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The Antibiotic Resistance Genes *bla*$_{SHV}$, *bla*$_{OXA-48}$, *bla*$_{TEM}$ and *bla*$_{IMP}$ in *Pseudomonas aeruginosa* Isolated from Respiratory Tract Infections in Baghdad, Iraq

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Abstract

***Background*:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common pathogen associated with respiratory tract infections. Our study aimed to detect the antibiotic resistance profile and some antibiotic resistance genes of local isolates of P. aeruginosa from respiratory tract infections and to determine the biofilm formation by these isolates.

***Methods and Results*:** Two hundred sputum samples were obtained from patients with CF from different hospitals in Baghdad from November 2022 to February 2023. Biochemical tests and the VITEK-2 system were used to identify P. aeruginosa isolates. The disc diffusion technique was used in the antibiotic susceptibility test, and the results were identified according to CLSI guidelines 2020. Biofilm formation was performed by the microtiter-plate method and determined using an ELISA reader at OD570. The PCR was performed to detect the *bla*$_{SHV}$ gene, *bla*$_{TEM}$ gene, *bla*$_{IMP}$ gene, and *bla*$_{OXA-48}$ gene.

Sixty (30%) isolates of P. aeruginosa were identified from 200 sputum samples.The results showed that 93.4% of the isolates were resistant to Amoxicillin-Clavulanic acid, 90% to Nitrofurantoin and Cefepime, 88.4% to Cefotaxime, 85% to Doxycycline, 83.4% to Tobramycin, 81.7% to Tetracycline and 80% to Meropenem. In comparison, 91.6% were sensitive to Ofloxacin, 68.3% to Azithromycin, and 36.6% to Chloramphenicol. All P. aeruginosa isolates were identified as MDR. The results revealed that 55% of the isolates produced strong biofilms, 38.3% produced moderate biofilms, and 6.7% produced weak biofilms. The rates of *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{IMP}$, and *bla*$_{OXA-48}$ genes were 28.3%, 60%, 26.6%, and 68.3%, respectively.

***Conclusion*:** This study revealed that all isolates showed MDR phenotype. Biofilm formation by P. aeruginosa isolates and the variation in the incidence of antibiotic resistance encoding genes, in addition to the abuse and overuse of antibiotics, are significant reasons for the progress and spread of antibiotic resistance.**(International Journal of Biomedicine. 2023;13(4):341-344.)**

Keywords: *P. aeruginosa* • cystic fibrosis • antibiotic resistance • biofilm

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Abbreviations

CF, cystic fibrosis; **AR**, antibiotic resistance; **MDR**, multidrug resistant; **PCR**, polymerase chain reaction

Introduction

The opportunistic *Pseudomonas aeruginosa* (*P. aeruginosa)* is a common bacterium that can survive in various environments but prefers humid conditions. It is a common bacterium found in hospitals that causes nosocomial severe infections, specifically in severely sick and immuno-compromised patients. *P. aeruginosa* is implicated in various diseases, such as respiratory and urinary tract infections, infection of individuals with thermal burns, and wound and soft tissue infections.[(1)] *P. aeruginosa* is a prevalent infection in the lungs of patients suffering from cystic fibrosis (CF), and it is linked to frequent pulmonary exacerbations as well as significant mortality and morbidity. This bacterium can live in the lungs of CF patients for years.[(2)] Many of the antimicrobial medications that are currently used for treating severe

infections caused by multidrug-resistant (MDR) strains, such as carbapenems, fluoroquinolones, and third generation cephalosporins, have been found to be ineffective against *P. aeruginosa* strains.[3,4] The treatment of *P. aeruginosa* infections can become progressively challenging as *P. aeruginosa* antibiotic resistance (AR) increases. *P. aeruginosa* possesses many resistance mechanisms that contribute to eradication failure and persistent infections, including porin loss and efflux pump overexpression, as well as the synthesis of inactivating enzymes such as ß-lactamases.[5,6] Clinically approved antibacterial agents may be inefficient in treating infections by *P. aeruginosa* due to the bacterium's capacity to produce biofilms. The production of biofilms allows *P. aeruginosa* to survive externally in hostile conditions and improves colonization inside the host.[7] Additionally, biofilms serve as diffusion barriers, preventing antibiotics from entering bacterial cells.[8,9]

Our study aimed to detect the AR profile and some AR genes of local isolates of *P. aeruginosa* from respiratory tract infections and to determine the biofilm formation by these isolates.

Materials and Methods

Two hundred sputum samples were obtained from patients with CF from different hospitals in Baghdad from November 2022 to February 2023. They were plated on McConkey and cetrimide agar for 24 hours at 37°C. Biochemical tests and the VITEK-2 system were used to identify *P. aeruginosa* isolates. The disc diffusion technique was used in the antibiotic susceptibility test, and the results were identified according to CLSI guidelines 2020. The antibiotics used were Azithromycin (AZM) 30 µg, Doxycycline (DOX) 30 µg, Chloramphenicol (CHL) 30 µg, Tetracycline (TE) 30 µg, Nitrofurantoin (NF) 100 µg, Amoxicillin-Clavulanic acid (AMC) 20/10 µg, Ofloxacin (OF) 5 µg, Norfloxacin (NOR) 10 µg, Meropenem (MEM) 10 µg, Ticarcillin (TC) 75 µg, Cefepime (FEP) 30 µg, Tobramycin (TOB) 10 µg, Cefotaxime (CTX) 30 µg and Imipenem (IMP) 10 µg. Biofilm formation was performed by the microtiter-plate method and determined using an ELISA reader at OD570. The PCR was performed to detect the bla_{SHV} gene, bla_{TEM} gene, bla_{IMP} gene, and bla_{OXA-48} gene (Table 1).

Table 1.

Primer sequence.

Primer	Sequence: 5' → 3'	Amplicon size (bp)	Ref.
bla_{SHV}	F: TCAGCGAAAAACACCTTG R: TCCCGCAGATAAATCACC	472	(10)
bla_{TEM}	F: TTTCGTGTCGCCCTTATTCC R:ATCGTTGTCAGAAGTAAGTT	403	(11)
bla_{IMP}	F:ACCGCAGCAGAGTCTTTGCC R:ACAACAAGTTTTGCCTTACC	587	(12)
bla_{OXA-48}	F:GCGTGGTTAAGGATGAACAC R:CATCAAGTTCAACCCAACCG	438	(13)

Ref -Reference

Results

Sixty (30%) isolates of P. aeruginosa were identified from 200 sputum samples. The pattern of distribution of *P. aeruginosa* isolates based on gender and age groups is presented in Figure 1. The results showed that 93.4% of the isolates were resistant to Amoxicillin-Clavulanic acid (AMC), 90% to Nitrofurantoin (NF) and Cefepime (FEP), 88.4% to Cefotaxime (CTX), 85% to Doxycycline (DOX), 83.4% to Tobramycin (TOB), 81.7% to Tetracycline and 80% to Meropenem. In comparison, 91.6% were sensitive to Ofloxacin (OF), 68.3% to Azithromycin (AZM), and 36.6% to Chloramphenicol (CHL) (Table 2). All *P. aeruginosa* isolates were identified as MDR. The results revealed that 55% of the isolates produced strong biofilms, 38.3% produced moderate biofilms, and 6.7% produced weak biofilms. The rates of bla_{SHV}, bla_{TEM}, bla_{IMP}, and bla_{OXA-48} genes were 28.3%, 60%, 26.6%, and 68.3%, respectively (Table 3). Table 4 indicates the relation between biofilm formation and antibiotic resistance.

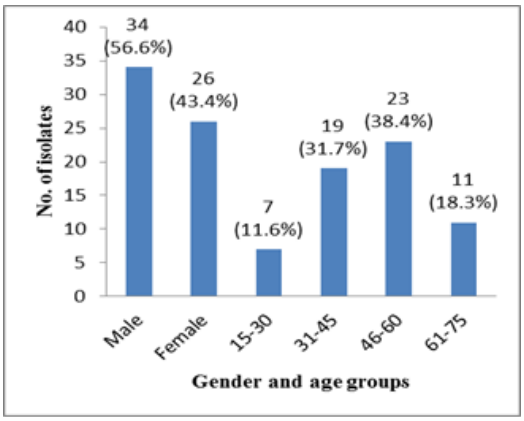

Fig. 1. *Distribution of P. aeruginosa isolates.*

Table 2.

Antimicrobial resistance of P. aeruginosa isolates.

Class	Antibiotic	Sensitive n (%)	Resistance n (%)
Macrolides	AZM	41 (68.3)	19 (31.7)
Tetracyclines	DOX	9 (15)	51 (85)
	TE	11 (18.3)	49 (81.7)
Beta-lactam and Beta-Lactamase Inhibitor	AMC	4 (6.6)	56 (93.4)
	TC	14 (23.3)	46 (76.7)
Nitrofurantoin	NF	6 (10)	54 (90)
Fluroquinolones	OF	55 (91.6)	5 (8.4)
	NOR	17(28.4)	43 (71.6)
Carbapenems	MEM	12 (20)	48 (80)
	IMP	17 (28.4)	43 (71.6)
Cephalosporin	FEB	6 (10)	54 (90)
	CTX	7 (11.6)	53 (88.4)
Chloramphenicol	CHL	22 (36.6)	38 (63.4)
Aminoglycosides	TOB	10 (16.6)	50 (83.4)

Table 3.
The antibiotic resistance genes among P. aeruginosa isolates.

Genes	No. of isolates	%
bla_{SHV}	17	28.3
bla_{TEM}	36	60
bla_{IMP}	16	26.6
bla_{OXA-48}	41	68.3
bla_{TEM}, bla_{OXA-48}	36	60
bla_{TEM}, bla_{OXA-48}, bla_{SHV}, bla_{IMP}	11	18.3

Table 4.
The relation between antibiotic resistance and biofilm formation in P. aeruginosa isolates.

Antibiotic	Strong (n=33)	Moderate (n=23)	Weak (n=4)
	n (%)	n (%)	n (%)
AZM	11 (33.3)	7 (30.4)	1 (25)
DOX	29 (87.8)	20 (86.9)	2 (50)
TE	27 (81.8)	18 (78.2)	4 (100)
AMC	31 (93.9)	21 (91.3)	4 (100)
TC	23 (69.7)	20 (86.9)	3 (75)
NF	29 (87.8)	21 (91.3)	4 (100)
OF	3 (9)	2 (8.6)	0 (0)
NOR	23 (69.7)	18 (78.2)	2 (50)
MEM	26 (78.7)	19 (82.6)	3 (75)
IMP	24 (72.7)	16 (69.5)	3 (75)
FEB	29 (87.8)	21 (91.3)	4 (100)
CTX	31 (93.9)	18 (78.2)	4 (100)
CHL	22 (66.6)	13 (56.5)	3 (75)
TOB	27 (81.8)	20 (86.9)	3 (75)

Discussion

P. aeruginosa is the most common pathogen in the respiratory tract of patients who suffer from CF and other chronic infections. The low antibiotic sensitivity is one of the most concerning aspects of this bacterium.[14] In the current study, 60(30%) of 200 sputum samples tested positive for *P. aeruginosa*, whereas 70% were positive for other bacterial isolates. This agreed with a study by Wang et al., who reported that *P. aeruginosa* was the pathogen with the most significant sputum isolation rate (23.8%).[15] Distribution of *P. aeruginosa* isolates among males was 56.6%, compared to females (43.4%); males were more susceptible to bacterial infection than females due to higher exposure to numerous unfavorable environmental variables.[16] The age group 46-60 years had the highest percentage of the AR isolates (38.4%). The isolates were highly susceptible to Ofloxacin (91.6%) and highly resistant to Amoxicillin-Clavulanic acid (93.4%). This result was close to that of a study by Motbainor et al., who showed that 100% of *P. aeruginosa* isolates were Amoxicillin-Clavulanic acid resistant.[17] All isolates were MDR, and this result agreed with Abbas et al., who reported that 100% of *P. aeruginosa* isolates were MDR.[18] AR may be linked to alterations in bacterial enzymes as well as patients' overuse and abuse of antibiotics.[19,20] The bla_{SHV} gene was detected in 28.3% of the isolates, bla_{TEM} in 60%, bla_{IMP} in 26.6%, and bla_{OXA-48} in 68.3% of the isolates. At the same time, other studies show 6.6% for bla_{SHV},[21] 100% for bla_{TEM},[22] 42.8% for bla_{IMP},[23] and 36.1% for bla_{OXA-48}.[24] The incidence of resistance genes varies greatly between studies, which may lead to variation in infection management guidance. The current study showed a correlation between biofilm formation and resistance to antibiotics. These findings were consistent with previous studies, which indicated that bacteria in planktonic form are less resistant to antibiotics than bacteria in biofilm form.[25] Horizontal gene exchange, on the other hand, is considerably increased in biofilms because resistant bacteria can transfer resistance genes to other bacteria.[26,27]

In conclusion, this study revealed that all isolates showed MDR phenotype. Biofilm formation by *P. aeruginosa* isolates and the variation in the incidence of AR encoding genes, in addition to the abuse and overuse of antibiotics, are significant reasons for the progress and spread of AR. The elevated prevalence of MDR *P. aeruginosa* emphasizes the critical importance of establishing alternate treatment methods.

Competing Interests

The authors declare that they have no competing interests.

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