics at concentrations of 8–128 μg/mL. The antibiotics were β-lactams (cefotaxime (Ctx), ceftriaxone (Cro), ceftazidime (Caz), cefixime (Cef), cefazolin (Cfz) imipenem (Ipm),

Table 1 | Sequences of primers and thermal condition used in PCR amplifications

Gene	Primer sequence (5′ to 3′)	Product size (bp)	Annealing temperature (°C)	References
27 F **1429 R**	AGAGTTTGATCCTGGCTCAG TACGGYTACCTTGTTACGACTT	**1,420 bp**	95 °C 5 min → 37_[95 °C 60 sec, 52 °C 30 sec, 72 °C 1 min, 30 s] → 72 °C 5 min	Asghari *et al.* (2013)
bla*SHV*	GGTGACGAACAGCTGGAGCG GAGTTCGCCGACCGTCATGC	**108 bp**	94 °C 5 min → 40_[94 °C 60 sec, 62 °C 30 sec, 72 °C 10 sec] → 72 °C 5 min	Designed for this study
bla_{CTX}	TGGTGAYVTGGMTBAARGGCA TGGGTRAARTARGTVACCAGAA	**175 bp**	94 °C 5 min → 35_[94 °C 45 sec, 53 °C 45 sec, 72 °C 1 min] → 72 °C 5 min	Deccache *et al.* (2015)
bla_{TEM}	GCKGCCAACTTACTTCTGACAACG CTTTATCCGCCTCCATCCAGTCTA	**247 bp**	94 °C 5 min → 40_[94 °C 60 sec, 62 °C 30 sec, 72 °C 10 sec] → 72 °C 5 min	Rodriguez-Mozaz *et al.* (2015); Pallares-Vega *et al.* (2019)
bla_{VIM}	GTTTGGTCGCATATCGCAAC AATGCGCAGCACCAGGATAG	**382 bp**	94 °C 5 min → 35_[94 °C 60 sec, 60 °C 30 sec, 72 °C 10 sec] → 72 °C 5 min	Aruhomukama *et al.* (2019)
SulI	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	**163 bp**	94 °C 5 min → 35_[94 °C 60 sec, 62 °C 30 sec, 72 °C 10 sec] → 72 °C 5 min	Awad *et al.* (2015)
QnrS	GACGTGCTAACTTGCGTGAT TGGCATTGTTGGAAACTTGC	**118 bp**	94 °C 5 min → 35_[94 °C 60 sec, 60 °C 30 sec, 72 °C 10 sec] → 72 °C 5 min	Calero-Cáceres *et al.* (2014)

gentamicin (Gm), sulfamethoxazole (SMX) and ciprofloxacin (CIP)) that were purchased from Sigma Chemical Company (Sigma Aldrich, UK).

PCR[1] genome detection

DNA extraction

The DNA was extracted and purified using a bacterial genomic DNA extraction kit (TIANGEN, Beijing, China), according to manufacturer's instructions. DNA extraction was performed with three replicates of samples to compensate for heterogeneity. Then, the isolated DNA was stored at −20 °C until use.

DNA quantification

DNA concentration and purity were determined using a NanoDrop spectrophotometer by placing a drop (approximately 2 μL) on the sample space and analyzing using the software. (NanoDrop spectrophotometer, Thermo Scientific, Wilmington, USA).

Identification of bacteria using 16S rRNA

At the first step of the polymerase chain reaction (PCR), isolated bacteria were confirmed by amplification of the 16S rRNA gene. So primer set Eubac 27F and 1492R was used to amplify the 1,420 bp fragment of the 16S rRNA gene region of bacteria to check the nucleic acid extraction and the identification of inhibitors.

For this purpose, PCRs were carried out in a volume of 25 μL with 1 μL of genomic DNA, 0.50 mM of each primer, 12.5 μL master mix with amplification conditions given in Table 1. All PCR assays contained positive and negative controls.

PCR products were resolved by electrophoresis on 2.5% (wt/vol) agarose gels containing SYBR® Safe Stain together with a DNA molecular weight marker. Gels were viewed on a UV transilluminator (UV Tec., Vilber, France), and DNA fragment sizes were compared against the 1,000 bp ladder.

Identification ARB by DNA sequencing

PCR products were analyzed for DNA sequencing and DNA sequences analysis was undertaken using BLAST[2] algorithms and databases from the National Center for Biotechnology (www.ncbi.nlm.nih.gov).

[1] Polymerase chain reaction.

[2] Basic Local Alignment Search Tool.

Table 2 | Average values of physicochemical characteristics in hospital wastewater

Parameter	Amount
pH	7.7–8
BOD5, mg L^{-1}	190 ± 35
COD, mg L^{-1}	350 ± 67
SS, mg L^{-1}	150 ± 20

PCR sensitivity

McFarland turbidimetric standards were used to evaluate PCR sensitivity, which for this suspensions of all bacteria with concentration equivalent to 10^8 cells per mL were prepared. Then DNA was extracted from serial dilutions prepared from the suspensions and tested by PCR.

Detection of ARGs

The occurrence of genes resistant to β-lactams (bla$_{TEM}$, bla*SHV*, bla$_{CTX}$, bla$_{VIM}$), fluoroquinolones (*QnrS*) and sulfonamides (*SulI*) was tested using standard PCR reactions.

Specific primers to amplify the selected genes and thermal conditions used in PCR amplifications are presented in Table 1. For detection of these genes, PCR reactions were carried for example for 16S rRNA and the PCR products were resolved by electrophoresis on 1.5% (wt/vol) agarose gels.

Wastewater characterization

Physicochemical parameters including pH, biochemical oxygen demand (BOD_5), COD chemical oxygen demand (COD) and total suspended solids (TSS) were determined in samples in accordance with *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2005).

The results of the physicochemical analysis of samples are presented in Table 2.

Statistical analysis

Analysis of variance (ANOVA) was used to compare bacteria grown in different concentrations of antibiotics. Also the relationship between the number of bacteria isolated and physicochemical parameters was evaluated using the Pearson correlation matrix. All statistical analyses were performed in R version 3.5.0. *P*-values were used to assess significant differences between samples (*P*-value of <0.05).

RESULTS AND DISCUSSION

Isolation of ARB in hospital wastewater

In this study, all bacteria grown on EMB and cetrimide agar without antibiotics were identified and counted. Five types of bacteria were isolated from EMB agar and five types of bacteria were isolated from cetrimide agar based on morphology.

Also since significant associations between the amount of HPC and nosocomial infection pathogen contamination have been reported in studies (Asghari *et al.* 2013), then, in this research HPCs also were examined in addition to the detection of *Escherichia coli* and *Pseudomonas aeruginosa.* Bacteria identified in hospital wastewater are presented in Table 3 and statistic estimation by Pearson correlation between numbers of isolated microorganisms from hospital effluents and these parameters is presented in Table 4.

Detected bacteria were confirmed by PCR method and amplification of the 16S rRNA gene region. Based on BLAST, the two types of colonies identified on EMB agar were *Escherichia coli* and the five colonies identified on cetrimide agar were *Pseudomonas aeruginosa* (Table 5).

Bacterial resistance at different concentrations of antibiotics

The resistance of the identified bacteria at different concentrations of antibiotics 8–128 μg/mL was examined and the ability of these bacteria to grow at different concentrations was investigated.

Table 3 | Identified bacteria in hospital wastewater

Type of bacteria	(CFU/100 mL)
HPC	$1.8 \times 10^8 \pm 3.5 \times 10^7$
Detection of bacteria in antibiotic-free EMB agar	$1.2 \times 10^7 \pm 2.4 \times 10^6$
Detection of bacteria in antibiotic-free Cetrimide agar	$5.4 \times 10^6 \pm 4.8 \times 10^5$

Table 4 | Pearson correlation matrix among the isolated microorganism and physicochemical parameters

Variable	BOD	COD	ARB	Total bacteria/antibiotic-free/EMB agar	Total bacteria/antibiotic-free/cetrimide agar
BOD		0.9			
COD					
HPC	0.83		0.87	0.73	0.9
ARB	0.94				
SS	0.93	0.87			
Bacteria/antibiotic-free/EMB agar	0.91		0.96		0.71
Bacteria/antibiotic-free/cetrimide agar	0.8		0.86		

Table 5 | Sequence based identification of hospital wastewater bacteria by BLAST searches

Closest match in GenBank			Identity	Accession nos.
Bacteria grown on EMB agar	E1	*Acinetobacter baumannii*	98.95%	FJ907197.1
	E2	*Enterobacter cloacae* subsp. *dissolvens*	99.65%	MN545624.1
	E3	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	98.84%	LN624806.2
	E4	*Escherichia coli*	99.27%	CP007390.1
	E5	*Escherichia coli*	97.02%	MN094132.1
Bacteria grown on cetrimide agar	P1	*Pseudomonas aeruginosa*	99.43%	MN700178.1
	P2	*Pseudomonas aeruginosa*	98.96%	CP030328.1
	P3	*Pseudomonas aeruginosa*	97.86%	KY885175.1
	P4	*Pseudomonas aeruginosa*	97.81%	EU263017.1
	P5	*Pseudomonas aeruginosa*	98.67%	CP028959.1

Based on gene sequencing, two types of *Escherichia coli* were identified. These *Escherichia coli* had the ability to grow at concentrations of 8, 16 and 32 μg/mL of antibiotics and even the ability to grow at concentrations of 64 μg/mL of sulfamethoxazole, cefotaxime and ceftazidime antibiotics and only were able to grow at a concentration of 128 μg/mL sulfamethoxazole.

All the five species of *Pseudomonas aeruginosa* grew at concentrations of 8, 16 and 32 μg/mL of antibiotics. However, some of them (P1, P2, P3) were able to grow at a concentration of 64 μg/mL of sulfamethoxazole, cefotaxime, ceftazidime and cefixime antibiotics. Some of the isolated species (P1, P2) also grew at concentrations of 128 μg/mL of sulfamethoxazole.

It should be noted that all of identified bacteria were able to grow at concentrations of 8 and 16 μg/mL gentamicin and 8–32 μg/mL ciprofloxacin.

The results of this study are consistent with other studies on antibiotic resistance for different antibiotic members and the highest minimum inhibitory concentration (MIC) values for beta-lactam antibiotics are similar (Korzeniewska & Harnisz 2013; Akther *et al.* 2018). Also in a study by Pontes *et al.* on bacteria resistance to antibiotics, it was shown that bacteria are more sensitive to gentamicin, which is consistent with the present study (Pontes *et al.* 2009).

Based on our research on the ability of bacteria to be detected at different concentrations of antibiotic, all identified bacteria were able to grow at a concentration of 32 μg/mL antibiotics except for gentamicin. Figure 1 shows the ratio between the amount of bacteria grown in EMB and cetrimide agar with an antibiotic concentration of 32 μg/mL (16 μg/mL for gentamicin) to the amount of bacteria grown in both culture media without antibiotics.

As Figure 1 shows, the identified bacteria for the sulfamethoxazole antibiotic are more resistant to this than to other antibiotics and are more sensitive to gentamicin. Among the antibiotics of the beta-lactam group, the resistance of bacteria to ceftazidime was higher than to other antibiotics.

Analysis of variance (ANOVA) showed significant differences between the number of grown *Escherichia coli* (both species) at concentrations of 8 and 128 μg/mL sulfamethoxazole, 8 and 64 μg/mL ceftazidime and cefotaxime, but only there were significant differences between the number of the grown *Pseudomonas aeruginosa* at concentrations of 8 and 128 sulfamethoxazole (ANOVA, $P < 0.05$).

Antibiotic resistance gene detection

The PCR results for resistance genes are shown in Figure 2. PCR sensitivity was measured for all genes. According to the results of the PCR sensitivity tests, all the result were positive up to 1 CFU dilution.

The presence of genes encoding antibiotic resistance determinants in all isolated bacteria was assessed by PCR (Figure 3).

In this study, five types of bacteria were extracted from EMB agar, and were examined for the presence of resistance genes. Since two types of these bacteria were *Escherichia coli*, *Escherichia coli* ARGs are shown separately in this figure. The identified *Escherichia coli* both have the *SulI*, bla*SHV*, bla$_{TEM}$ genes and only one type of *Escherichia coli* had the bla$_{CTX}$ and *QnrS* genes. And as shown in the figure, all the bacteria grown on the EMB agar had the *SulI* gene.

The highest abundance of ARGs, among identified *Pseudomonas aeruginosa* was the sulfamethoxazole resistance gene, only one type of identified *Pseudomonas aeruginosa* had the *QnrS* gene and only one type of identified *Pseudomonas aeruginosa* had only one resistance gene.

It should be noted that 60% of identified *Pseudomonas aeruginosa* had the *bla*$_{VIM}$ resistance gene, 60% of *Pseudomonas aeruginosa* had the bla$_{TEM}$ and bla*SHV* resistance genes, and 40% of identified *Pseudomonas aeruginosa* had the bla$_{CTX}$ resistance gene. Table 6 shows the bacteria with the resistance genes.

Table 6 shows the presence of resistance genes in identified *Pseudomonas aeruginosa* and *Escherichia coli.* In different studies, similar results were obtained compared with the results of this study. Ranking of abundance of ARGs in our samples

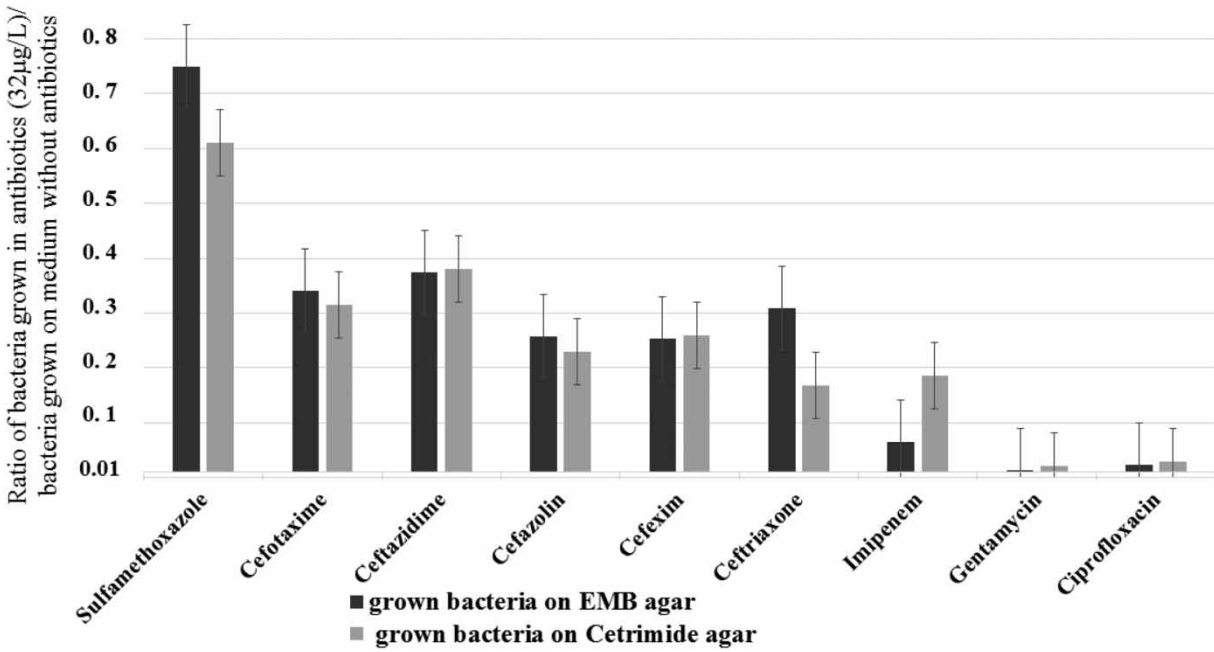

Figure 1 | Ratio of bacteria grown on medium with antibiotics/bacteria grown on medium without antibiotics.

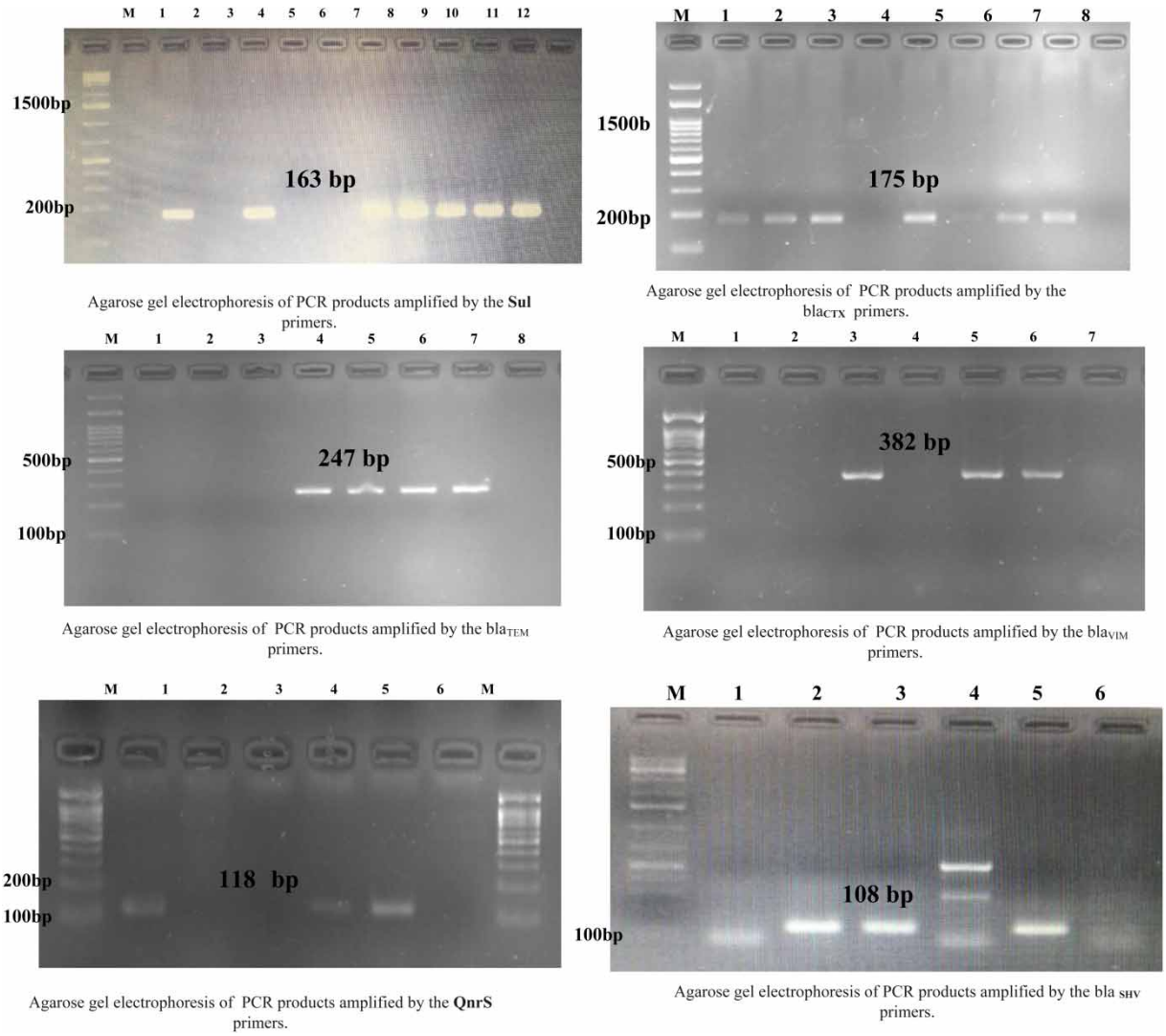

Figure 2 | Results of multiplex PCR for amplification of antibiotic-resistance genes.

is almost similar to the other studies, so that genes encoding bla$_{TEM}$, bla$_{CTX}$ and bla*SHV* were the most common types of β-lactams in bacteria and bla*SHV* has been the most prevalent in most studies (Uemura *et al.* 2010; Korzeniewska *et al.* 2013; Piotrowska *et al.* 2019).

In this study, the presence of ARGs was examined before the bacteria were exposed to antibiotics. The results of this stage of the study are shown in Table 7.

The results indicated that some extracted bacteria before being exposed to antibiotics, had *SulI*, bla$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{VIM}$ genes and that among these, the *SulI* gene was observed in most samples before contact with antibiotics.

CONCLUSION

Findings of our study showed a high prevalence of *Pseudomonas aeruginosa* and *Escherichia coli* carrying ARGs in hospital wastewater and is a serious cause for concern. Since antibiotic resistance is a growing global public health threat the identification, treatment and management of these effluents are necessary. Because hospitals effluents without treatment are discharged to the sewer systems, they carry significant quantities of antibiotic-resistant bacteria to municipal sewage that is considered a source of ARBs and create problems such as public health risks and imbalance of the microbial community in the sewage systems, which in turn affects the biological treatment process. Hospital effluents have high concentrations

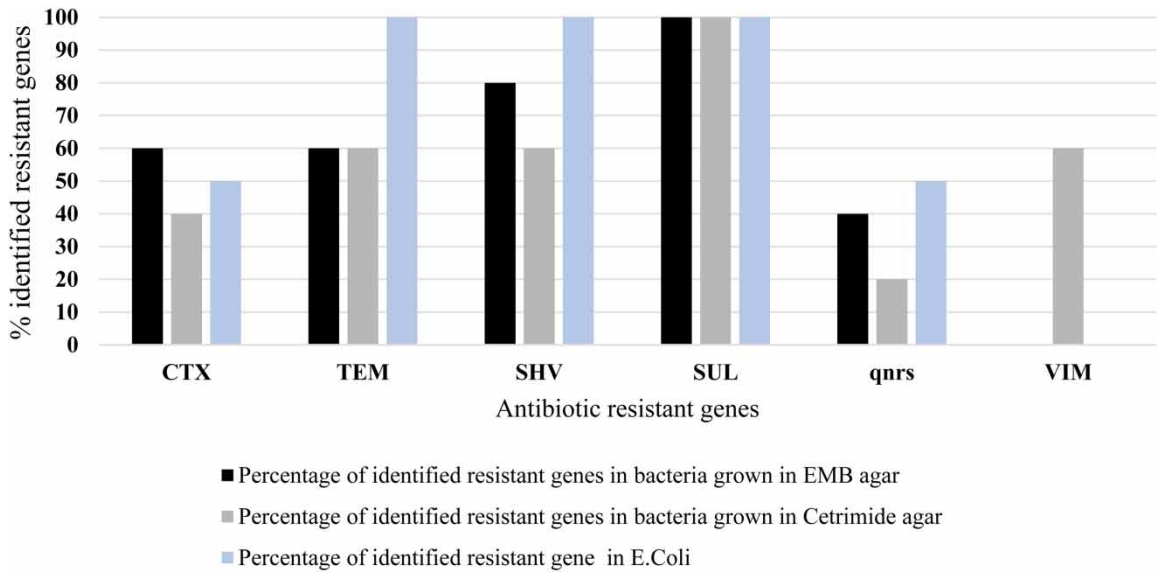

Figure 3 | Prevalence of antibiotic-resistance genes in hospital wastewater samples.

Table 6 | Resistant genes in isolated bacteria

	Resistance gene pattern						Antibiotic pattern
Isolated bacteria	**Ctx**	**Tem**	**Shv**	**Vim**	**Sul**I	***QnrS***	**MDR**
P1	+	+	+	+	+	+	MDR
P2	+			+	+		MDR
P3		+	+	+	+		MDR
P4		+	+		+		MDR
P5					+		MDR
E3		+	+		+		MDR
E4	+	+	+		+	+	MDR

Table 7 | Resistant genes in isolated bacteria before contact with antibiotics

	Resistance gene					
Isolated bacteria	**Ctx**	**Tem**	**Shv**	**Vim**	**Sul**I	***QnrS***
P1	−	−	−	+	+	−
P2	−	−	−	−	+	−
P3	−	+	−	−	−	−
P4	−	−	−	−	−	−
P5	−	−	−	−	−	−
E3	−	−	−		+	−
E4	−	−	+		+	−

of antibiotic groups and their metabolites, which could be the reason for the development of environmentally resistant genes. As hospital effluents are usually not treated and conventional treatment plants are inefficient in removing these pollutants, so hospital wastewater treatment at the source can be a good option for managing these pollutants and this option has

advantages such as preventing dilution due to mixing with municipal wastewater, prevention of losses in the environment due to leakage and overflow of mixed wastewater and prevention of pathogens and ARBs entering the environment. In some cases it is necessary that new wastewater treatment processes be assessed for their capability to eliminate ARBs. Also in order to minimize the dispersion of ARBs and ARGs through effluents, it is necessary to implement effective methods of wastewater disinfection and surveillance programs.

This research was based on the results of previous studies and according to the studies performed on major antibiotics used in the studied hospital, to identify the antibiotic resistance of the extracted bacteria. Real conditions were prepared for the bacteria but, due to financial constraints, other antibiotics that were not widely used in this hospital were not studied; bacteria may be resistant to those antibiotics and may have other resistance genes. Generally new data and results generated in this study will help towards a better understanding of the diversity of ARBs and ARGs. Also the publication of such papers could help to amend legislation in countries with the aim to treat hospital wastewater before it is discharged into the sewage system or to use primary treatment of hospital effluents before their discharge into the main wastewater flow for later treatment in treatment plants.

However, given that results, patterns and the potential human risk of hospital wastewater discharges is still unclear, reporting standards need to be developed and adhered to in order to enable consolidation of data across studies in meta-analysis, which are essential for evidence-based policy decision making.

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DECLARATION OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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