

Antibiotic resistance of *Pseudomonas aeruginosa* and *Escherichia coli* isolated from untreated water of hospitals in Baghdad city

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Abstract : Antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in untreated hospital wastewater form a major environmental and public health concern. The aim of this study was to investigate the antibiotic resistance patterns of *Pseudomonas aeruginosa* and *Escherichia coli* isolated from untreated wastewater of hospitals in Baghdad. These bacteria were selected to represent the most prevalent gram-negative bacteria found in hospital wastewater, and they have the potential to generate resistance and spread resistance genes to antibiotics. A total of 150 samples were collected during January 2024- May 2024. VITEK 2 system correctly identified and 16S rRNA gene confirmed 10 (6.7%) and 12 (8%) isolates of *P. aeruginosa* and *E. coli* respectively. All (100%) *P. aeruginosa* isolates and 10 (83%) of *E. coli* isolates were positive for *bla*_{CTX-M} gene while all isolates were negative for other genes (*bla*_{SHV}, *bla*_{VIM} and *bla*_{TEM}). *P. aeruginosa* and *E. coli* isolates were found to show the highest sensitivity to Colistin and the highest resistance to Ticarcillin, Piperacillin, Imipenem and Meropenem. The findings of this study indicate that wastewater of hospitals requires additional treatment to reduce discharged ARB and ARGs in the receiving water bodies. As a result, the efficiency of the quality should be continuously monitored and assessed.

Keywords : Antibiotic resistance genes, *Pseudomonas aeruginosa*, *Escherichia coli*, untreated hospital wastewater

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Introduction

Many antibiotic-resistant bacteria have been found in environmental samples, such as untreated wastewater from hospitals. Hospital wastewater are highly selective environments, and they contribute to the high rates of resistant bacteria that are being discharged in the natural environment (1). The most common bacterial pathogens found in hospital wastewater are Salmonella, Shigella, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Vibrio, Clostridium, Yersinia, Campylobacter, Leptospira and groups of total coliforms consisting of Serratia, Enterobacter, Klebsiella, and *Escherichia coli* (2). Antibiotic resistance is growing global threat to public health and according to previous studies, infections caused by antibiotic resistant species are expected to cause over 10 million deaths globally by 2050, resulting in significant economic

damage if the threat is not considered (3)(4). 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. The resistance to β -lactam antibiotics can be due to the expression of a single mechanism of resistance or to the effects of several mechanisms (5). 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 (6).

Materials and Methods

Samples collection

A total of 150 samples were collected during January 2024- May 2024 from untreated wastewater of hospitals in Baghdad, Iraq. Samples were collected in sterilized glass bottles and then these bottles were

refrigerated at 4 °C until processing in laboratory.

Bacterial identification

VITEK 2 system and molecular markers based on the amplification of 16S rRNA were used to characterize and confirm the identification of *P. aeruginosa* and *E. coli* isolates.

DNA extraction and quantification

From bacterial growth, genomic DNA was isolated according to the protocol of ABIOPure extraction. Quantus Fluorometer was used to detect the concentration of extracted DNA to detect the quality of samples for downstream applications. For 1 µl of DNA, 200µl of diluted Quantifluor Dye was mixed. After 5 min incubation at room

temperature, DNA concentration values were detected.

Polymerase chain reaction (PCR) cycle

The DNA of isolates was targeted for *bla_{SHV}*, *bla_{VIM}*, *bla_{TEM}* and *bla_{CTX-M}* genes using primers listed in Table (1). A reaction mixture (20 µl) contained 10 µl of Master Mix, 6 µl of nuclease free water, 2 µl of DNA, and 1 µl of each primer. The experiment was continued according to the following program: initial denaturation at 95°C for 5 min, 30 cycles at 95°C for 30 Sec, 55, 60, 62, or 68°C for 30 Sec, 72°C for 30 Sec. and then a final extension at 72°C for 7 min as shown in Table (2). The analysis of PCR products was done using 1.5% agarose gel electrophoresis and by using gel imaging system, the ethidium bromide stained bands in gel were visualized.

Table 1: Primer sequences used for PCR.

Genes	Primer sequence (5'-3')	Product size (bp)	Annealing Temp. (°C)	References
<i>bla_{SHV}-F</i>	GGTGACGAACAGCTGGAGCG	108	62	20
<i>bla_{SHV}-R</i>	GAGTTCGCCGACCGTCATGC			
<i>bla_{VIM}-F</i>	GTTTGGTCGCATATCGCAAC	382	60	20
<i>bla_{VIM}-R</i>	AATGCGCAGCACCAGGATAG			
<i>Bla_{TEM}-F</i>	ATGAGTATTCAACATTTC	858	68	19
<i>Bla_{TEM}-R</i>	CCAATGCTTAATCAGTGAGG			
<i>Bla_{CTX-M}-F</i>	ACCGCGATATCGTTGGT	551	55	19
<i>Bla_{CTX-M}-R</i>	CGCTTTGCGATGTGCAG			

Table 2: PCR program

Steps	°C	m:s	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:30	
Annealing	55, 60, 62, or 68	00:30	30
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Antibiotic susceptibility test

Susceptibility to antibiotics was determined by Vitek 2 system. The Vitek 2 system automatically measures a turbidity signal for each test well containing an antibiotic, every 15 minutes for up to 18 hours. These data are used to generate growth curves and by comparing with a control, the minimum inhibitory concentration (MIC) of each antibiotic is estimated (Nation *et al.*, 2014). MIC results ($\mu\text{g/ml}$) were translated into clinical categories (Susceptible, Intermediate, and Resistant) by comparing with Breakpoints for susceptibility category determination recommended by the clinical and laboratory standards institute (CLSI) guidelines.

Results and Discussion

Identification of bacteria

The VITEK 2 system correctly identified and 16S rRNA gene confirmed 10 (6.7%) and 12 (8%) isolates of *P. aeruginosa* and *E. coli* respectively. Morphological characterization and biochemical tests still fall short in terms

of ease, rapidity, and reliability therefore the use of automated systems targeted to solve these challenges is necessary. 16S rRNA gene is present in all bacteria and subject to different evolutionary rates depending on the gene region considered so it has historically been used for classification of isolates (7). There are studies that have previously shown that multidrug resistant *P. aeruginosa* is the main pathogen that is found in the discharge of the hospital wastewater treatment plants. This is an indication that the bacterium may play a major role in the transmission and the spread of antimicrobial resistance, which globally is an increasing healthcare problem (8). Higher and different prevalence rates were reported by Karungamye *et al.*⁹, who stated that *E. coli* was the second common isolate detected, with a prevalence of (27.9%) followed by *pseudomonas* ssp. (18 %).

Detection of antibiotic-resistant genes

All (100%) *P. aeruginosa* isolates and 10 (83%) of *E. coli* isolates were positive for *Bla_{CTX-M}* gene Figure (1) as well as all isolates were negative for other genes

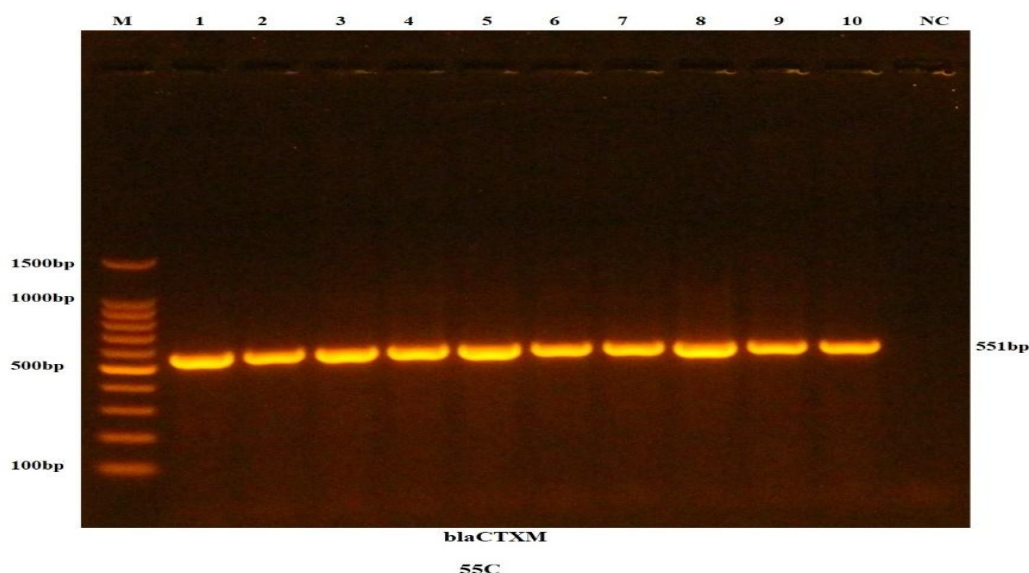


Figure (1): Results of the amplification of *Bla_{CTX-M}* gene of bacterial species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 551bp PCR products.

The results of this study are inconsistent with an Iranian study which stated that all *E. coli* and *P. aeruginosa* isolates were negative for *Bla_{CTX-M}* gene, 50% of *E. coli* isolates were positive for *bla_{SHV}* gene, and 20% of *P. aeruginosa* isolates were positive for both *bla_{VIM}* and *Bla_{TEM}* genes. All isolates were obtained from untreated hospital wastewater (6). Another study reported that the most prevalent extended spectrum beta lactamase (ESBL) encoding gene is *bla_{CTX-M}* (10)

bla_{CTX-M}, *bla_{SHV}*, and *bla_{TEM}* genes which encode three main types of ESBLs CTX-M, SHV and TEM respectively, can be plasmid mediated or expressed chromosomally. CTX-M, which has become more prevalent than SHV and TEM, includes a rapidly expanding family which has spread among a wide range of clinically important bacteria and over wide geographic areas (10) (11).

Antimicrobial resistance profile of isolates

Colistin which is considered the last recourse for the treatment of severe infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, was the most effective antibiotic against isolates (100% isolates of *P. aeruginosa* and *E. coli* were susceptible) as shown in Figure (2) and Figure (3). This might be attributed to the less frequent use of this antibiotic because of the unsustained availability in local markets and hospitals. Nowadays resistance to Colistin is low, however, there are some studies reporting high resistance rates. This prevalence may vary between geographical regions with different treatment strategies. Some countries, like Japan and South Africa, do not have access to Colistin while in a few areas of the world, like Europe and Australia, have only the parenteral formulation of Colistin and in the United States, Brazil, Malaysia, and Singapore, can use either Colistin or the polymyxin B parenteral for treatment (12).

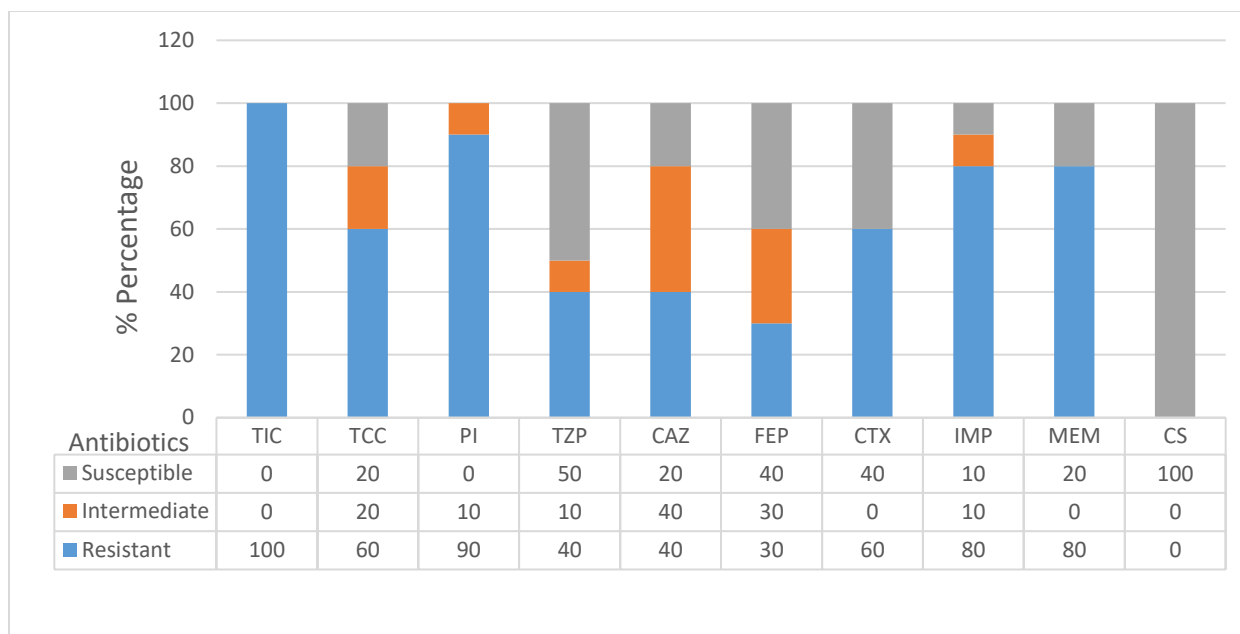


Figure (2): Antimicrobial resistance pattern of *P. aeruginosa* isolates, Ticarcillin (TIC), Ticarcillin/Clavulanic acid (TCC), Piperacillin (PI), Piperacillin/Tazobactam (TZP), Ceftazidime (CAZ), Cefepime (FEP), Cefotaxime (CTX), Imipenem (IMP), Meropenem (MEM).

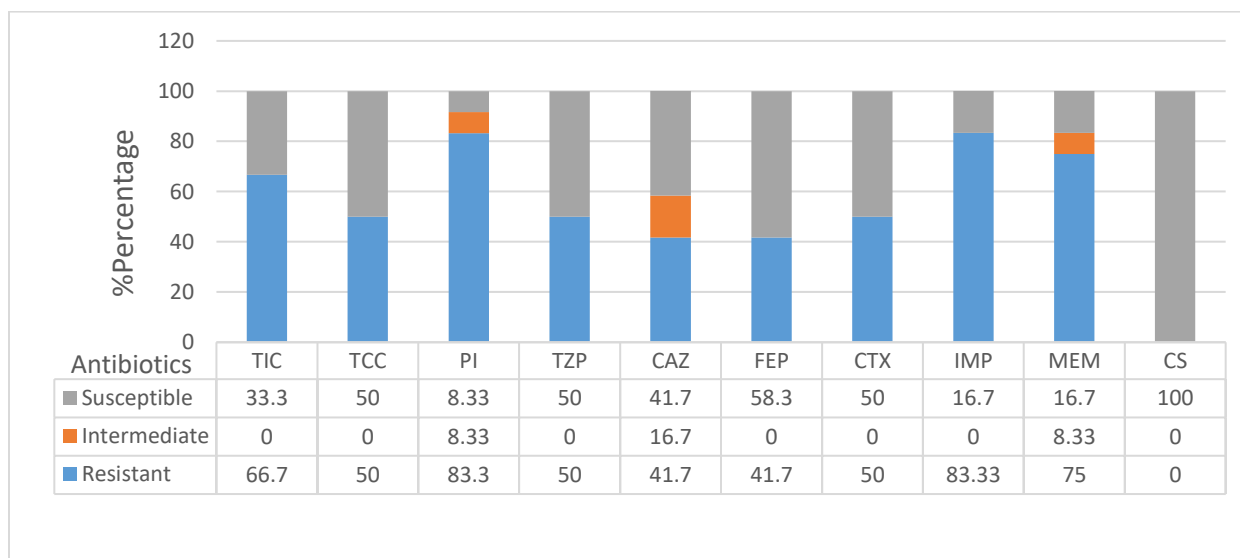


Figure (3): Antimicrobial resistance pattern of *E. coli* isolates, Ticarcillin (TIC), Ticarcillin/Clavulanic acid (TCC), Piperacillin (PI), Piperacillin/Tazobactam (TZP), Ceftazidime (CAZ), Cefepime (FEP), Cefotaxime (CTX), Imipenem (IMP), Meropenem (MEM).

In the current study, High resistant percentages were recorded against ticarcillin in both *P. aeruginosa* and *E. coli* isolates. As well as relatively high resistant percentages were recorded against ticarcillin/clavulanic acid. These bacteria are resistant mainly because of a group of enzymes that lead to

resistance increase in Aztreonam, Ceftazidime, Cefotaxime, related Oxyimino- β -lactams, cephalosporins, and penicillins, known as ESBLs, but Clavulanic acid inhibits them (11) (13). Resistant percentages against cefotaxime recorded in the current study represent a challenge as

cefotaxime is a third-generation cephalosporin that is the most active cephalosporin available. Although cefotaxime is one of the cephalosporins which can be hydrolyzed by Cefotaximase (class A β -lactamases that encoded by *CTX-M* gene and presents higher level of hydrolytic activity against cefotaxime than against ceftazidime, this study showed that 40% of *CTX-M* Positive *P. aeruginosa* isolates and 50% *CTX-M* Positive *E. coli* isolates were susceptible. This finding may be due to a low level of expression of *CTX-M* gene (14) (15). Very high resistant percentages were recorded against carbapenems (imipenem and meropenem) as shown in Figure (2) and Figure (3). Carbapenem resistant strains emerged because of the extensive use of carbapenem antibiotics (. Actually, for facing the increased antimicrobial resistance to broad spectrum cephalosporins, the use of more powerful β -lactam antibiotics (carbapenems) became routine (16). The mechanisms of resistance to carbapenems in

Gram-negative bacteria including: the outer membrane protein (OprD) loss or reduced expression that could confer a resistance to imipenem and a reduced susceptibility to meropenem; acquired Amber class B metallo-beta-lactamase (MBLs) production that lead to resistance to imipenem and meropenem; and overexpression activity of the efflux pumps MexAB-OprM that may confer a reduced susceptibility to meropenem, but don't affect susceptibility to imipenem (17) (18).

Conclusion

The findings of this study indicate that wastewater of hospitals requires additional treatment to reduce discharged ARB and ARGs in the receiving water bodies. As a result, the effluent quality should be continuously monitored and assessed, and further developments for treating the final effluent are necessary.

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