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Original Article

Presence of T3SS (exoS, exoT, exoU and exoY), susceptibility pattern and MIC of MDR-*Pseudomonas aerug*inosa from burn wounds

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Abstract

Introduction: The emergence of resistance is a major public health and clinical issue, particularly in pathogens causing nosocomial infections. Recently, there is the emergence of *Pseudomonas aeruginosa* resistance to different broad-spectrum antibiotics.

Methodology: The current study was designed to find out the prevalence of multi-drug resistant (MDR) *P. aeruginosa* in burn patients, the antibiotic susceptibility pattern of MDR *Pseudomonas,* and to determine the Minimum Inhibitory Concentration (MIC) of the effective antimicrobials. The assessment of virulence genes (*exoT*, *exoS*, *exoY* and *exoU*) was also achieved through PCR. In the current study wound swabs were collected from 160 burn patients from two burn units (MTI-Govt. Lady Reading Hospital and MTI-Khyber Teaching Hospital).

Results: Out of these 160 samples, 26 samples (16.25%) were positive for *P. aeruginosa.* Per patients, one isolate was included in the current study. Antibiotic susceptibility pattern showed all *P. aeruginosa* isolates were 100% resistant to amoxicillin-clavulanic acid, 84.62% resistance to Cefepime, and Ceftazidime, and 76.92% resistance to Amikacin, Aztreonam, and Ciprofloxacin. Whereas the lowest resistance was observed to Imipenem and Piperacillin-Tazobactam (53.85%), Colistin Sulfate (23.08%), and Polymyxin-B (15.38%). Regarding the prevalence of MDR, 22 (84.61%) isolates out of 26 were found to be MDR-*P. aeruginosa*. For MDR-*P. aeruginosa*, the MIC range was 1-2 µg/mL against Polymyxin-B, 2-8 µg/mL against Colistin sulfate, 16-1024 µg/mL against Imipenem and 128-1024 µg/mL against Piperacillin-Tazobactam. 100% of the isolates carried *exoT*, 88.46% carried *exoY*, and 57.69% and 38.46% carried *exoU* and *exoS*, respectively.

Conclusions: These findings further emphasize the need for antibiotic discipline and to follow the recommended hospital antibiotic policy to prevent the proliferation of MDR strains of *P. aeruginosa* in the community.

Key words: Pseudomonal infections; burn patients; antibiotic resistance; multi-drug resistance; exoenzymes; MIC.

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Introduction

P. aeruginosa is an opportunistic pathogen responsible for causing 10-20% of severe acute and chronic nosocomial infections such as septicemia, cystic fibrosis, burn and wound infections, [1] pneumonia, catheter-related infection, bloodstream infections, and suppurative thrombophlebitis, [2] iatrogenic infections, [3] endocarditis, multi-organ failure, gastrointestinal infections, dermatitis, bacteremia, bone and joint infections, [4] acute ulcerative keratitis [5] as well as meningitis, skin and soft-tissue infections [6]. It also causes infections in immunocompromised and burn patients [2]. Burn wards often harbor MDR *P. aeruginosa,* which can be a source of infection [5]. MDR *P. aeruginosa* is most common in burn patients because of the presence of denatured and dead burn eschar, and moist environment [7]. This makes the burn wounds vulnerable to infections by *P. aeruginosa* [8].

MDR *P. aeruginosa* is an emerging cause of burn morbidity and mortality and is difficult to eradicate [1]. It is estimated that at least 50% of all the deaths caused by burns are the results of untreatable infections of *P. aeruginosa* [9]. In another report, it is estimated that 75% of all the deaths following burn injuries are related to infections [7]. Furthermore, patients infected with

drug-resistant and MDR *P. aeruginosa* have a higher mortality rate of 34% as compared to patients infected with drug-susceptible *P. aeruginosa* where the mortality rate is 22% [10].

P. aeruginosa infections are problematic due to their intrinsic and acquired resistance to many effective antimicrobial classes [2]. *P. aeruginosa* naturally shows resistance to penicillin and most beta-lactam antibacterial. Therefore, carbapenems are the drug of choice for MDR-*P. aeruginosa*, however, the increasing frequency of carbapenem-resistant *P. aeruginosa* has recently become a serious concern globally [8]. The main reason for increasing drug-resistant *P. aeruginosa* strains is the inappropriate use of antibiotics [11]. The overall prevalence of antibiotic-resistant *P. aeruginosa* is increasing, with up to 10% of global isolates found to be MDR [4]. A study from Pakistan revealed that 99% of the clinical isolate of *P. aeruginosa* were resistant to most commonly used anti-pseudomonal drugs [12].

The treatment for the infections caused by *P. aeruginosa* is frequently complicated due to limited susceptibility patterns to different antibiotics and the emergence of antibiotic resistance during therapy [13]. Eradication of MDR *P. aeruginosa* from hospital burn wards is a demanding task, therefore is preferred to use minimum inhibitory concentration and combination antibiotics therapy to provide broader spectrum antimicrobial effects and to prevent the rapid emergence of resistance in nosocomial infections caused by *P. aeruginosa* [14].

P. aeruginosa pathogenesis is linked with the production of different virulence factors; rhamnolipids, pyocyanin, exotoxin A, elastase, phospholipase C, and Type-III Secretion System (T3SS) [15]. Through T3SS *P. aeruginosa* injects 04 effector proteins into host cytosol: Exo-enzyme Y, Exo-enzyme S, Exo-enzyme T, and Exo-enzyme U encoded by the genes *exoY*, *exoS*, *exoT,* and *exoU*, respectively. *exoY* and *exoT* play a minor role in virulence, *exoY* and *exoT* are present in most of the clinical isolates [16], while *exoU* are generally variably distributed and *exoS* are the more prevalent ones among the isolates [17]. Previous reports showed that *exoS* in burn cases is associated with increased virulence [18]. *ExoU* displays high-level cytotoxicity in various cell lines including fibroblast, epithelial, and macrophages [19,20].

Studies regarding the MICs of various drugs against *P. aeruginosa* and also the presence of different virulence-associated genes in burn patients will increase our understanding for timely treatment strategies in burns to avoid the risk for complications. Limited data is available in this regard from developing countries, especially Pakistan. Keeping in view the above, the present study is carried out with the aim of determining the prevalence of MDR *P. aeruginosa* in burn patients admitted to MTI-Lady Reading Hospital and MTI-Khyber teaching hospital, Peshawar. Isolates will also be checked for their antibiotic susceptibility patterns and MICs of effective antibiotics against MDR strains. Furthermore, enhanced virulence due to the presence of T3SS genes will also be evaluated by amplification of *exoS*, *exoT*, *exoU* and *exoY.*

Methodology

Sample collection

The current experimental study was conducted at the Burn Unit and Microbiology Department of two hospitals (MTI-LRH and MTI-KTH, Peshawar). A total of 160 non-duplicate different clinical pus samples were collected from burn patients at the burn unit of these hospitals using standard microbiological techniques. These clinical samples include 76 males and 84 females, aged between (9 months and 80 years) who were admitted to or attended burn units, over a period of six months. Patients were distributed according to age into the following groups: group 1 (≤ 1 year), age group 2 (2-20 years), age group 3 (21-40 years), age group 4 (41-60 years), and age group 5 (61-80 years).

Isolation and Identification of P. aeruginosa Isolates

Samples were inoculated on MacConkey agar (Oxoid, UK), Blood agar (Oxoid, UK), and *Pseudomonas* cetrimide agar (PCA) plates followed by incubation at 37 ºC for overnight. Phenotypic identification of *P. aeruginosa* was performed using standard laboratory techniques, observing colony morphology on MacConkey and blood agar plates, and biochemical tests (urease, oxidase, motility test, and fermentation of different sugars).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility test of *P. aeruginosa* isolates was performed by Kirby Bauer's Disc Diffusion method using 10 different antibiotics including cell wall inhibitors (Piperacillin-tazobactam, Cefepime, Aztreonam, Ceftazidime, Amoxicillin-clavulanic acid, Imipenem), Cell membrane inhibitors (Colistin sulfate, Polymyxin-B), Protein synthesis inhibitor (Amikacin) and DNA replication inhibitor (Ciprofloxacin). *P. aeruginosa* ATCC 27853 was used as quality-control strain. Results were interpreted according to the guidelines of CLSI-2022 [32].

Table 1. Primers used for detection of virulence genes *exoS*, *exoT*, *exoY* and *exoU* by PCR.

Target Gene	Primer Sequence (5´–3´)	Product Size (bp)	Reference
exoS	F: GCGAGGTCAGCAGAGTATCG R: TTCGGCGTCACTGTGGATGC	118	[21]
exoU	F: CCGTTGTGGTGCCGTTGAAG R: CCAGATGTTCACCGACTCGC	134	
exoY	F: CGGATTCTATGGCAGGGAGG R: GCCCTTGATGCACTCGACCA	289	
exoT	F: AATCGCCGTCCAACTGCATGCG R: TGTTCGCCGAGGTACTGCTC	152	

Determination of Minimum Inhibitory Concentrations (MIC)

Stock solutions of selected antibiotics were prepared according to their labeled potencies and stored immediately at -70 °C. MDR *P. aeruginosa* were selected after antimicrobial susceptibility assay and their MICs of effective antibiotics such as Polymyxin-B, Colistin Sulfate, Imipenem, and Piperacillin-Tazobactam were performed by modified broth microdilution method [33] using Resazurin dye. The MIC was defined as the lowest antimicrobial concentration with no visible observed growth. The MIC_{50} and MIC_{90} were defined as the minimum antimicrobial amount that inhibits 50% and 90% of isolates respectively.

Preparation of DNA Templates and amplification of virulence genes

DNA from all MDR strains were extracted by ethanol precipitation method [34]. The extracted DNA was kept at -20°C before further processing. For the investigation of virulence genes (*exoS*, *exoT*, *exoU* and *exoY*) through PCR, specific primers were used given in the Table 1.

Table 2. Distribution of the Burn Patients and *P. aeruginosa* in terms of age, gender, burn cause, exposure, residence, and stay in hospital.

Factors	Burn cases n (%)	*P. aeruginosa* n (%)
Age (Years)		
≤ 1	10 (6.25)	2 (7.69)
2-20	100 (62.5)	22 (84.61)
21-40	28 (17.5)	-
41-60	20 (12.5)	2 (7.69)
61-80	2 (1.25)	-
Gender		
Male	76 (47.5)	16 (61.53)
Female	84 (52.5)	10 (38.46)
Residence		
Rural	98 (61.25)	14 (53.84)
Urban	62 (38.75)	12 (46.15)
Hospitalization Stay (days)		
1-7	106 (66.25)	16 (61.53)
8-14	18 (11.25)	6 (23.07)
15-21	8 (5)	-
22-28	22 (13.75)	2 (7.69)
29-35	6 (3.75)	2 (7.69)
Burn Cause		
Accidental	160 (100)	26 (100)
Exposure		
Boiled Water	28 (17.5)	4 (15.38)
Cooking	8 (5)	2 (7.69)
Electricity	28 (17.5)	6 (23.07)
Fire	28 (17.5)	2 (7.69)
Gas	12 (7.5)	-
Tea/Milk	34 (21.25)	12 (46.15)
Oil/Petrol	22 (13.75)	-

Results

Demographic data of burn patients

Out of 160 burn cases, 84 (52.5%) were females and 76 (47.5%) were males of age ranges from 1 to 76 years. Maximum number of observed cases were in the age group 2 to 20 years (n = 100, 62.5%). Majority of the burn patients were from rural areas (n = 98, 61.25%). All 160 cases (100%) were accidental, and no one was suicidal. Overall, 34 patients (21.25%) had tea/milk burn injuries followed by 28 patients (17.5%) had boiled water, fire, and electricity injuries. The rest patients were having 13.75%, 7.5%, and 5% burn injuries by oil, gas, and cooking respectively. In contrast to females, burns due to tea/milk exposures were more frequent in males (58.82%) (Table 2).

Isolation of P. aeruginosa from clinical samples

Of 160 clinical pus samples from a burn, 26 (16.25%) were positive for *P. aeruginosa*. Among these 26 *P. aeruginosa* isolates, 16 were collected from males and 10 were from females. Males were commonly affected by *P. aeruginosa* (16, 61.53%) compared to females (10, 38.46%). The prevalence of *P. aeruginosa* was more in age group 2 (22 isolates; 84.61) followed by age group 1 and 4 (2 isolates; 7.69% in each). In patients' distribution according to stay in the hospital, the highest incidence of *P. aeruginosa* was observed in 1-7 days stay (16 isolates; 61.53%) followed by 8-14 days stay (06 isolates; 23.07), 22-28 days stay (02 isolates; 7.69%), 29-35 days stay (02 isolates; 7.69%).

Table 3. Antibiotics susceptibilities of 26 *P. aeruginosa* isolates from burn patients against 10 antibiotics.

Antibiotics	Antibiotic Susceptibility (N = 26)					
	Sensitive - S		Intermediate - I		Resistant - R	
	n	%	n	%	n	%
Colistin Sulfate	20	76.92	0	-	6	23.08
Polymyxin-B	22	84.62	0	-	4	15.38
Amikacin	6	23.08	0	-	20	76.92
Ciprofloxacin	6	23.08	0	-	20	76.92
Piperacillin-Tazobactam	8	30.77	4	15.38	14	53.85
Cefepime	4	15.38	0	-	22	84.62
Ceftazidime	4	15.38	0	-	22	84.62
Ciprofloxacin	6	23.08	0	-	20	76.92
Aztreonam	4	15.38	2	7.69	20	76.92
Imipenem	12	46.15	0	-	14	53.85
Amoxicillin-clavulanic acid	0	-	0	-	26	100.00

Table 4. Distribution of resistance pattern among MDR *P. aeruginosa* isolates against different antibiotics.

Antibiotic resistant profile	Number of antibiotics	Number of antibiotic resistant isolates	Total number of resistant isolates (%)
AMC, ATM, CIP, CAZ, FEP, AK, IPM, TZP, CT, PB	10	1	9.09
AMC, ATM, CIP, CAZ, FEP, AK, IPM, TZP, CT	9	1	9.09
AMC, ATM, CIP, CAZ, FEP, AK, IPM, TZP	8	5	45.45
AMC, ATM, CIP, CAZ, FEP, AK AMC, ATM, CAZ, FEP, CT, PB	6	3	27.27
AMC, CIP, CAZ, FEP, AK	5	1	9.09

CT: colistin sulfate; AK: amikacin; TZP: piperacillin-tazobactam; PB: polymyxin-B; FEP: cefepime; CAZ: ceftazidime; ATM aztreonam; IPM: imipenem; AMC: amoxicillin-clavulanic acid; CIP: ciprofloxacin.

Table 5. Minimum inhibitory concentrations (MICs) of 4 antibiotics against 22 MDR-*Pseudomonas aeruginosa* isolates from burn.

Antibiotics	Number of isolates with MIC values (µg/ml)										
	1	2	4	8	16	32	64	128	256	512	1024
Polymyxin-B	6	16	-	-	-	-	-	-	-	-	-
Colistin Sulfate	-	8	12	2	-	-	-	-	-	-	-
Imipenem	-	-	-	-	4	4	10	-	-	2	2
Piperacillin -Tazobactam	-	-	-	-	-	-	-	8	10	2	2

Table 6. MIC Ranges, and MIC_{50}, MIC_{90} values of 22 MDR *P. aeruginosa* isolates from burn patients.

Antibiotic	MIC Range µg/mL	MIC_{50} µg/mL	MIC_{90} µg/mL
Polymyxin-B	1-2	2	2
Colistin Sulfate	2-8	4	4
Imipenem	16-1024	64	512
Piperacillin - Tazobactam	128-1024	256	512

MIC_{50}: MIC that inhibit 50% of strains; MIC_{90}: MIC that inhibit 90% of strains.

The highest incidence of *P. aeruginosa* was observed in burn cases of Tea/milk (n = 12/26, 46.15%) followed by electricity (n = 6/26, 23.07%) and boiled water (n = 4/26, 15.38%). The lowest incidence of *P. aeruginosa* was found in cooking and in fire cases which was 2/26 (7.69%) in each (Table 2).

Antimicrobial Susceptibility Profile of Pseudomonas aeruginosa

Table 3 shows the antibiotic susceptibility pattern of all *P. aeruginosa* isolates, all pseudomonal isolates were 100% resistant to amoxicillin-clavulanic acid, 84.62% resistance to Cefepime and Ceftazidime and 76.92% resistance to Amikacin, Aztreonam and Ciprofloxacin. Whereas low resistance was shown to Imipenem and Piperacillin-Tazobactam (53.85%), Colistin Sulfate (23.08%), and Polymyxin-B (15.38%). Among these 22 (84.61%) out of 26 are resistant to three or more antibiotics and were interpreted as MDR *P. aeruginosa*. While 4 (15.39%) strains are resistant to only one antibiotic, interpreted as non-MDR.

Table 4 shows the distribution of resistance/resistance pattern among MDR *P. aeruginosa* isolates against different antibiotics, one MDR *P. aeruginosa* isolate showed resistance to 10 antibiotics, one isolate showed resistance to 9 antibiotics, and five showed resistance to 8 antibiotics, three isolates showed resistance to 6 antibiotics, and one isolate showed resistance to 5 antibiotics. The most frequent resistance profile among isolates included resistance to 10 (9.09%), 9 (9.09%), 8 (45.45%), 6 (27.27%), and 5 (9.09%) antibiotics.

Minimum Inhibitory Concentration (MIC)

The MICs of each antibiotic alone for *P. aeruginosa* are shown in Table 5. The Polymyxin-B had low MICs for *P. aeruginosa* (MIC_{50} 2 µg/mL: MIC_{90} 2 µg/mL). The values of MIC_{90} of Polymyxin-B, Colistin Sulfate, Imipenem, and Piperacillin-Tazobactam for MDR- *P. aeruginosa* strains were 2, 4, 512 and 512 µg/mL, respectively. While the values of MIC_{50} of Polymyxin-B, Colistin Sulfate, Imipenem, and Piperacillin-Tazobactam for MDR- *P. aeruginosa* strains were 2, 4, 64 and 256 µg/mL, respectively. Table 6 shows MIC Ranges, and MIC_{50}, MIC_{90} values of all MDR *P. aeruginosa*.

Genotypic detection of virulence genes

Concerning the virulence genes, prevalent one was *exoT* (100%; 26/26) followed by *exoY* (88.46%; 23/26), *exoU* (57.69%; 15/26), and *exoS* (38.46%; 10/26). 4 (15.38%) of isolates carried both *exoU* and *exoY,* while 10 (38.46%) showed existence of *exoY* and *exoS,* and only two (7.69%) carried both *exoU* and *exoS* genes. Co-existence of *exoU*, *exoS*, and *exoY* was observed in 1(3.84%) isolate (Figure 1).

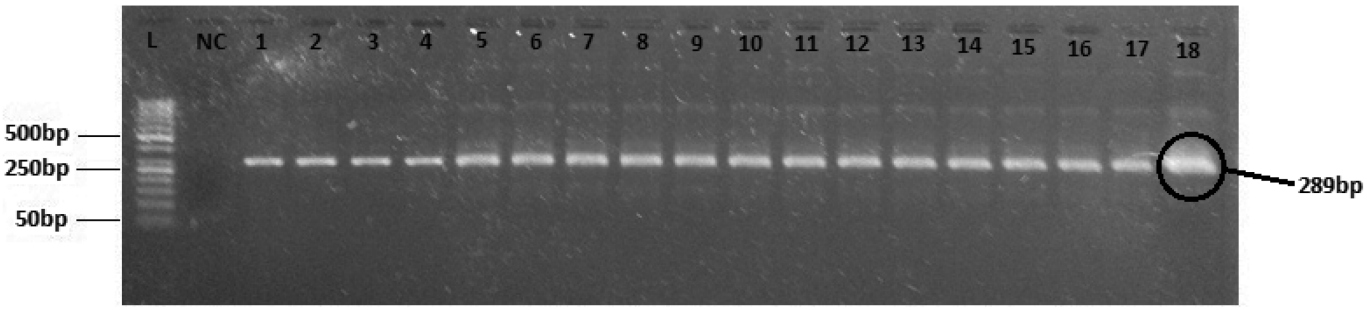
Discussion

P. aeruginosa is a serious nosocomial pathogen and the major cause of fatal infections in cystic fibrosis, hospitalized, immunocompromised, and especially in burn patients. The main causes of morbidity and mortality in burn wound infections are increased prevalence and drug resistance. Because of the increased prevalence rate of *P. aeruginosa,* they are exposed to different antibiotics and thus become MDR [22].

In the current study, the female (52.5%) population was affected more as compared to males (47.5%). This may be due to more involvement of females in household chores, which demand more exposure to fire (e.g., in cooking and heating). A similar pattern was observed by Anvarinejad *et al.* [9], females (53%) were affected more than males (47%).

In the current study, the most prevalent organism isolated from burn patients was *P. aeruginosa* (16.25%). Similar studies are available in literature that showed a high prevalence of *P. aeruginosa* in burn patients [2,23]. Naseer *et al.* from Egypt also reported *P. aeruginosa* as the most frequent isolate (21.6%) in their study [24]. The high prevalence of *P. aeruginosa* in the burn center is due to the presence of dead and

Figure 1. Amplification bands of isolate 1 to 18 *exoY* (289bp) on the agarose gel.



denatured burn eschar, and a moist environment which makes the burn wounds vulnerable to infections by *P. aeruginosa* [7,8].

The existence of MDR *P. aeruginosa* isolates leads to many problems, concerning the treatment of infections. Therefore, continuous surveillance to prevent the further spread of MDR *P. aeruginosa* isolates and inhibition of colonization in burn centers should be employed. In present study 22 (84.61%) isolates were MDR *P. aeruginosa*. A similar study was carried out by Anvarinejad *et al.* [9], which revealed that 63.50% of isolates were MDR *P. aeruginosa* among burn patients.

Pseudomonal infections in burn patients are highly resistant. So, it is important to develop treatment strategies against these highly resistant infections. For this, it is necessary to design such a type of research study which find out the accurate amount of antibiotics for the treatment of the patient. Keeping in view this, the present study was designed to find out antibiotic susceptibility patterns and MICs of effective drugs against Pseudomonal isolates.

In the present study, the resistance rate of *P. aeruginosa* isolates against investigated antibiotics was relatively high. All isolates were resistant to Amoxicillin-clavulanic acid (100%) followed by cefepime and ceftazidime (84.62%), aztreonam, ciprofloxacin and amikacin (76.92%). A similar study by Nikokar *et al.* [11], showed that *P. aeruginosa* acquired high-level resistance against Ceftazidime (68.6%), Piperacillin (69.7%), and Ciprofloxacin (63.3%). A high level of resistance to Gentamicin (80%), Amikacin (73%) was also reported by MR *et al.* [25]. Upadhaya *et al.* showed that the highest resistance was found against Piperacillin/Tazobactam (82.4%), Cefotaxime (76.5%) Ceftazidime (70.6%) [2]. Intermediate resistance was seen in imipenem and piperacillin-tazobactam (53.85%) and low-level resistance was seen in colistin sulfate (23.08%) and polymyxin-B (15.38%) in our study. While a study conducted by Upadhaya *et al.* [2], in Nepal, shows a low level of resistance for Gentamicin (37.2%) and Imipenem (23.3%).

In the current study out of 26 Pseudomonal isolates, 22 (84.61%) were found to be MDR. Goudarzi *et al.* in Iran also found 80.8% multidrug resistance in *P. aeruginosa* which is higher than the rate of MDR reported in Turkey (20.9%) and Brazil (71%) and lower than China (90.1%) and Thailand (100%) [26]. The main factors that play a crucial role in developing resistance are a poor hygienic statement, the difference in the type of strains, epidemiological conditions, the ability of strains the acquisition of resistant genes, unrestricted prescriptions, and frequent use of antibiotics for the treatment of burn infections.

In the present study, Polymyxin-B was the most effective antibiotic against *P. aeruginosa* which is consistent with other studies conducted at Iranian burn centers. A similar study by Upadhaya *et al.* [2], in Nepal, showed that Imipenem was the most effective antibiotic against *P. aeruginosa*. Similar studies [23], showed that Amikacin was the most effective drug against all *P. aeruginosa* isolates with maximum sensitivity (80.5%) followed by Imipenem (66.7%) and Gentamicin (56.1%).

In the present study, the MIC assay was done by the modified broth microdilution method. MIC_{50} and MIC_{90} values were 2 and 2 µg/mL for polymyxin B; 64 and 512 µg/mL for imipenem; 4 and 4 µg/mL for colistin sulfate; 256 and 512 µg/mL for piperacillin-tazobactam, respectively. A similar study was carried out by Nazli *et al.* [27], in which MIC_{50} and MIC_{90} values were 2 and 32 µg/mL for imipenem, and 1 and 1.5 µg/mL for colistin, respectively. Another study by Maeda *et al.* [28] reported that MIC_{50} and MIC_{90} values were 16 and 256 µg/mL for meropenem, 32 and 128 µg/mL for piperacillin-tazobactam respectively.

Concerning the virulence genes, 100% of the isolates carried *exoT*, 88.46% carried *exoY*, 57.69% and 38.46% carried *exoU* and *exoS*, respectively. The virulence gene *exoT* and *exoY* as compared to *exoS* and *exoU* were more prevalent in the isolates, confirming previous reports [17,29]. Cytotoxicity toward macrophages and epithelial cells triggered by *ExoU* has been previously reported [30,31]. The presence of *exoU* gene in MDR-*P. aeruginosa* is of great importance due to *exoU* association with a high level of cytotoxicity and very restricted therapeutic choices to treat burn patients. The *exoU* gene-carrying isolates showed resistance to ceftazidime, cefepime, carbapenems, gentamicin and piperacillin-tazobactam. This association between *exoU* gene and MDR-*P. aeruginosa* was also reported by Garey *et al.* [16].

Conclusions

The absence of new anti-pseudomonal agents against MDR *P. aeruginosa* has amplified the problem. Therefore, polymyxin B and colistin are becoming the last resort for the treatment of such infections. Consequently, these agents must be instituted early in the management of patients, who have as a consequence of their injury, a severely challenged renal function, and, therefore, contribute to increased morbidity and mortality. The use of these two agents was stopped

since 1980 due to dose-related nephrotoxicity. Colistin should mainly be used as salvage therapy in combination with one or more antimicrobials. In the present study, maximum strains were sensitive to polymyxin B (84.62%) and colistin sulfate (76.92%). These findings further emphasize the need for antibiotic discipline and to follow the recommended hospital antibiotic policy to prevent the proliferation of MDR strains of *P. aeruginosa* in the community.

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