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Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms

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Objectives: **To assess the prevalence, levels of antimicrobial susceptibility and resistance mechanisms of *Pseudomonas*.**

Methods: **A total of 445 clinical isolates and 200 environmental isolates were collected from three hospitals in Minia, Egypt. The MICs of different antibiotics were determined using the agar dilution method. The isolates were tested for β-lactamase production and for the presence of efflux pumps.**

Results: **Out of the 445 clinical specimens, 107 *Pseudomonas* strains (24%) and 81 *Pseudomonas aeruginosa* strains were isolated (18.2%). Out of the 200 environmental specimens, 57 *Pseudomonas* strains (28.5%) and 39 *P. aeruginosa* strains were isolated (19.5%). Amikacin was the most active drug against *P. aeruginosa* followed by meropenem, cefepime and fluoroquinolones. *P. aeruginosa* was highly resistant to all other antibiotics tested. The environmental isolates of *P. aeruginosa* exhibited higher antibiotic resistance than clinical isolates. Mechanisms of resistance used by *P. aeruginosa* included β-lactamase production and multiple drug resistance efflux pumps. Our results showed that 29 (36%) of the clinical *P. aeruginosa* isolates and 37 (95%) of the environmental *P. aeruginosa* isolates were β-lactamase producers. In addition, *P. aeruginosa* isolates effectively used an efflux-mediated mechanism of resistance against ciprofloxacin and meropenem, but not gentamicin or cefotaxime.**

Conclusions: **This study examined the prevalence of *P. aeruginosa*, and its susceptibility patterns to different antibiotics. The presence of antibiotic-resistant *P. aeruginosa* isolates could be attributed to β-lactamase production and the use of multiple drug resistance efflux pumps.**

Keywords: nosocomial infections, antibiotics, β-lactamases, efflux pumps

Introduction

The Gram-negative bacterium *Pseudomonas aeruginosa* is a ubiquitous aerobe that is present in water, in soil and on plants. Moreover, *P. aeruginosa* can be frequently isolated from tap water in patient rooms.[1] However, clinical isolates of *P. aeruginosa* appear to be more resistant to amoebal ingestion than environmental isolates.[2]

P. aeruginosa accounts for a significant proportion of nosocomial infections.[3] A general problem with nosocomial infections is the tendency of nosocomial pathogens to acquire new antibiotic resistance.[4] Multidrug-resistant (MDR) strains of *P. aeruginosa* are often isolated among patients suffering from nosocomial infections, particularly those in the intensive care unit (ICU).[5] Thus, infections caused by *P. aeruginosa* are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs.[6]

As an opportunistic infectious pathogen, *P. aeruginosa* can often lead to life-threatening diseases. For example,

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P. aeruginosa is the main cause of mortality in cases of polymicrobial bacteraemia,[7] and the second most common bacterium causing sepsis in the ICU.[8] In addition, *P. aeruginosa* has been implicated in urinary tract infections, burn wounds, ventilator-associated pneumonia and multi-organ system failure.[9–12]

Therefore, it was important to study the susceptibility patterns of *P. aeruginosa* isolates to some commonly used antibiotics in Egypt. Use of the antibiogram as an epidemiological indicator for our isolates can help us make the best use of these antimicrobial agents in the management of *P. aeruginosa* infections. We also studied the prevalence of *Pseudomonas* species infections, especially *P. aeruginosa*, in hospitalized patients and in the hospital environment in Egypt. The high intrinsic antibiotic resistance of this organism is attributed to factors such as active drug efflux and β-lactamase production.[13] Thus, we concluded the study by testing these two possible mechanisms of resistance of the isolated *P. aeruginosa* to different antibiotics.

Materials and methods

Study design

A total of 445 clinical specimens were examined; 100 urine specimens (from patients with urinary tract infection), 45 stool specimens (from patients with gastrointestinal track infections), 50 sputum specimens (from patients with respiratory tract infections), 8 ear swab specimens (from patients with ear infections), 100 wound swab specimens, 45 abscess swab specimens and 25 burn swab specimens (from patients with wounds, abscesses, or burns). All specimens were collected from El-Minia University Hospital, El-Minia General Hospital and El-Minia Chest Hospital in Egypt. In addition, 200 environmental specimens were randomly collected from furniture, medical appliances and the surroundings of the same hospitals such as patients' beds, tables, ward sinks and surgical equipment. All specimens were examined for the existence of *Pseudomonas* species, and *P. aeruginosa* by standard procedures.[14,15] Ethical approval to perform the study was obtained from the management boards of these hospitals and the Egyptian Ministry of Health and Population.

PCR detection of P. aeruginosa

This method was used before by Abd-El-Haleem *et al.*[16] for the detection of *P. aeruginosa*. Total bacterial DNA was prepared using the boiling approach. Bacterial cells were pelleted by centrifugation, resuspended in 50 μL of TE buffer and then lysed by boiling for 10 min. The lysate was centrifuged and the supernatant was transferred to a new tube. The crude cell lysate was used directly for PCR.

The primer pair PaLif (5′-ATGGAAATGCTGAAATTCGGC-3′) and PaLir (5′-CTTCTTCAGCTCGACGCGACG-3′) was selected in order to amplify conserved regions of a target gene in *P. aeruginosa* and thus generate a PCR amplicon with a certain molecular weight (504 bp) that can be identified by gel electrophoresis.

PCR assays were performed in a 50 μL volume with 2 U of DNA *Taq* polymerase (GIBCO PRL) in a thermal cycler (PTC-100 MJ Research, Watertown MA, USA). After initial denaturation for 2 min at 94°C, 30 cycles were performed (the conditions for each cycle were: 30 s at 94°C, 30 s at 51°C and 1 min at 72°C). The final cycle was followed by 72°C incubation for 7 min. A reaction mixture containing sterile water was included as a negative control and a purified DNA mixture of the targeted bacteria was included as a positive control. The amplified PCR products were analysed by gel electrophoresis in 2% agarose gels stained with ethidium bromide, and then visualized and photographed in a Multi-Image light cabinet (Alpha Innotech Corporation, USA).

Antibiotics

The following antibiotics were obtained from the Egyptian market: ampicillin (Nile Pharmaceutical Company, Cairo, Egypt), ampicillin/sulbactam (Medical Union Pharmaceutical Company, Cairo, Egypt), amoxicillin (Egyptian International Pharmaceutical Industries Company; EIPICO, Cairo, Egypt), amoxicillin/clavulanate (Medical Union Pharmaceutical Company), cefalexin (Glaxo Wellcome, Cairo, Egypt), cefuroxime (Glaxo Wellcome), cefotaxime (Aventis Pharma, Cairo, Egypt), cefoperazone (Pharco Pharmaceuticals, Cairo, Egypt), ceftriaxone (EIPICO), cefepime (Bristol Myers Squibb; BMS, Cairo, Egypt), meropenem (Astra-Zeneca, Cairo, Egypt), gentamicin (Memphis for Pharmaceutical Chemical Industries Co., Cairo, Egypt), amikacin (Bristol Myers Squibb) and chloramphenicol (Chemical Industries Development; CID, Cairo, Egypt). Tetracycline, ciprofloxacin, levofloxacin, ofloxacin, norfloxacin and azithromycin were obtained from Sigma-Aldrich (St Louis, MO, USA).

Determination of MICs

The MICs of different antibiotics were determined by the agar dilution method, according to the CLSI (formerly known as the NCCLS) (1997), on Mueller–Hinton agar (MHA). Overnight cultures of tested organisms on Mueller–Hinton broth (MHB) were diluted to the initial cell density of $\sim 10^7$ cfu/mL with fresh MHB. Inocula of $\sim 10^5$ cfu per spot were applied to the surface of dry MHA plates containing graded concentrations (from 1–1024 mg/L) of the respective antibiotics. Plates were incubated at 37°C for 18–24 h and MICs were calculated. Spots with the lowest concentrations of antibiotic showing no growth were defined as the MIC.

β-Lactamase detection

The tested isolates were tooth-picked onto the surface of nutrient agar plates. After overnight incubation at 37°C, the plates were overlaid with 1% molten agarose containing 0.2% soluble starch and 1% penicillin. The plates were incubated for 15 min at room temperature to solidify, and iodine solution was poured onto the agar plates. After 10 s, the residual iodine solution was damped out and the plates were incubated at room temperature until a discolouration zone appeared around β-lactamase-producing colonies.[17] Thus, the presence of a clear zone around bacterial growth is indicative of β-lactamase production.

Study of efflux system

We employed the method of Lomovskaya *et al.*[18] to study the efflux system of *P. aeruginosa* isolates. The MICs of four antibiotics of different groups (ciprofloxacin, meropenem, cefotaxime and gentamicin) for 25 MDR *P. aeruginosa* isolates were examined in the presence and absence of 10 μM of the efflux inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Sigma). The reduction in MIC of a certain antibiotic with CCCP is an indication of resistance to this antibiotic mediated by an efflux system.

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Table 1. Prevalence of *Pseudomonas* species in clinical specimens

Type of specimen	Number of specimens	*Pseudomonas* isolates, no. (%)[a]	*P. aeruginosa* isolates, no. (%)[a]
Urine	100	29 (29)	22 (22)
Stool	45	3 (6.7)	2 (4.4)
Sputum	50	4 (8)	3 (6)
Ear discharge	80	21 (26.3)	16 (20)
Wound exudate	100	16 (16)	11 (11)
Abscess exudate	45	13 (28.8)	9 (20)
Burn exudate	25	21 (84)	18 (72)
Total	445	107 (24)	81 (18.2)

[a]Percentage of the number of isolates with respect to the total number of specimens.

Table 2. Prevalence of *Pseudomonas* species in environmental specimens

Source of specimens	Number of specimens	*Pseudomonas* isolates, no. (%)[a]	*P. aeruginosa* isolates, no. (%)[a]
El-Minia University Hospital	90	32 (35.6)	24 (26.7)
El-Minia General Hospital	70	18 (25.7)	12 (17.1)
El-Minia Chest Hospital	40	7 (17.5)	3 (7.5)
Total	200	57 (28.5)	39 (19.5)

[a]Percentage of the number of isolates with respect to the total number of specimens.

Results

Prevalence of Pseudomonas species in clinical and environmental specimens

Out of the 445 clinical specimens, 107 *Pseudomonas* strains (24%) and 81 *P. aeruginosa* strains were isolated (18.2%). Out of the 200 environmental specimens, 57 *Pseudomonas* strains (28.5%) and 39 *P. aeruginosa* strains were isolated (19.5%), as shown in Tables 1 and 2. From Table 1, the percentage of *P. aeruginosa* isolates with respect to the total number of clinical *Pseudomonas* isolates can be calculated as $81/107 \times 100 = 75.7\%$. From Table 2, the percentage of *P. aeruginosa* isolates with respect to the total number of environmental *Pseudomonas* isolates can be calculated as $39/57 \times 100 = 68.4\%$.

To further examine the percentage of *P. aeruginosa* isolates with respect to the total number of *Pseudomonas* isolates, 40 random *Pseudomonas* strains (20 clinical and 20 environmental) were examined biochemically and by PCR followed by gel electrophoresis and results were comparable (Figure 1). The average percentage of *P. aeruginosa* isolates with respect to the total number of clinical *Pseudomonas* isolates was 80%, whereas the average percentage of *P. aeruginosa* isolates with respect to the total number of environmental *Pseudomonas* isolates was 57.5%. Thus, the biochemical and molecular methods for identification of *P. aeruginosa* did not show a significant difference from our standard procedures for detecting the bacterium.[14,15]

Antibiotic susceptibility and determination of MICs

Tables 3 and 4 show the respective MIC distributions of different antibiotics for clinical isolates (81 isolates) and environmental isolates (39 isolates) of *P. aeruginosa*. Tables 5 and 6 show the

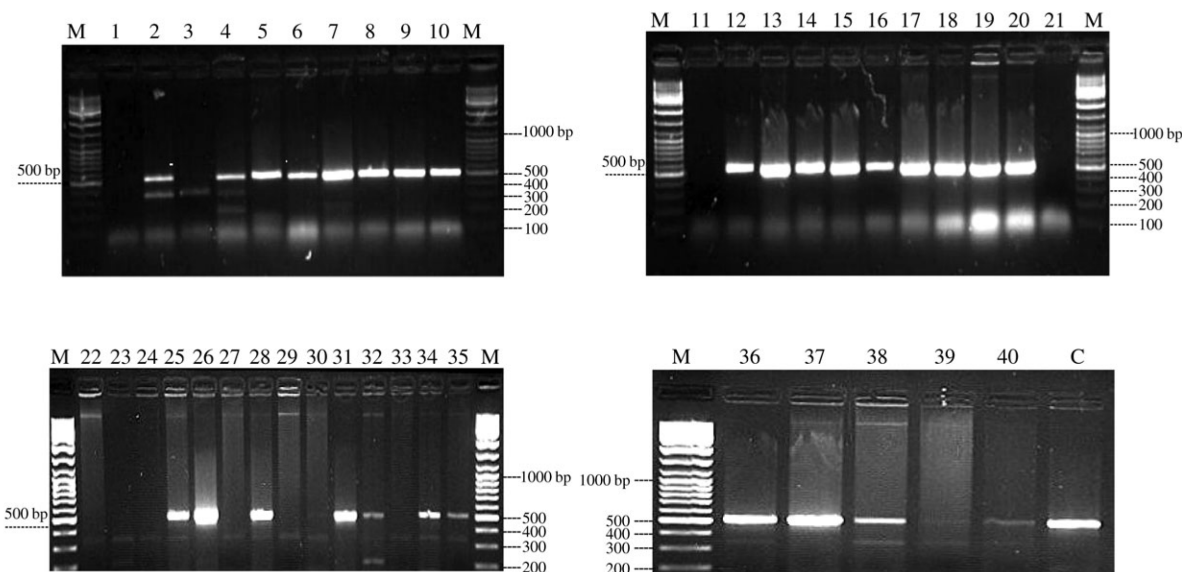

Figure 1. Gel electrophoresis of the PCR-amplified products for detection of clinical (lanes 1–20) and environmental (lanes 21–40) *Pseudomonas* isolates.

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Table 3. MICs of different antibiotics for clinical *P. aeruginosa* isolates (81 isolates)

Antibiotic	Breakpoint[a] (mg/L)	Number of isolates with MICs (mg/L) of											
		1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	8	0	0	0	0	0	5	2	8	6	20	30	10
Ampicillin/sulbactam	8	0	0	0	11	5	4	15	15	22	4	5	0
Amoxicillin	8	0	0	0	0	0	2	7	3	8	12	31	18
Amoxicillin/clavulanate	8	0	0	0	3	4	2	8	8	23	27	6	0
Cefalexin	16	0	0	0	2	5	4	2	12	3	14	38	1
Cefuroxime	16	0	0	0	5	5	3	2	3	9	24	30	2
Cefoperazone	16	0	0	0	15	16	21	8	9	7	5	0	0
Cefotaxime	8	0	0	0	8	18	18	12	9	9	6	1	0
Ceftriaxone	8	0	0	2	19	20	15	6	10	4	3	2	0
Cefepime	8	0	0	16	28	14	11	4	5	1	2	0	0
Meropenem	4	28	16	13	6	6	8	3	1	0	0	0	0
Chloramphenicol	8	0	0	0	3	8	6	7	6	10	13	23	5
Tetracycline	4	0	0	0	4	13	10	24	19	3	8	0	0
Gentamicin	4	0	2	19	12	15	10	7	10	3	3	0	0
Amikacin	16	0	3	19	26	21	6	4	1	1	0	0	0
Ciprofloxacin	1	33	24	7	5	5	1	2	3	1	0	0	0
Levofloxacin	2	16	29	11	6	3	8	5	2	1	0	0	0
Norfloxacin	4	9	26	25	0	7	5	1	1	3	4	0	0
Ofloxacin	2	32	21	8	7	4	2	3	3	0	1	0	0
Azithromycin	4	0	4	6	6	19	16	14	8	8	0	0	0

[a]Breakpoints of different antibiotics according to NCCLS (1997).

Table 4. MICs of different antibiotics for environmental *P. aeruginosa* isolates (39 isolates)

Antibiotic	Breakpoint[a] (mg/L)	Number of isolates with MICs (mg/L) of											
		1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	8	0	0	0	0	0	0	0	0	3	7	16	13
Ampicillin/sulbactam	8	0	0	0	0	0	0	1	1	14	11	12	0
Amoxicillin	8	0	0	0	0	0	0	0	0	0	9	7	23
Amoxicillin/clavulanate	8	0	0	0	0	0	0	0	0	11	20	8	0
Cefalexin	16	0	0	0	0	0	2	0	1	3	6	27	0
Cefuroxime	16	0	0	0	0	1	2	4	0	3	12	17	0
Cefoperazone	16	0	0	0	0	2	2	3	12	11	6	3	0
Cefotaxime	8	0	0	0	0	0	1	4	12	11	5	6	0
Ceftriaxone	8	0	0	0	3	0	6	9	8	6	4	3	0
Cefepime	8	0	0	0	8	11	10	2	4	3	1	0	0
Meropenem	4	9	3	5	4	5	2	6	3	0	0	0	0
Chloramphenicol	8	0	0	0	0	0	0	0	1	4	14	12	8
Tetracycline	4	0	0	0	1	5	18	15	0	0	0	0	0
Gentamicin	4	0	0	2	4	6	8	7	4	2	6	0	0
Amikacin	16	0	0	5	11	4	12	3	4	0	0	0	0
Ciprofloxacin	1	9	6	3	2	3	5	6	4	1	0	0	0
Levofloxacin	2	2	3	9	4	6	4	8	2	1	0	0	0
Norfloxacin	4	1	3	4	4	6	5	4	3	6	3	0	0
Ofloxacin	2	6	4	2	3	5	7	4	6	2	0	0	0
Azithromycin	4	0	0	1	4	9	17	3	5	0	0	0	0

[a]Breakpoints of different antibiotics according to NCCLS (1997).

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Table 5. Antibiotic susceptibility of clinical *P. aeruginosa* isolates

Antibiotic	Susceptible		Intermediate		Resistant		MIC_{90}
	Number	%[a]	Number	%[a]	Number	%[a]	
Ampicillin	0	0	0	0	81	100	>1024
Ampicillin/sulbactam	11	14	5	6	65	80	512
Amoxicillin	0	0	0	0	81	100	>1024
Amoxicillin/clavulanate	3	4	4	5	74	91	512
Cefalexin	7	9	4	5	70	86	1024
Cefuroxime	10	12	3	4	68	84	1024
Cefoperazone	31	38	21	26	29	36	256
Cefotaxime	8	10	18	22	55	68	256
Ceftriaxone	21	26	20	25	40	49	256
Cefepime	44	54	14	17	23	29	128
Meropenem	57	71	6	7	18	22	32
Chloramphenicol	3	4	6	8	71	88	1024
Tetracycline	0	0	4	5	77	95	256
Gentamicin	21	26	12	15	48	59	128
Amikacin	69	85	6	7	6	8	32
Ciprofloxacin	33	41	24	30	24	29	16
Levofloxacin	45	56	11	13	25	31	32
Norfloxacin	50	62	0	0	31	38	64
Ofloxacin	53	65	8	10	20	25	64
Azithromycin	10	12	6	7	65	80	128

[a]Percentage of the number with respect to the total number of clinical *P. aeruginosa* isolates (81 isolates).

Table 6. Antibiotic susceptibility of environmental *P. aeruginosa* isolates

Antibiotic	Susceptible		Intermediate		Resistant		MIC_{90}
	Number	%[a]	Number	%[a]	Number	%[a]	
Ampicillin	0	0	0	0	39	100	>1024
Ampicillin/sulbactam	0	0	0	0	39	100	1024
Amoxicillin	0	0	0	0	39	100	>1024
Amoxicillin/clavulanate	0	0	0	0	39	100	1024
Cefalexin	0	0	2	5	37	95	1024
Cefuroxime	1	3	2	5	36	92	1024
Cefoperazone	2	5	2	5	35	90	512
Cefotaxime	0	0	0	0	39	100	1024
Ceftriaxone	3	8	0	0	36	92	512
Cefepime	8	21	11	28	20	51	256
Meropenem	17	44	4	10	18	46	64
Chloramphenicol	0	0	0	0	39	100	>1024
Tetracycline	0	0	1	3	38	97	64
Gentamicin	2	5	4	10	33	85	512
Amikacin	20	51	12	31	7	18	128
Ciprofloxacin	9	23	6	15	24	62	128
Levofloxacin	5	13	9	23	25	64	64
Norfloxacin	8	21	4	10	27	69	256
Ofloxacin	10	26	2	5	27	69	128
Azithromycin	1	3	4	10	34	87	128

[a]Percentage of the number with respect to the total number of environmental *P. aeruginosa* isolates (39 isolates).

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Table 7. Effect of adding CCCP on antibiotic resistance pattern of *P. aeruginosa* isolates

	Ciprofloxacin		Gentamicin		Cefotaxime		Meropenem	
Isolate number	MIC (mg/L)	MIC in presence of 10 μM CCCP (mg/L)	MIC (mg/L)	MIC in presence of 10 μM CCCP (mg/L)	MIC (mg/L)	MIC in presence of 10 μM CCCP (mg/L)	MIC (mg/L)	MIC in presence of 10 μM CCCP (mg/L)
1	16	16	256	256	512	512	8	2
2	8	8	16	16	64	64	1	1
3	64	16	128	128	256	256	32	32
4	64	64	32	32	512	512	64	4
5	8	8	32	32	256	256	16	4
6	16	2	128	128	512	512	2	2
7	8	2	64	64	128	128	1	1
8	64	8	128	128	512	512	8	1
9	16	16	64	64	256	256	64	64
10	32	32	256	256	512	512	32	32
11	128	32	512	512	512	512	64	4
12	32	32	32	32	128	128	1	1
13	64	64	256	256	512	512	1	1
14	32	4	128	128	512	512	8	2
15	128	128	256	256	256	256	32	32
16	256	8	128	128	512	512	64	8
17	2	2	16	16	64	64	1	1
18	32	4	64	64	512	512	1	1
19	32	32	256	256	128	128	32	4
20	128	2	32	32	512	512	128	32
21	32	16	16	16	64	64	16	16
22	1	1	32	32	64	64	16	4
23	64	16	128	128	128	128	1	1
24	128	16	64	64	128	128	64	16
25	128	64	256	256	512	512	64	64

MIC_{90}s (MIC required to inhibit the growth of 90% of organisms) of each antibiotic, and whether the bacteria were susceptible, intermediately susceptible or resistant to each antibiotic.

The breakpoint MIC of a drug is the highest concentration that can be safely attained in blood using the recommended dosing regimen. Organisms are considered susceptible to a drug if the MIC is below the breakpoint MIC. Organisms characterized by intermediate susceptibility are inhibited at concentrations that approach breakpoint. The MIC for a resistant organism surpasses the breakpoint MIC of the drug, and for that drug the risk of toxicity outweighs the potential benefits of therapy.

Among the antibiotics tested, amikacin was the most active drug against *P. aeruginosa* followed by meropenem, cefepime and fluoroquinolones. *P. aeruginosa* was highly resistant to all other antibiotics tested. In addition, the environmental isolates of *P. aeruginosa* exhibited higher antibiotic resistance than clinical isolates.

Resistance through β-lactamase production

All *P. aeruginosa* isolates were subjected to β-lactamase detection. The presence of a clear zone around bacterial growth was indicative of β-lactamase production as described in the Materials and methods section. Our results showed that 29 (36%) of the clinical *P. aeruginosa* isolates and 37 (95%) of the environmental *P. aeruginosa* isolates were β-lactamase producers.

Resistance through the efflux system

MDR nosocomial infections by *P. aeruginosa* are increasing worldwide.[19] The evolution of MDR bacteria can be attributed to the uncontrolled extensive use of antibiotics in hospitals and the community.[20] The evolution of MDR strains can be caused by an active efflux system that expels antibiotics from the cell.[21] Table 7 shows the MICs of four antibiotics (ciprofloxacin, gentamicin, cefotaxime and meropenem) for 25 different MDR *P. aeruginosa* isolates in the presence and absence of the efflux inhibitor (CCCP). As can be seen from the table, the addition of CCCP enhanced the activities of ciprofloxacin and meropenem, but not gentamicin or cefotaxime. These results emphasized the presence of an efflux-mediated resistance in the tested strains to ciprofloxacin and meropenem, but not to gentamicin or cefotaxime.

Discussion

In this study, *P. aeruginosa* was the most prevalent species in the isolated strains. *P. aeruginosa* represented 75.7% of clinical

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Pseudomonas isolates and 18.2% of all clinical specimens. It also represented 68.4% of environmental *Pseudomonas* isolates and 19.5% of all environmental specimens.

The effectiveness of amikacin against *P. aeruginosa* is corroborated by data from other groups.[22,23] One earlier study reported that meropenem was the most effective antibiotic against *P. aeruginosa*.[24] However, more recent studies demonstrated the evolution of meropenem-resistant strains of *P. aeruginosa*.[25,26] Our study revealed moderate activity of quinolones towards *P. aeruginosa*. Whereas others reported similar rates of resistance to quinolones,[22,27] Corona-Nakamura *et al*.[28] showed that *P. aeruginosa* was absolutely susceptible to ciprofloxacin. This discrepancy can be attributed to the continuous development of MDR strains of *P. aeruginosa* in different parts of the world.

The resistance pattern of *P. aeruginosa* to cephalosporins was consistent with the one reported by Yetkin *et al*.[23] who showed that the percentage of resistance to cephalosporins was in the range of 27% to 88%. Results in Tables 5 and 6 demonstrated that cefepime was the most active cephalosporin against *P. aeruginosa*. This is consistent with reports from several groups.[24,28,29] Additionally, the *P. aeruginosa* resistance pattern to gentamicin was close to the one reported by Muller-Premru and Gubina.[30]

In order to define the main mechanisms used by *P. aeruginosa* to resist antibiotics, we tested for β-lactamase production and for the possess of efflux-mediated resistance. *P. aeruginosa* were previously shown to use β-lactamase-mediated resistance to antibiotics.[31–33] We observed high levels of β-lactamase production in *P. aeruginosa* isolates (36% in clinical isolates and 95% in environmental isolates) in Minia similar to what has been reported elsewhere.[29] *P. aeruginosa* isolates in Minia also used efflux-mediated resistance to ciprofloxacin and meropenem, but not gentamicin or cefotaxime. Efflux-mediated fluoroquinolone resistance of *P. aeruginosa* was reported in several studies.[34–36] Moreover, Pai *et al*.[37] reported that overproduction of the MexAB-OprM efflux system was associated with clinical episodes of carbapenem resistance in *P. aeruginosa*. Therefore, mechanisms of resistance used by *P. aeruginosa* isolates from Minia included β-lactamase production and the use of multiple drug resistance efflux pumps.

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Transparency declarations

None to declare.

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