Original Article

Occurrence of Class 1, 2, and 3 Integrons among Multidrug-resistant *Pseudomonas aeruginosa* in Babylon Province, Iraq

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Abstract

Background: Clinical management of bacterial infections has faced significant difficulties in recent years due to the advent and spread of multiple drug-resistant (MDR) bacteria. Worldwide, nosocomial infections are brought on by *Pseudomonas aeruginosa*, a clinically significant *Pseudomonas* species. **Objectives:** This research aimed to identify class 1, 2, and 3 integrons in *P. aeruginosa* in Babylon, Iraq. **Materials and Methods:** From February 2022 to October 2022, 131 isolates from various sites including (burn, wound, and urine) were collected from different hospitals in Babylon Province for both genders and ages. These isolates were identified using traditional techniques as well as the Vitek 2 system (bioMerieux, France). *Pseudomonas aeruginosa* isolates were subjected to disc diffusion antimicrobial susceptibility testing. Class 1, 2, and 3 integron-specific primers were used in the polymerase chain reaction technique for the molecular identification of integron genes. **Results:** *Pseudomonas aeruginosa* isolates that were 131 (100%) had integron class 1. On the contrary, only five (3.81%) contained a class 2 integron. There was no presence of class 3 integron in any isolate. **Conclusion:** The MDR *P. aeruginosa* was highly prevalent (100%) and this suggested that the availability of class 1 integrons in our area was alarmingly high, showing the need for epidemiological monitoring.

Keywords: Antibiotic resistance, integron, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa frequently infects people with cancer, burn injuries, cystic fibrosis, and other severe illnesses.[1] Burns are not specific to any given population, age, gender, occupation, or ethnic group; every individual is at risk of any type of burns and in any location.[2] *Pseudomonas aeruginosa* infection in burn patients is prevalent and ranks among the most serious life-threatening illnesses in burn units because burn wounds generally serve as a good site for bacterial multiplication due to immune system suppression.[3] The use of antimicrobial agents is of great importance in the treatment of bacterial infections.[4] *Pseudomonas aeruginosa* is typically less resistant to numerous antibiotics by nature as compared with other gram-negative bacilli. Strains of *P. aeruginosa* that are multiple drug resistant (MDR) are difficult to treat clinically.[5]

Pseudomonas aeruginosa isolates that are resistant to medication frequently cause burn infections, which can be fatal in medical facilities.[6] MDR *P. aeruginosa* outbreaks in healthcare situations are most likely a result of increased antibiotic use. Bacterial resistance is typically explained by the transferable genetic elements' plasmids, transposons, and integrons or by gene mutation.[7] Part of antibiotic resistance is due to three different kinds of integrons, known as class 1, class 2, and class 3. They can be recognized by differences in the integrase gene's *intI* structure.[8]

The integron is a genetic component that can play a significant part in the spread of multidrug resistance in gram-negative bacteria, particularly in *Pseudomonas*.[9] The integrase genes (*intI2* and *intI3*) are found in class 2 and 3 integrons, and their corresponding produces are 46%

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and 61% identical to class 1 integrase.[10] Antimicrobial resistance is attributable to several mechanisms of integrons, beta-lactamases, and efflux pumps.[11] Integrons have a significant role in the spread of antibiotic resistance; therefore, this study sought to determine the molecular relationships and presence of integrons with multiple drug resistance patterns in *P. aeruginosa* isolates from medical samples in Babylon Province, Iraq.

Materials and Methods

Study subjects

A cross-sectional study was conducted at various hospitals in Babylon Province from February 2022 to October 2022 using clinical specimens, as listed in Table 2, and transported to the laboratory for additional examination using common microbiological and biochemical characterization, then confirmed by the Vitek2 system to identified *P. aeruginosa* isolates.

Susceptibility testing for antimicrobials

Relative to CLSI, 2022,[12] antibiotic susceptibility was tested by the disk diffusion test. As a control positive, *P. aeruginosa* ATCC 27853 (ATCC/USA) was also examined as a reference strain for antibiotic resistance. However, isolates that were multiple-drug resistant were distinct as being resistant to at least three different antibiotic groups.[13]

Extraction of genomic DNA

Genomic DNA was extracted from *P. aeruginosa* isolates. The genomic DNA extraction procedure was provided by Favorgen DNA Extraction KIT, Korea and stored at –20°C. DNA concentration and purity of the samples were assessed using Nano-drop (Analytik Jena, Germany).

Polymerase chain reaction assay

The presence of class 1, 2, and 3 integrases was examined in all isolates. For each gene, specific primers were constructed [Table 1]. A final volume of 25 µL was used for the polymerase chain reaction (PCR). Under the following conditions, PCR amplification for the identification of integrase genes *intI1* and *intI*2 was performed as reported,[14] PCR products were separated on 1% agarose gel, and their expected sizes were checked.[15]

Statistical analysis

Chi-square at a probability of 0.05 was applied to assess the differences of tested variables using statistical package for the social sciences, SPSS version 25.0 (SPSS, IBM, Chicago, Illinois) and the odds ratio was calculated.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and written approval before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by University of Babylon ethical committee according to document number B220101 (including the number and the date in 17/01/2022) to get this approval.

Results

Isolates

From diverse clinical samples taken from several hospitals in the Babylon Province of Iraq, 131 verified *P. aeruginosa* isolates were found. Total *P. aeruginosa* isolates were 51.9 % (68/131) from male and 48.1% (63/131) from female hospitalized patients. The mean (±standard deviation [SD]) age of the group was 34.1 ± 20.6 years. From 385 diverse clinical samples taken from several hospitals in the Babylon province of Iraq, 131 isolates were obtained [Table 2].

Resistances patterns

All 131 *P. aeruginosa* isolates were tested for susceptibility to 22 antibiotics [Table 3]. The findings suggested that different isolates' rates of susceptibility to various antibiotics may be observed.

Table 2: Distribution of *Pseudomonas aeruginosa* in patients

Source of sample	No. of isolates	Percentage of isolates (%)
Burn	103	78.63
Wound	7	5.34
Urine	9	6.87
Others	12	9.16
Total	131	100

Table 1: Primers for polymerase chain reaction amplification of integrase genes

Gene	Sequence	Product size (bp)	Reference
Int1: F	5′-GCA TCC TCG GTT TTC TGG-3′	457	[14]
Int1: R	5′-GGT GTG GCG GGC TTC GTG-3′		
Int2: F	5′-CAC GGA TAT GCG ACA AAA AGG T-3′	789	
Int2: R	5′-GTA GCA AAC GAG TGA CGA AAT G-3′		
Int3: F	5′-ATC TGC CAA ACC TGA CTG-3′	922	
Int3: R	5′-CGA ATG CCC CAA CAA CTC-3′		

Figure 1 shows that all *P. aeruginosa* isolates were resistant to ampicillin, cefixime, and cefotaxime in (100%). The isolates were resistant to Ceftizoxime (99.23%), Cefepime (93.90%), Tetracycline (91.60%), Gentamicin (83.97%), Doxycycline (90.84%), Cefoperazone (84.73%), Tobramycin and Meropenem (81.68%) respectively, Ticarcillin clavulanic (80.91%), Amikacin (79.39 %) Piperacillin (77.86%), Imipenem (74.80%), Levofloxacin (75.57%), Aztreonam (74.05%), Ciprofloxacin (73.28%), Norfloxacin (71.75%), Piperacillin-tazobactam PIT (64.12%), and Doripenem

Table 3: Concentrations of antibiotics according to CLSI 2022

Class	Antibiotic	Symbol	Concentration	Company/origin
Aminoglycosides	Amikacin	AK	30 µg	Bioanalyse(Turkey)/ Liofilchem (Italy)
	Gentamicin	CN	10 µg	
	Tobramycin	TOB	10 µg	
Beta-Lactam	Piperacillin-tazobactam	PIT	100/10 µg	
	Ticarcillin-clavulanate	TCC	75/10 µg	
Carbepenems	Imipenem	IMP	10 µg	
	Meropenem	MEM	10 µg	
	Doripenem	DOM	10 µg	
Cephems	Ceftizoxime	CZX	30 µg	
	Cefixime	CFM	5 µg	
	Cefoperazone	CFP	75 µg	
	Cefotaxime	CTX	30 µg	
	Cefepime	FEP	30 µg	
Fluoroquinolone	Ciprofloxacin	CIP	5 µg	
	Norfloxacin	NOR	10 µg	
Macrolides	Azithromycin	AZM	30 µg	
Monobactams	Aztreonam	ATM	30 µg	
Penicillins	Ampicillin	AMP	10 µg	
	Piperacillin	PRL	100 µg	
Tetracyclines	Tetracycline	TE	30 µg	
	Doxycycline	DO	30 µg	
Quinolones	Levofloxacin	LEV	5 µg	

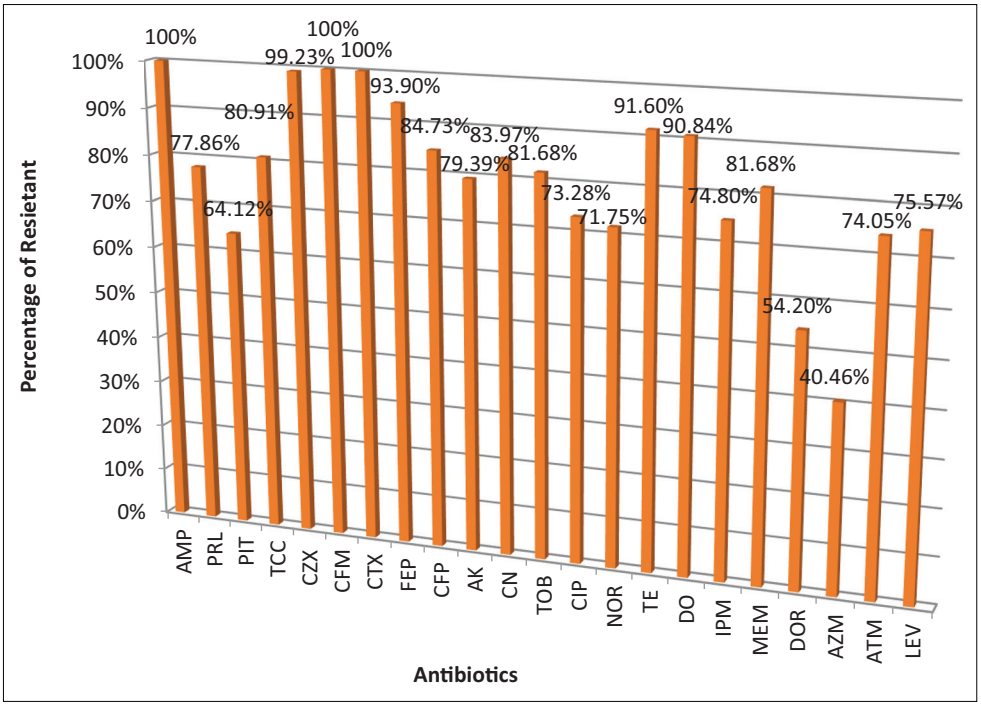

Figure 1: Antibiotics susceptibility of *P. aeruginosa* isolates

(54.20%). Whereas (40.46%) of the isolates were sensitive to Azithromycin. All isolates (100%) showed MDR resistance to at least three groups of antibiotics, with some isolates displaying resistance to nearly all classes.

Molecular profiles of intgrons

The PCR result showed 100% isolates, tested positive for the class 1 and 3.82% of isolates tested positive for two integron cassettes [Table 4]. However, none of the isolates had any class 3 integrons [Figures 2–4].

Association of isolates sources, antibiotic resistances with types of integrons

The occurrence of integrons, class 2 and resistance to antibiotics listed in Tables 4 and 5, was found to be significantly correlated.

Discussion

Pseudomonas aeruginosa-MDR isolates have emerged and spread around the world, raising serious concerns that could make it more difficult to choose empirical agents.

Table 4: Frequency of integron-positive *Pseudomonas aeruginosa* isolates based on clinical specimens

Clinical sample	Integron-1 positive no. (%)	Integron-2 positive no. (%)	Integron-2 negative no. (%)
Burn	103 (100)	2 (1.94)	101 (98.06)
Wound	7 (100)	0	7 (100)
Urine	9 (100)	1 (11.11)	8 (88.88)
Others	12 (100)	2 (16.66)	10 (88.33)
Total	131 (100)	5 (3.82)	126 (96.18)
P Value		0.158	

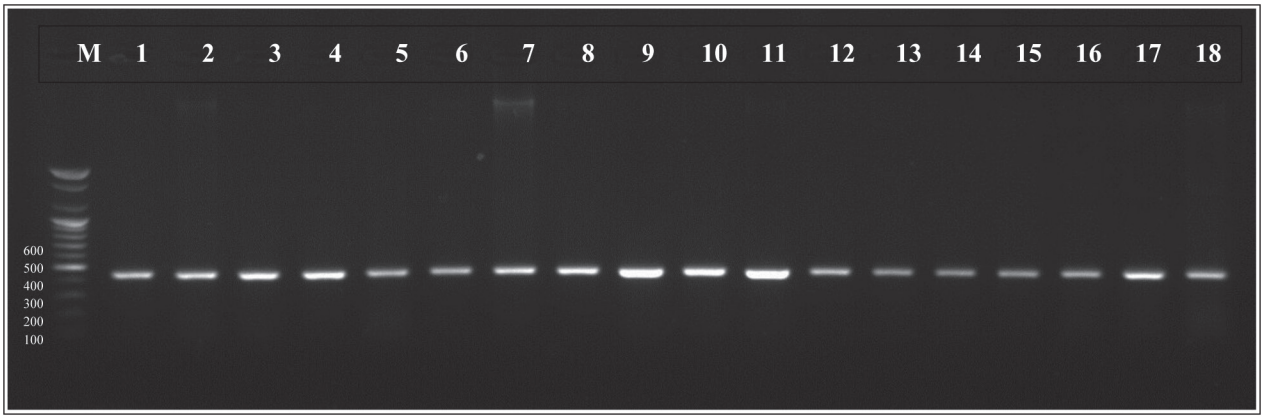

Figure 2: PCR amplification of *intI1* gene (457 bp) in *P. aeruginosa* isolates, on 1.5% agarose at 70 V for 2 h. Positive isolates

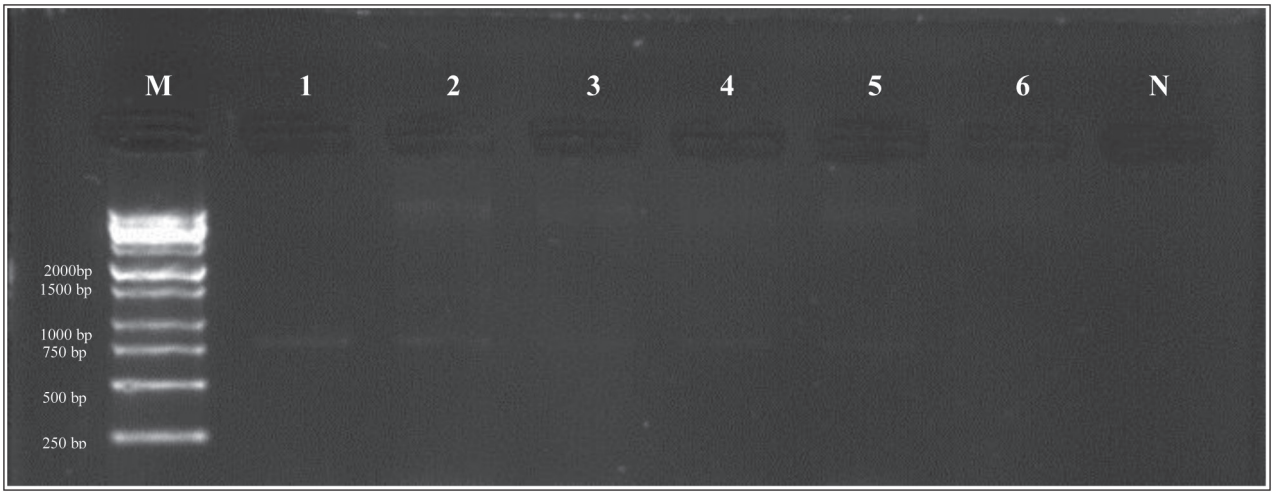

Figure 3: PCR amplification of *intI2* gene (789 bp) in *P. aeruginosa* isolates, on 1.5% agarose at 70 V for 2 h. Lanes (1–5) positive isolates, lane (6) negative isolates. N: negative control

Figure 4: PCR amplification of *intI3* gene (922 bp) in *P. aeruginosa* isolates, on 1% agarose at 70 V for 2 h. All isolates were negative, N: negative control

Table 5: Comparison of the antibiotic resistance between class 2 integron—positive and class 2 integron—negative of *Pseudomonas aeruginosa*

Antibiotics	Resistance, No. (%)	Integron 2-negative isolates R, No. (%)	Integron 2-positive isolates R, No. (%)	*P*-value	OR
Ampicillin (AMP)	131 (100%)	126 (96.18)	5 (3.82)	≤0.0001	0.040
Piperacillin (PRL)	102 (77.86%)	99 (97.06)	3 (2.94)	≤0.0001	0.030
Piperacillin-tazobactam (PIT)	84 (64.12%)	83 (98.81)	1 (1.19)	≤0.0001	0.012
Ticarcillin-Clavulanate (TCC)	106 (80.91%)	103 (97.17)	3 (2.83)	≤0.0001	0.029
Ceftizoxime (CZX)	130 (99.23%)	125 (96.15)	5 (3.85)	≤0.0001	0.040
Cefixime (CFM)	131 (100%)	126 (96.18)	5 (3.82)	≤0.0001	0.040
Cefotaxime (CTX)	131 (100%)	126 (96.18)	5 (3.82)	≤0.0001	0.040
Cefepime (FEP)	123 (93.90%)	119 (96.75)	4 (3.25)	≤0.0001	0.034
Cefoperazone (CFP)	111 (84.73%)	107 (96.40)	4 (3.60)	≤0.0001	0.037
Amikacin (AK)	104 (79.39%)	101 (97.12)	3 (2.88)	≤0.0001	0.030
Gentamicin (CN)	110 (83.97%)	107 (97.27)	3 (2.73)	≤0.0001	0.028
Tobramycin (TOB)	107 (81.68%)	104 (97.2)	3 (2.8)	≤0.0001	0.029
Ciprofloxacin (CIP)	96 (73.28%)	93 (96.88)	3 (2.12)	≤0.0001	0.022
Norfloxacin (NOR)	94 (71.75%)	91 (96.81)	3 (3.19)	≤0.0001	0.033
Tetracycline (TE)	120 (91.60%)	115 (95.83)	5 (4.17)	≤0.0001	0.044
Doxycycline (DO)	119 (90.84%)	114 (95.80)	5 (4.20)	≤0.0001	0.044
Imipenem (IPM)	98 (74.80%)	96 (97.96)	2 (2.04)	≤0.0001	0.021
Meropenem (MEM)	107 (81.68%)	104 (97.2)	3 (2.8)	≤0.0001	0.029
Doripenem (DOR)	71 (54.20%)	68 (95.77)	3 (4.23)	≤0.0001	0.044
Azithromycin (AZM)	53 (40.46%)	50 (94.34)	3 (5.66)	0.002	0.060
Aztreonam (ATM)	97 (74.05%)	95 (97.94)	2 (2.06)	≤0.0001	0.021
Levofloxacin (LEV)	99 (75.57%)	96 (96.97)	3 (3.03)	≤0.0001	0.031

It is huge significant pathogens producing a variety of diseases in hospitals and healthcare situations.[16,17] Many resistance genes can be found as gene cassettes within integrons, that are found on transmissible plasmids and transposons.[18] Integrons have been found as a major source of resistance genes and were thought to act as antimicrobial resistance gene reservoirs in microbial communities. Antibiotic resistance mediated by integrons was reported in *P. aeruginosa* isolates.[19,20] Azithromycin had the highest antibiotic susceptibility rate in the current study, with a susceptibility rate of 59.54%, while the majority of *P. aeruginosa* isolates displayed significant antibacterial agent resistance rates. According to the results of the current study, patients are becoming more resistant (100%) to several categories of antibiotics like ampicillin, cefixime, and cefotaxime. Mutations in the class A -lactamases are the primary source of the resistance to third-generation cephalosporins.[21] In the middle of the 1990s, the fourth-generation Cephalosporin Cefepime was released into clinical use.[22] Furthermore, 83.97% and 79.39% of our isolates were resistant to aminoglycoside groups gentamicin and amikacin, respectively. Resistance of *P. aeruginosa* isolates to aminoglycoside antibiotics in this study was parallel to the study of Poonsuk *et al*.[23] In the current work, three classes of integrase genes were screened using PCR amplification, 100% and 3.81% of *P. aeruginosa* isolates harboring class 1 and 2 integron genes, respectively, but none carried class 3 integron genes among all *P. aeruginosa* isolates.

Many research has reported on the existence of the intI1 gene in tested isolates of *P. aeruginosa*.[24-26] The significance of classes 1 integrons in relative to highly antibiotic-resistant *P. aeruginosa* isolates was shown in the current investigation and agreed with,[27] which also highlighted their prevalence and importance. The int2 gene frequency has been reported.[26,28] Resistance to several β-lactams, including aminoglycosides, rifamycin, chloramphenicol, and quinolones, is conferred by the class 1 integrons. The *attI* recombination site, which is recognized by the integrase and serves as a receptor site for incoming gene cassettes, and the intI gene, which codes for a site-specific recombinase, are the two key parts of an integron.[29] On the basis of the existence of class 1 integrons, the establishment of a high frequency of intI1 in our area raises severe future concerns and has the potential to increase and spread antibiotic resistance. Previous studies found that the intI2 and intI3 genes were absent.[23,30] The rise in MDR strains observed in the current investigation may be related to the recent, unrestricted usage of antibiotics in healthcare. The primary cause of MDR, which results in the selection and spread of bacteria resistant to antibiotics in clinical medicine, is the reckless use of antibiotics without antibiotic sensitivity testing.

Conclusions

In this investigation, we found that MDR *P. aeruginosa* was highly associated with class 1 integrons (100%) suggesting that the frequency of class 1 integrons in our area is alarmingly high, demonstrating the need for epidemiological monitoring.

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Nil.

Conflict of interests

There are no conflicts of interest.

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