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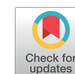
The correlation between biofilm formation capability and antibiotic resistance pattern in *Pseudomonas aeruginosa*



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ABSTRACT

The infections caused by *Pseudomonas aeruginosa* are life-threatening, due to high intrinsic antimicrobial resistance of this microorganism. The integrons and biofilm formation of *P. aeruginosa* have a significant role in antibiotic resistance. Therefore, this study aimed to evaluate antibiotic resistance pattern in *Pseudomonas aeruginosa* isolates with biofilm formation ability. This cross-sectional study from January 2017 to December 2017 was conducted on 78 isolates (58 clinical and 20 environmental) of *P. aeruginosa* recovered from the 547 samples (439 of clinical and 108 of environmental samples). The isolates were identified by phenotypic and genotypic tests. Kirby-Bauer disk diffusion method was used for susceptibility testing. The prevalence of class 1, 2 and 3 Integrons, *rhlA*, and *lasB* genes were determined using Polymerase Chain Reaction (PCR). Biofilm formation was determined using the microtiter plate method. Data analyzed using Stata 14 software and Chi-Square test.

The most prevalent resistance was observed against Ticarcillin/Clavulanic Acid (55%). Generally, 56.4% of isolates were producers of strong biofilm in both environmental and clinical isolates. The prevalence of strong biofilm producers in clinical isolates was more than environmental. A significant correlation was observed between *Int1*, *Int2*, and *rhlA* genes with biofilm formation capability ($P = 0.02$).

Regarding > 50% of both environmental and clinical isolates were producers of strong biofilm and because the source of clinical isolates may be from the environment, the necessary hygiene measurements should be taken. No significant correlation was observed between *lasB* gene with biofilm formation capability.

1. Introduction

Pseudomonas aeruginosa is a Gram-negative microorganism that is the third most common cause of nosocomial infections after *Staphylococcus aureus* and *Escherichia coli* (Abbasi et al., 2017). As well as, it is the main causes of hospital infections, especially in patients with cystic fibrosis (CF), burns or immunodeficiency and those who receive artificial ventilation (Arvanitidou et al., 2005).

The widespread use of antibiotics in recent years has caused the *P. aeruginosa* be resistant to broad-spectrum antibiotics from different groups (Khaledi and Meskini, 2018), where currently existence of strains with multi-drug resistance (MDR) is a major concern in countering with this bacterium in main wards of the hospital, such as the Burn and Intensive Care Units (ICU) (Nakhjavani, 2008). Nosocomial infection is one of the main problems of medicine in developed and also developing countries, which promotes the spread of infectious diseases in the community (Abbasi et al., 2017; Khaledi et al., 2016b). Patients who have burn wound have a higher sensitivity to infection with microorganisms due to the weakened immune system results from loss of skin defenses and burn injuries (Rossolini and Mantengoli, 2005).

In recent years, a wide prevalence of MDR *P. aeruginosa* strains has been reported worldwide (Magiorakos et al., 2012). Different factors play a role in the resistance of bacteria against antibiotics such as; the permeability of microorganisms to antibiotics, the existence of efflux pumps, the change in receptors of microorganisms for antibiotics, and the production of beta-lactamase enzymes (Fajardo and Martínez,

Abbreviations: CF, cystic fibrosis; MDR, multi-drug resistance; ICU, Intensive Care Unit; TSB, Trypticase soy broth

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2008). It has been well established that a large spectrum of these resistance patterns is generated by the acquisition of other genetic elements such as transposon, phage, and mobile gene cassettes containing resistant genes (Khaledi et al., 2016a). These mobile genetic elements have a high potential for transferring drug resistance genes (Svara and Rankin, 2011). There are several classes of integrons, among which classes 1, 2, and 3, especially class 1 have a significant role in the antibiotic resistance (Hall and Collis, 1995).

In *P. aeruginosa*, Metallo-β-lactamase (MBL) are encoded by mobile genetic elements, including class 1 and 2 integrons (Pournaras et al., 2002). Another cause of resistance to *P. aeruginosa* is the biofilms formation. The biofilms do not degrade with antibacterial agents such as disinfectants, heating, drying, and remain on the surfaces, in particular, in hospitals and can cause contamination and transmission of infectious diseases. Biofilm acts as a barrier to the immune system and antibiotics, which is the main cause of infectious diseases (Branda et al., 2005). The genes such as *rhlA* and *lasB* are accounted for Quorum Sensing and the biofilm formation of *P. aeruginosa* (Khairy et al., 2013). As the beginning of *lasB* activity is linked with the start of the initial development of biofilm (Sauer et al., 2002). The next step in biofilm expansion was maturation. In which, the second quorum-sensing regulon (*rhlA*) have a significant role (Davies et al., 1998). Because *P. aeruginosa* has a chief role in producing hospital infections, especially in burn patients, knowledge of how these strains are disseminated has a special epidemiological importance to find the source of infection.

Accordingly, this study aimed to evaluate antibiotic resistance pattern in *Pseudomonas aeruginosa* isolates with biofilm formation ability.

2. Material and methods

2.1. Ethics statement

The Ethics Committee of Hamadan University of Medical Sciences approved the current study protocol (IR.UMSHA.REC.1395.109).

2.2. Collection of specimens

This cross-sectional study with consecutive sampling from January 2017 to December 2017 was conducted. In total, 439 clinical specimens (urine, blood, wounds, Cerebrospinal fluid) of patients with nosocomial (Urinary tract infection, Wound infection, soft tissue and skin infections, Bacteremia) infections admitted in the burn ward of Besat Hospital of Hamadan, Iran, were achieved. Also, 108 environmental samples were collected from the environment (beds, patients' clothing, tap water, and sink). Samples were transferred to the Microbiology Laboratory of the Faculty of Medicine. Finally, isolates were detected and identified by microbiology and biochemical tests (Alikhani et al., 2014). In addition to phenotypic methods, genotypic confirmation of isolates was done by the presence of toxA gene (Frank et al., 1989). In total, 78 (58 clinical and 20 environmental isolates) of *P. aeruginosa* were retrieved.

2.3. Susceptibility testing

Antimicrobial susceptibility was determined using the Kirby-Bauer agar disk diffusion method (Goel et al., 2013). Briefly, pure colonies were taken and inoculated to broth culture (Soybean-Casein Digest Medium). Inoculation was made with a broth culture Soybean-Casein Digest Medium (HiMedia BioSciences Company) diluted to match a 0.5 McFarland turbidity standard. Using an aseptic technique, a sterile swab placed into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube. Using the swab, the Mueller-Hinton agar plate streaked to form a bacterial lawn. To obtain uniform growth, the plate streaked with a swab in one direction, rotated the plate 90° and streaked the plate again in that direction. Repeat this rotation 3 times. Allow the plate to dry for approximately 5 min. An antibiotic disc dispenser was used to dispense discs containing specific antibiotics onto the plate. Using a flame-sterilized forceps gently pressed each disc to the agar to ensure that the disc was attached to the agar. Then, plates were incubated overnight at an incubation temperature of 37 °C. The diameter of the zones of inhibition was measured using a ruler. Finally, the results interpreted as susceptible, intermediate, and resistance according to the Clinical and Laboratory Standard Institute guidelines. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains. The Susceptibility profiles were determined for Ceftazidime (30 μg), efepime (30 μg), Ciprofloxacin (5 μg), Imipenem (10 μg), Meropenem (10 μg), Piperacillin-tazobactam (30 μg), Ticarcillin-clavulanic acid (75/10 μg), Tobramycin (10 μg), Colistin (10 μg), Aztreonam (30 μg), Amikacin (30 μg), and Gentamicin (30 μg) antibiotics (Mast Company, UK).

2.4. Detection of genes by PCR technique

DNA of clinical isolates was extracted using a DNA extraction kit (Merck Company, German) and the frequency of classes 1, 2, and 3 integrons, *rhlA*, and *lasB* genes were determined in isolates using PCR. Briefly, PCR assays for detection of class 1, 2, 3 integrons, *toxA*, *rhlA*, and *lasB* genes were performed using suitable primers (Gu et al., 2007) as listed in Table 1. PCR was conducted in 25 μL of final reaction volume. The reaction contained 2 μL of DNA template, PCR Master Mix (12.5 μL), each primer (10 pmol) with volume 1 μL. PCR conditions were; Initial denaturation (94 °C for 5 min), followed by 35 cycles of denaturation (94 °C for 60 s), an annealing temperature for each gene (according to Table 1) for 1 min, an extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min.

2.5. Biofilm production assay

The biofilm formation was determined using a microtiter plate

Table 1
The primers used in this study for detection of *toxA*, Integrons, and biofilm-related genes.

Genes	(Sequence) 5′–3′	Length (bp)	Annealing temperature	Reference
toxA	F: 5′-GGTAGTTGGTCGCTGAAC-3′ R:5′-GACGAAGAAGGTGGCATC-3′	177	62 °C	This study
Int1	F: 5′- CACGGATATGCGACAAAAAG-3′ R: 5′-GATGACAACGAGTGACGAAATG_3′	160	51 °C	Gu et al. (2007)
Int2	F: 5′- CACGGATATGCGACAAAAAG-3′ R: 5′-GATGACAACGAGTGACGAAATG_3′	787	51 °C	
Int3	F: 5′-GCCTCCGGCAGCGACTTTCAG_3′ R: 5′-ACGGATCTGCCAAACCTGACT_3′	980	51 °C	
lasB	F: 5′-AATGACAAAGTGGAACTGGTGATCC-3′ R: 5′-GTAGGTGTACTTGCCGATCTTCTGG-3′	213	62 °C	Van Belkum et al. (1998)
RhlA	F: 5′- TGCTGATGGTTGCTGGCTTTC -3′ R: 5′- CTCGGTGGTGATGGCATTCG-3′	89	52 °C	This study

method. In brief, after adding bacteria to micro-plates containing 200 uL of Luria Bertani medium (HiMedia BioSciences Company) incubated overnight. Then, Safranin dye was used for staining (O'Toole et al., 1999). The absorbance was measured at a wavelength of 560 nm. The Trypticase soy broth (HiMedia BioSciences Company) with no bacterium was used as a negative control. Finally, for the interpretation of biofilm formation, the criteria of Stepannovic et al. was used (Bardbari et al., 2017). For determining biofilm, the following values were applied:

Non-biofilm producer: OD 560 < 0.275.
Weak biofilm producer: 0.275 ≤ OD 560 < 0.55.
Medium biofilm producer: 0.55 ≤ OD 560 < 0.825.
Strong biofilm producer: 0.825 ≤ OD560.

2.6. Statistical analysis

Statistical analysis was performed using Stata 14 software. The rate of some parameters such as positive cultures of *P. aeruginosa*, susceptibility, Integrons type genes, and biofilm-related genes were categorized. The relationship between biofilm formation capability, the occurrence of biofilm-related genes of *P. aeruginosa* in both of two groups or between of them and relationship between the origin of *P. aeruginosa* isolates and certain features (e.g. resistance pattern) were assessed by Chi-Square. Statistically significant level for all of the testes was considered at $P < 0.05$.

3. Results

3.1. Distribution of isolates in clinical and environmental samples

In total, 78 isolates of *P. aeruginosa* were recovered from both clinical wards (n = 58) and environment (n = 20). Distribution of clinical specimens was as follows; Urine (n = 24), blood (n = 12), wound (n = 13), CSF (n = 9). As well, frequency of environmental samples came from close proximity to the infected patients was as follows; beds (n = 5), patients' clothing (n = 4), tap water (n = 5), and sink (n = 6).

Thirty-four (58.6%) and 24 (41.4%) of patients were males and females, respectively. The mean age of males and females was 39.7 and 25.3 years, respectively.

3.2. Results of susceptibility testing

As presented in Table 2, > 50% of clinical and environmental isolates were resistant to all of the used antibiotics. In clinical isolates, the most resistance has been observed against Ticarcillin/Clavulanic Acid (75.8%) followed by Aztreonam with 72.4% resistance. Also, in environmental isolates, the most prevalent resistance was seen against mentioned antibiotics (55%). 62.8% of isolates (67.2% clinical and 50% of environmental) were MDR and 37.2% were Non-MDR (32.8% and 50% of isolates were from clinical and environmental, respectively).

Table 3
Correlation between MDR and Non-MDR strains and biofilm formation ability by the phenotypic method.

Types of biofilm	Clinical or environmental	MDR	Non-MDR	Total
Strong biofilm producer	Clinical	34 (58.6%)	1 (1.7%)	35 (60.3%)
	Environmental	7 (35%)	1 (5%)	8 (40%)
Moderate biofilm producer	Clinical	3 (5.2%)	12 (20.8%)	15 (26%)
	Environmental	2 (10%)	4 (20%)	6 (30%)
Weak biofilm producer	Clinical	2 (3.4%)	5 (8.6%)	7 (12%)
	Environmental	1 (5%)	3 (15%)	4 (20%)
No biofilm producer	Clinical	0 (0%)	1 (1.7%)	1 (1.7%)
	Environmental	0 (0%)	2 (10%)	2 (10%)
Total	Clinical	39 (67.2%)	19 (32.8%)	58 (100%)
	Environmental	10 (50%)	10 (50%)	20 (100%)
P-value		0.00	0.2	–

3.3. Association of biofilm formation with antibiotic resistance

According to data abstracted in Table 3, most of both clinical and environmental isolates were biofilm producers. Accordingly, biofilm formation ability of the isolates was categorized as the strong, moderate, weak, and non-biofilm producers. In total, 43 of 78 (55.1%) isolates were producers of strong biofilm in both environmental and clinical isolates. About 35 (60.3%) of clinical isolates were strong biofilm producers, and also, 8 (40%) of environmental isolates were producers of strong biofilm. Only 3 (3.8%) of both clinical and environmental isolates were non-biofilm producers.

Of total 78 isolates, 49 (62.8%) were MDR, and remaining 29 (37.2%) of isolates were Non-MDR. Overall, 46.8% of MDR isolates were producers of strong biofilm, of which, 34 (58.6%) were MDR clinical isolates, and also 7 (35%) were environmental isolates with the capability to produce biofilm. A significant association between MDR and biofilm formation ability was reported ($P = 0.00$).

3.4. Biofilm-related genes, integrons, and correlation with biofilm formation

Among clinical isolates of *P. aeruginosa*, the prevalence of genes; *lasB*, *rhlA*, *Int1*, *Int2*, and *Int3* were 54 (93.1%), 56 (96.5%), 36 (63.1%), 16 (27.5%), and 4 (6.9%), respectively. Also, for the same genes in environmental isolates, the prevalence has reported 20 (100%), 17 (85%), 13 (65%), 6 (30%), and 7 (35%), respectively (Fig. 1).

43 (97.7%) of clinical and environmental isolates containing *lasB* gene were strong biofilm producers. Also, 44 (100%) of clinical and environmental isolates which were positive for *rhlA* had a strong biofilm formation ability. Overall, producing the strong biofilm between both types of isolates containing *Int1*, *Int2* and, *Int3* was 38 (88.3%), 18 (40.9%), and 8 (18.1%), respectively. Regarding the biofilm formation capability of most environmental and clinical isolates, a significant correlation was observed between *Int1*, *Int2*, and *rhlA* genes with biofilm formation capability of isolates ($P = 0.02$). Also, no correlation was found between *lasB* and *Int3* with biofilm formation capability ($P > 0.05$), Table 4.

Table 2
The antimicrobial resistance pattern in clinical and environmental isolates of *P. aeroginosa*.

	Co	CPM	ATM	AK	PRL	CIP	CAZ	IMI	GM	MEM	TN	TIM
Clinical												
Susceptible	58 (100)	27 (46.5)	16 (27.5)	30 (51.7)	24 (41.3)	21 (36.2)	30 (51.7)	27 (46.5)	32 (55.1)	28 (48.2)	30 (51.7)	14 (24.1)
Resistance	–	31 (53.4)	42 (72.4)	28 (48.2)	34 (58.6)	37 (63.7)	28 (48.2)	31 (53.4)	26 (44.8)	30 (51.7)	28 (48.2)	44 (75.8)
Environmental												
Susceptible	20 (100)	10 (50)	9 (45)	11 (55)	11 (55)	10 (50)	11 (55)	10 (50)	9 (45)	12 (60)	12 (60)	9 (45)
Resistance	–	10 (50)	11 (55)	9 (45)	9 (45)	10 (50)	9 (45)	10 (50)	11 (55)	8 (40)	8 (40)	11 (55)

Note: CO: Colistin, CPM: Cefepime, ATM: Azteronam, AK: Amikacin, PRL: Piperacilin, CIP: Ciprofloxacin, CAZ: Ceftazidime, IMI: Imipenem, GM: Gentamicin, MEM: Meropenem, TN: Tobramycin, TIM: Ticarcilin/Calvulanic Acid.

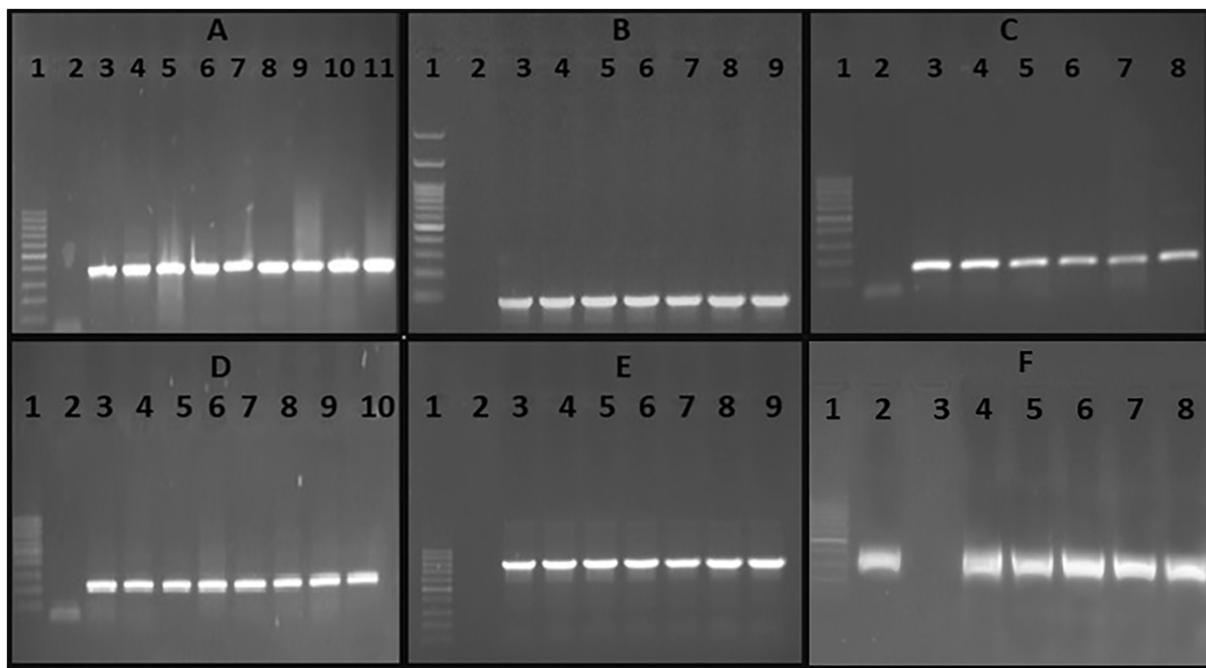

Fig. 1. PCR amplification of *toxA*, *rhlA*, *lasB*, *Int1*, *Int2*, and *Int3* genes. A: lane1:50 bp DNA ladder, lane 2: Negative control, lane 3: Positive control, lanes 4–11: PCR product from *toxA* positive isolates. B: lane 1:100 bp DNA ladder, lane 2: Negative control, lane 3: Positive control, lanes 4–9: PCR product from *rhlA* positive isolates. C: lane1:100 bp DNA ladder, lane 2: Negative control, lane 3: Positive control, lanes 4–8: PCR product from *lasB* positive isolates. D: lane1:100 bp DNA ladder, lane 2: Negative control, lane 3: Positive control, lane 4–10: PCR product from *intI* positive isolates. E: lane1:100 bp DNA ladder, lane 2: Negative control, lane 3: Positive control, lanes 4–9: PCR product from *Int2* positive isolates. F: lane 1:100 bp DNA ladder, lane 2: Positive control, lane 3: Negative control, lanes 4–8: PCR product from *Int3* positive isolates.

Table 4
The frequency distribution of biofilm formation ability and biofilm-related genes in clinical and environmental isolates of *P. aeruginosa.*

Biofilm	*LasB*		*RhlA*		*Int1*		*Int2*		*Int3*	
	No	%	No	%	No	%	No	%	No	%
No-biofilm	3	100	2	66.7	0	0	1	33.3	0	0.0
Weak biofilm	9	90.0	6	60.0	4	40.0	0	0.0	1	10.0
Moderate biofilm	19	90.4	21	100	7	33.4	3	14.2	2	9.5
Strong biofilm	43	97.7	44	100	38	88.3	18	40.9	8	18.1
P-value	0.52		0.00		0.00		0.02		0.66	

3.5. Correlation between class 1, 2, and 3 integrons with antibiotic resistance

As shown in Table 5, there was a significant relationship between *Int1* and antibiotic resistance against Cefepime, Aztreonam, Amikacin, Piperacillin, Ciprofloxacin, Ceftazidime, Imipenem, Gentamicin, Meropenem, and Tobramycin in clinical isolates ($P < 0.05$), this mode there was in environmental isolates ($P < 0.05$), except such correlation, was not observed against Tobramycin in environmental isolates ($P > 0.05$), also, this correlation was not reported between prevalence of class1 integrons and resistance against Ticarcillin/Clavulanic Acid in both clinical and environmental isolates ($P > 0.05$).

Our results showed only statistically significant association between the presence of *Int2* and resistance against Aztreonam ($P < 0.045$), while, didn't show any association between existence of *Int2* and resistance against other antibiotics used in the current study in clinical isolates ($P > 0.05$), but in environmental isolates, such a correlation was observed for Meropenem ($P < 0.011$), and Tobramycin ($P < 0.011$). Also, no relationship between the existence of *Int3* and antibiotics resistance was observed ($P > 0.05$).

4. Discussion

As presented in the Results section, over than 50% of clinical/environmental isolates were MDR. In clinical isolates, the most resistance has been observed against Ticarcillin/Clavulanic Acid (75.8%), followed by Aztreonam with a resistance of 72.4%. Also, in environmental isolates, a highest resistance was observed against mentioned antibiotics (55%). The results of antibiotic resistance in clinical isolates are similar to a study conducted in the north of Iran (Nikokar et al., 2013). Interestingly, in most environmental isolates, the resistance rate against mentioned antibiotics was higher than clinical isolates. Therefore, the transmission of these isolates to patients leads to higher resistance.

For our knowledge, Carbapenems are the most effective antibiotics for the treatment of infections resulted from MDR strains; recently, the increasing occurrence of carbapenem-resistant isolates of *P. aeruginosa* has become a global concern (Rossolini et al., 2007). In the current study, the frequency of resistance against Imipenem and Meropenem was > 50%, but was not reported any resistance to Colistin in both clinical and environmental strains. Thus, this antibiotic was an effective agent against *P. aeruginosa* in our area. Inconsistent with this finding, in the research conducted by Josef Yayan et al. in 2015, no resistance was described against Colistin in *P. aeruginosa* isolated of patients suffered from pneumonia (Yayan et al., 2015). In compared to our findings, in a study carried out in Turkey, a total of 60 *P. aeruginosa* achieved from clinical samples, the maximum susceptibility reported to Colistin (96%), and Piperacillin/Tazobactam (93%) (Gu et al., 2007).

Biofilm formation of *P. aeruginosa* leads to loss of antibacterial susceptibility and the use of more concentrations of antibiotics in the treatment of infections caused by such isolates. Here we focused on the correlation between biofilm formation ability and the presence of related genes, including *lasB*, *rhlA*, and class 1, 2, and 3 integrons.

Different classes of integrons, particularly; class 1 are involved in resistance to various antibiotics that yields multi-drug resistance (MDR) in *P. aeruginosa*. In our study, the prevalence of *Int1*, *Int2*, and *Int3* in

Table 5
Correlation between classes 1, 2, and 3 integrons and antibiotic resistance.

Antibiotics	Clinical						Environmental					
	IntI		*IntII*		*IntIII*		*IntI*		*IntII*		*IntIII*	
	N (%)	*P*-value	N (%)	*P*-value	N (%)	*P*-value	N (%)	*P*-value	N (%)	*P*-value	N (%)	*P*-value
CO	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–	0 (0)	–
CPM	27 (46.4)	0.001	11 (19)	0.239	3 (5.2)	0.615	8 (40)	0.35	4 (20)	0.628	4 (20)	1.000
ATM	30 (52.6)	0.017	15 (25.9)	0.045	4 (6.9)	0.567	10 (50)	0.017	5 (25)	0.157	5 (25)	0.374
AK	24 (42.1)	0.001	19 (15.5)	0.561	3 (5.2)	0.344	9 (45)	0.005	5 (25)	0.050	5 (25)	0.160
PRL	25 (43.9)	0.028	11 (19)	0.385	3 (5.2)	0.635	8 (40)	0.070	5 (25)	0.050	4 (20)	0.642
CIP	28 (49.1)	0.004	11 (19)	0.764	3 (5.2)	1.000	10 (50)	0.003	5 (25)	0.141	5 (25)	0.350
CAZ	25 (43.9)	0.001	11 (19)	0.079	3 (5.2)	0.344	7 (35)	0.370	4 (20)	0.336	6 (30)	0.117
IMI	24 (42.1)	0.007	9 (15.5)	1.000	3 (5.2)	0.615	10 (50)	0.003	5 (25)	0.141	5 (25)	0.350
GM	24 (42.1)	0.001	10 (17.2)	0.140	3 (5.2)	0.316	10 (50)	0.017	5 (25)	0.157	5 (25)	0.374
MEM	25 (43.9)	0.001	10 (17.2)	0.385	3 (5.2)	0.612	8 (40)	0.015	5 (25)	0.018	3 (15)	1.000
TN	26 (45.6)	0.001	9 (15.5)	0.561	3 (5.2)	0.333	8 (40)	0.015	5 (25)	0.018	4 (20)	0.356
TIM	29 (50.9)	0.34	14 (24.1)	0.308	4 (6.9)	0.563	9 (45)	0.16	5 (25)	0.157	5 (25)	0.374

Note: CO: Colistin, CPM: Cefepime, ATM: Azteronam, AK: Amikacin, PRL: Piperacilin, CIP: Ciprofloxacin, CAZ: Ceftazidime, IMI: Imipenem, GM: Gentamicin, MEM: Meropenem, TN: Tobramycin, TIM: Ticarcilin/Calvulanic Acid.

clinical isolates were 36 (63.1%), 16 (27.5%), and 4 (6.9%), respectively. Also, in environmental isolates for the same genes, the prevalence has reported 13 (65%), 6 (30%), and 7 (35%), respectively. Concerning the obtained results, the highest prevalence in clinical/environmental isolates was related to the class 1 integrons, which in the other studies from different regions of the world showed this issue (Kor et al., 2013). Of course, this point should be noted that among integrons, class 1 has a more remarkably role in resistance (Manchanda et al., 2010). Like our findings, in a study conducted by Esmaeili et al., in Iran on the strains isolated from patients admitted in the burn ward, over than 90% of isolates had the class 1 integrons. But in contrast to our findings, they didn't find any correlation between this class of integrons and antimicrobial resistance.

The increasing in clinically-resistant strains likely is elucidated by antibiotic selective pressure and the predominant existence of integrons (Tenover, 2006). However, this is not always absolute and other mechanisms such as; the presence of purines, efflux pumps, plasmid acquisition, and chromosomal mutation can cause antibiotic resistance (Chen et al., 2009).

Generally, 43 (55.1%) of isolates were producers of strong biofilm in both environmental and clinical isolates. The prevalence of strong biofilms producers in clinical and environmental isolates were 35 (60.3%), and 8 (40%), respectively.

Generally, 96.2% of isolates (both of MDR, and Non-MDR) of clinical and environmental isolates could produce the biofilm. In concordant to our results, the studies conducted by Jabalameli et al. and Ghanbarzadeh et al. reported the 96.9%, and 92.4% of biofilm formation in *P. aeruginosa* isolates, respectively (Corehtash et al., 2015; Jabalameli et al., 2012). Of total 78 isolates, 49 (62.8%) were MDR, and remaining 29 (37.2%) were Non-MDR. This high rate of MDR isolates in the present study might due to the inappropriate use of antibiotics.

Overall, 46.8% of MDR isolates were producers of strong biofilm, of which, 34 (58.6%) were clinical isolates, and also 7 (35%) were environmental isolates. It showed a significant correlation between MDR form and biofilm formation ($P = 0.00$). But this association was not found with Non-MDR isolates ($P = 0.2$). In line with our study, others showed a significant correlation between MDR isolates with biofilm formation capability compared to susceptible strains (Abidi et al., 2013; Corehtash et al., 2015).

Integrons like to plasmids and transposons have a great role in the spreading of multi-drug resistance through mobile gene cassettes that may contain resistance genes (Alikhani et al., 2017). As we know, the class 1 integrons chiefly encode the resistance against *Beta*-lactamases, Metallo-*beta*-lactamases, and aminoglycosides through mobile gene cassettes that may contain resistance genes (Oteo et al., 2014). Similarly, in the current study, a significant correlation was found between *Int1* and antibiotic resistance against Cefepime, Aztreonam, Amikacin, Piperacillin, Ciprofloxacin, Ceftazidime, Imipenem, Gentamicin, Meropenem, and Tobramycin in both environmental and clinical isolates ($P < 0.05$), except such correlation was not found against Tobramycin in environmental isolates ($P > 0.05$). Also, this correlation was not reported between the prevalence of class 1 integrons with resistance against Ticarcillin/Clavulanic Acid in both clinical and environmental isolates ($P > 0.05$). Our data are inconsistent with the results of Gu et al. in which isolates with positivity for integrons showed resistance of 88.9%, 92.6%, 88.9%, 48.1%, 59.3%, 25.9%, 63%, 66.7%, and 92.6% against Ceftriaxone, Gentamicin, Ceftazidime, Ciprofloxacin, Imipenem, Cefepime, Amikacin, and Cefotaxime, respectively. As well as, in agreement with our findings, Moradian et al. showed that the attendance of class1 integrons statistically had a significant relationship with resistance to Gentamicin, Ticarcillin, Imipenem, Amikacin, Cefotaxime, and Ofloxacin (Shahandashti et al., 2012).

The class 1 integron is accountable for maximum cases of antibiotic resistance and relatedness to the maximum variety of gene cassettes in a broad range of species (Boucher et al., 2007). Nonetheless, there are other classes of integrons including classes 2 and 3 with conferring the antibiotic resistance. As we know, the class 1 integrons chiefly encode the resistance against *Beta*-lactamases, Metallo-*beta*-lactamases, and aminoglycosides through mobile gene cassettes that may contain resistance genes (Gaze et al., 2005). Classes 2 and 3 integrons share their cassette pool with the class1 integrons (Michael et al., 2014).

Our results showed only statistically significant association between the presence of Int2 and resistance against Aztreonam ($P < 0.045$), while, didn't show any association between the presence of Int2 and resistance against other antibiotics in clinical isolates used in current study ($P > 0.05$). But in environmental isolates, such a correlation was seen for Meropenem ($P < 0.011$), and Tobramycin ($P < 0.011$). While, about 41% of isolates were positive for presence of Int2, but they didn't show any correlation with resistance against most of the antibiotics used. This subject possibly referred to this fact that the integron integrase gene of clinical class 2 integrons is silent, this limits the capability of the integrons to obtain and reorganize gene cassettes (White et al., 2001), and that their range of cassette functions than those of class 1 integrons is greatly more restricted (Biskri and Mazel, 2003).

Our results reported an occurrence of 18.1% of Int3 in isolates producing the strong biofilm, while, were not found a significant association between antibiotics resistance and the existence of Int3 in clinical and environmental isolates. The prevalence of class 3 integrons was reported high in the current study as compared with other studies

(Shibata et al., 2003), although we not found a significant difference between the presence of class 3 integrons and drug resistance.

Our findings showed that source of clinical isolates is possibly from the environment, so, appropriate disinfection should be taken to prevent the transmission of environmental strains to patients and transmission of pathogens from patients to the environment and hospital devices. However, once such resistant strains have emerged, either the host strains can spread among patients and the hospital environment or the resistance can disseminate among bacterial isolates (Livermore, 2002).

Also, a significant correlation was observed between *Int1*, *2*, and *rhlA* genes with biofilm formation capability of isolates ($P = 0.02$). Based on results, among the isolates involved in producing biofilm, the rate of these genes was high. It is known that antibiotic concentration needed to overcome colonization of bacteria in the biofilm is 100 to 1000 fold more than their planktonic type (Whiteley et al., 2001). Another study suggests that antibiotic resistance in non-integrons-encoding isolates may be owing to biofilm formation (Aryanezhad et al., 2016). As well, an interesting finding in our study was that we did not found any correlation between the presence of *lasB* gene and biofilm formation. This difference possibly attributed to this fact that other classes of las operon such as *lasR-lasI* and other genes may be involved in this subject (Pye, 2013). The *lasR-lasI* QS system in *Pseudomonas aeruginosa* will be activated when attachment becomes irreversible and *rhlR-rhlI* will be activated during the first maturation stage (Finelli et al., 2003).

Finally, our results may be useful in the development of disinfection policies to control hospital-acquired infections.

5. Conclusions

In total, > 50% of both environmental and clinical isolates were producers of strong biofilm. Also, a significant correlation was observed between *Int1*, *Int2*, and *rhlA* genes with biofilm formation capability of isolates. This fact showed that the source of clinical isolates may be from the environment. Therefore, the necessary hygiene measurements should be taken for the prevention of transferring the environmental isolates to hospitalized patients, especially in critical wards such as ICU and burn units. No significant correlation was observed between *lasB* gene with biofilm formation capability, So, other genes may be involved in initiating biofilm formation.

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Declaration of competing interest

None declared.

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