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Alarming Trend of Antibiotic Resistance in *Pseudomonas aeruginosa* Isolates#

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Pseudomonas aeruginosa **is a concerning opportunistic pathogen frequently causing nosocomial and life-threatening infections. The present study was thus conducted to determine the prevalence of antibiotic resistance in clinical isolates of *Pseudomonas aeruginosa* in Zagazig, Egypt. From 250 clinical specimens, 86 isolates of *P. aeruginosa* (34.4%) were recovered to assess the level of antimicrobial susceptibility and to determine the possible existing resistance mechanisms to commonly used antibiotics. It was found that piperacillin, meropenem, amikacin and polymyxin B were the most effective antibiotics against *P. aeruginosa* followed by imipenem, ticarcillin, ciprofloxacin, ceftazidime, cefipime, gentamicin and norfloxacin. *P. aeruginosa* isolates were found to be highly resistant to all other antibiotics tested. The identified mechanisms of resistance of *P. aeruginosa* isolates included β-lactamase production and involvement of multiple drug resistance efflux. The present results showed that 42 (48.8%) of the clinical *P. aeruginosa* isolates were β-lactamase producers. Efflux pump was identified in 34 (39.5%) of the isolates that effectively mediated resistance to cefotaxime, ticarcillin, azetreonam, meropenem and norfloxacin but not to streptomycin. In conclusion, antibiotic resistance in clinical isolates of *P. aeruginosa* could be attributed to β-lactamase production and the activity of multiple drug resistance efflux pumps.**

Key words: *Pseudomonas aeruginosa*, Multidrug resistance, β-lactamases, Efflux pumps.

Pseudomonas aeruginosa is a versatile Gram negative opportunistic human pathogen, which is becoming increasingly more prevalent. It is associated with infections of immuno-compromised individuals, as a result of burns or other severe trauma, underlying diseases, including cancer, diabetes, cystic fibrosis, and deliberate immunosuppression.[32] The pathogenesis of *P. aeruginosa* is multifactorial as underlined by the large number of virulence factors and the broad spectrum of diseases the bacterium causes.[8] *P. aeruginosa* causes both community acquired infections as well as severe nosocomial infections, life threatening infections in immunocompromised persons, and chronic infections in cystic fibrosis patients.[26, 27] A major reason for its prominence is its high intrinsic resistance to antimicrobial agents, such that even for the most recent chemotherapeutic antimicrobial agents, a modest change in susceptibility can prevent their effectiveness.[39] The prevalence of *P. aeruginosa* in hospitals owes much to the intrinsic resistance of the organism to multiple antimicrobial agents [21] and the ability to acquire resistance to most of them.[13, 38]

Therefore, it is important to study the resistance patterns of *P. aeruginosa* isolates to commonly used antibiotics. The use of antibiogram as an epidemiological indicator can help us make the best use of antibiotics in the treatment of *P. aeruginosa* infections. The prevalence of *P. aeruginosa* infection in hospitalized patients was studied. The increasing antibiotic resistance of this organism is attributed to multiple factors including active drug efflux and β-lactamase production. [16]

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Thus, the study was conducted by testing these two possible mechanisms of resistance in *P. aeruginosa* isolates.

MATERIAL AND METHODS

Bacterial isolates

Two hundred and fifty clinical specimens were collected from inpatients and outpatients admitted to the Zagazig University Hospitals and Zagazig Chest Hospital over a fifteen-month period from March 2002 until May 2003. All samples were collected from patients with clinically diagnosed urinary tract infections (UTI), respiratory tract infections (RTI), wounds and ear infections. Specimens comprised urine, pus swabs or sputum according to type of infection. The specimens were used for isolation and identification of *P. aeruginosa* according to standard microbiological and biochemical procedures.

Antibiotics

The following antibiotics were used: ampicillin, amoxicillin, cefotaxime, tetracycline, norfolxacin, ceftriaxone , and erythromycin (from Egyptian International Pharmaceutical Industries, Cairo, Egypt), amoxicillin/clavulanate (Medical Union Pharmaceutical, Ismailia, Egypt), streptomycin and doxycycline (Nile Pharmaceutical, Cairo, Egypt), kanamycin (Misr Pharmaceutical, Cairo, Egypt), cefuroxime and ceftazidimie (Glaxo Wellcome, Cairo, Egypt), aztreonam, cefepime, amikacin (Bristol Myers Squibb, Cairo, Egypt), gentamicin (Memphis Pharmaceutical and Chemical, Cairo, Egypt), cefoperazone, azithromycin (Pfizer, Egypt), imipenem (Merck Sharp and Dohme, Hertfordshire, U.K.), meropenem (Astra-Zeneca, Cheshire, U.K.), and chloramphenicol (Chemical Industries Development, Cairo, Egypt). Cloxacillin, ticarcillin, piperacillin, and potassium clavulanate (Sigma-Aldrich, Saint Louis, MO, USA), polymyxin B (Novo Industry A/S, Copenhagen, Denmark), and ciprofolxacin (Bayer AG, Wuppertal, Germany).

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) (µg/mL) of different antibiotics were determined using agar dilution method using Mueller-Hinton agar (MHA),[4] according to Clinical Laboratory Standards Institute guidelines (CLSI).[42] Briefly, overnight cultures of bacteria in Mueller-Hinton broth (MHB) were diluted to contain approximately 10^7 cfu/mL with fresh MHB. Aliquots containing approximately 10^5 cfu/µL were spotted onto the dried surface of MHA plates containing different concentrations of the selected antibiotics (0.125 to 1024 µg/ml). Plates were incubated at 37°C for 18-20 h and MICs were determined. MIC was defined as the lowest concentration of antibiotic showing no visible growth.

Detection of β-lactamase

β-lactamase activity was detected using nitrocefin. A 0.5 mM nitrocefin solution was prepared by dissolving the powder (Glaxo, Middlesex, U.K.) in 0.1 M phosphate buffer saline (PBS), pH 7 containing dimethylsulfoxide (DMSO). Colonies of the test isolates were scraped from nutrient agar plates and suspended into 20 µL of PBS to produce a dense suspension on a glass slide followed by the addition of 20 µL of 0.5 mM nitrocefin solution. β-lactamase activity was indicated by the development of red color within 1-2 min.

Study of efflux systems

The existence of efflux mechanism in *P. aeruginosa* isolates was determined by testing the accumulation of ethidium bromide in the presence or absence of efflux inhibitors.[29] Overnight cultures were adjusted to approximately 10^5 cfu/µL. Washed cells were resuspended in 20 µL of 1 µg/ml ethidium bromide with or without either 100 µM dinitrophenol (DNP, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 0.4 % glucose or 0.1 % of toluene and were incubated at 37^0C for 15 min. Cells were collected by centrifugation at 1200 *x g* for 5 minutes and re-suspended in 10 µL of PBS. Aliquots of cell suspensions (5 µL) were spotted onto the surface of 1% agarose gel and examined over ultra violet transilluminator. Drug accumulation in *P. aeruginosa* cells was observed as bright fluorescence of ethidium bromide.

To study the efflux system of *P. aeruginosa* isolates, the MICs of antimicrobial agents for 37 MDR *P. aeruginosa* isolates were determined in the presence and absence of 100 µM of the efflux pump inhibitor DNP and dicyclohexylcarbodiimide (DCCD, Sigma-Aldrich, Steinheim, Germany).[4] The reduction in MIC of a certain antibiotic with DNP or DCCD is an indication of resistance to this antibiotic mediated by an efflux system.

RESULTS

Prevalence of *P. aeruginosa* in clinical specimens

Out of the 250 clinical specimens, 86 *P. aeruginosa* isolates (34.4%) were isolated and identified biochemically using standard procedures for detection.

As shown in Table 1, 20 (23.8%) isolates were from 84 specimens of sputum (33.6%), 23 (51.1%) were from 45 wound exudates (18%), 30 (53.6%) from 56 urine in case of UTI (22.4%), and 13 (20%) were from 65 ear discharges (26%).

Antibiotic susceptibility and determination of MICs

Table 2 showed the respective MIC distributions of different antibiotics for 86 clinical isolates of *P. aeruginosa*. Table 3 showed the MIC_{90} of each antibiotic, and whether the bacteria were susceptible, intermediately susceptible or resistant to each antibiotic.

Among the antibiotics tested, piperacillin, meropenem, amikacin and polymyxin B were the most effective antibiotics against clinical isolates of *P. aeruginosa* followed by imipenem, ticarcillin, ciprofloxacin, ceftazidime, cefipime, gentamicin and norfloxacin. *P. aeruginosa* isolated strains were highly resistant to all other antibiotics tested. As shown in Table 3, all of 86 clinical isolates of *P. aeruginosa* were resistant to more than three classes and were defined as MDR.[25, 30]

Resistance through β-lactamase production

Forty two (48.8%) of *P. aeruginosa* isolates were found to be β-lactamase producers.

Resistance through the efflux system

The activity of drug efflux in *P. aeruginosa* isolates was tested by testing the accumulation of ethidium bromide. Reduction in fluorescence intensity was observed with MDR isolates in the absence of efflux pump inhibitor and in presence of glucose (an energizer of efflux pump). In the presence of efflux pump inhibitor or toluene (a membrane permeabilizer) there was an increase in fluorescence intensity. *P. aeruginosa* ATCC 90271 was used as negative control. It was found that 34 isolates (39.5%) of clinical *P. aeruginosa* (86 total isolates) were positive for efflux pump activity.

Thirty seven (37) MDR *P. aeruginosa* isolates were tested for the effect of efflux pump inhibitor on the MIC. Table 4 showed the MICs of six antibiotics (cefotaxime, ticarcillin, azetreonam, meropenem, norfloxacin and streptomycin) in the presence and absence of the efflux inhibitors (DNP, and DCCD). The addition of DNP and DCCD enhanced the activities of selected antibiotics by lowering the MIC as observed in the reduction of MIC. In the presence of DNP and DCCD, highest effect was observed with ticarcillin and norfloxacin (32 folds decrease in MIC) followed by aztreonam and cefotaxime (16 folds decrease in MIC). Intermediate effect was obtained with meropenem (8 folds decrease in MIC). Little effect was obtained with streptomycin (2-4 folds decrease in MIC).These results emphasized the existence of an efflux-mediated resistance in the tested isolates to cefotaxime, ticarcillin, azetreonam, meropenem and norfloxacin but not to streptomycin.

DISCUSSION

P. aeruginosa is an emerging pathogen of concern due to its increasingly reported resistance. *P. aeruginosa* isolates show steady escalation in resistance to an increasing number of antimicrobial agents and the emergence of MDR *P. aeruginosa* infections is increasingly recognized.

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

Type of infections (Specimen)	Number of Specimens (%)[a]	*P. aeruginosa* isolates, no. (%)[b]
Respiratory Tract Infection (Sputum)	84 (33.6)	20 (23.8)
Wound Infection (Exudate or Pus)	45 (18)	23 (51.1)
Urinary Tract Infection (Urine)	56 (22.4)	30 (53.6)
Ear Infections (Discharge)	65 (26)	13 (20)
Total	250 (100) 86	(34.4)

[a]Percentage of the number of isolates with respect to the total number of specimens
[b]Percentage of the number of isolates with respect to the total number of specimens of each group

The present study found that *P. aeruginosa* represented 34.4% of clinical specimens collected (Table 1). All tested isolates were completely susceptible to polymyxin B, amikacin, meropenem, and pipracillin (Table 3) in agreement with previously reported data.[11, 33, 43]

The resistance rate to imipenem was 1% similar to other studies [3, 18] while different resistance rates were reported in different studies. Higher resistance rates to imipenem were reported from 9.5% in a study in Japan [31] to 42.3% in Poland [37]. All tested isolates were sensitive to meropenem, although higher resistance rate (45.5%) was reported in studies in Bulgaria [37] and 46% in a study in USA.[36] Similarly, all *P. aeruginosa* isolates in the present study were sensitive to piperacillin while other reports showed higher resistance rates.[10, 23] Other studies showed an emergence of resistance of *P.aeruginosa* isolates to polymyxin B.[17, 22] Also resistance rates to amikacin were reported in previous studies ranging from 4.2 % in Saudi Arabia [2] to 59.1% in Bulgaria,[37] 64% in USA,[36] and 77.1% in Iran.[19]

The present data revealed resistance rates against aztreaonam, ticarcillin, and gentamicin similar to other studies.[43] In the present study, ceftazidime was the most active cephalosporins with a susceptibility rate of 58% and this is consistent with other reports.[12, 34] This was followed by cefepime with a susceptibility rate of 40% as similar to previous studies.[23, 36] Ceftraixone showed resistance rate of 29% which was lower than that

Table 2. MICs of different antibiotics for 86 clinical *P. aeruginosa* isolates

Antibiotic	Breakpoint[a]	Number of isolates with MICs (µg/ml)										
	(µg/ml)	1	2	4	8	16	32	64	128	256	512	≤1024
Ampicillin	8	0	0	0	0	0	0	0	0	0	0	86
Amoxicillin	8	0	0	0	0	0	0	0	14	5	0	67
Amoxicillin/clavulanate	8	0	0	0	0	0	0	0	0	7	0	79
Aztreonam	8	0	0	0	0	6	26	38	16	0	0	0
Carbenicillin	128	0	0	0	0	0	0	0	0	0	0	86
Ticarcillin	64	0	0	0	1	1	2	65	17	0	0	0
Piperacillin	64	0	0	3	61	13	7	2	0	0	0	0
Imipenem	4	0	0	85	0	1	0	0	0	0	0	0
Meropenem	4	0	86	0	0	0	0	0	0	0	0	0
Cefoperazone	16	0	0	0	0	0	0	0	2	13	0	71
Cefuroxime	16	0	0	0	0	0	0	12	20	4	0	50
Cefotaxime	8	0	0	0	0	0	36	27	9	6	0	8
Ceftraixone	8	0	0	0	0	27	34	9	7	1	0	8
Ceftazidime	8	0	0	1	49	25	5	6	0	0	0	0
Cefepime	8	0	0	0	34	24	17	11	0	0	0	0
Streptomycin	NA	0	0	0	0	0	0	0	0	0	0	86
Kanamycin	NA	0	0	0	0	0	0	0	0	0	0	86
Gentamicin	4	0	0	8	7	5	3	15	8	7	33	0
Amikacin	16	0	0	4	82	0	0	0	0	0	0	0
Tetracycline	4	0	0	0	0	0	0	0	0	0	0	86
Doxyciline	4	0	0	0	0	0	0	0	0	0	0	86
Erythromycin	4	0	0	0	0	0	0	0	0	0	0	86
Azithromycin	4	0	0	0	0	0	0	0	8	19	0	59
Nalidixic acid	NA	0	0	0	0	0	0	0	0	0	0	86
Norfloxacin	4	0	0	4	5	0	0	59	16	2	0	0
Ciprofloxacin	1	51	5	0	0	1	0	11	18	0	0	0
Chloramphenicol	8	0	0	0	0	0	0	0	0	0	0	86
Polymyxin	B	0	86	0	0	0	0	0	0	0	0	0

[a]Breakpoints of different antibiotics according to NCCLS (2004).
NA, not applicable

reported by other studies.[6, 23] The highest resistance rate to Cefotaxime (58%) was similar to other reports.[15, 28] All isolates were resistant to cefoperazone and cefuroxime. The resistance pattern to cephalosporins was consistent with Yetkin *et al.*[43] who reported that the resistance rate to cephalosporins was in the range of 27% to 88%.

The present results revealed that resistance rate of *P. aeruginosa* to ciprofloxacin was 34%, by contrast, a previous study [7] reported that *P. aeruginosa* was fully susceptible to ciprofloxacin. In another study, the reported resistance rate to ciprofloxacin was 11.9% in 1999 and 20.6% in 2006. [34] In the present study, resistance rate to norfloxacin reached 86.5% similar to other studies.[6, 35] This discrepancy of resistance rates can be attributed to the continuous development of MDR strains of *P. aeruginosa* in different parts of the world.

In order to determine the possible mechanisms by which *P. aeruginosa* isolates resist antibiotics, the isolates were tested for β-lactamase production and efflux-mediated resistance. *P. aeruginosa* is known to possess β-lactamase-mediated resistance to antibiotics.[5, 40, 41] High levels of β-lactamase production was fond in *P. aeruginosa* clinical isolates (48.8%). This was similar to the data previously reported.[11, 14] The reduction of MICs of cefotaxime, ticarcillin, aztreaonam, meropenem in the presence of efflux pump inhibitors (DNP and DCCD) indicates the involvement of efflux-mediated resistance in tested *P. aeruginosa* isolates. This finding is consistent with other reports which showed major contribution

Table 3. Antibiotic susceptibility of clinical *P. aeruginosa* isolates

Antibiotic	Susceptible		Intermediate		Resistant		
	Number	%[a]	Number	%[a]	Number	%[a]	MIC_{90}
Ampicillin	0	0	0	0	86	100	>1024
Amoxicillin	0	0	0	0	86	100	>1024
Amoxicillin/clavulanate	0	0	0	0	86	100	>1024
Aztreonam	0	0	6	7	80	93	128
Carbenicillin	0	0	0	0	86	100	>1024
Ticarcillin	69	80	0	0	17	20	128
Piperacillin	86	100	0	0	0	0	32
Imipenem	85	99	0	0	1	1	4
Meropenem	86	100	0	0	0	0	2
Cefoperazone	0	0	0	0	86	100	>1024
Cefuroxime	0	0	0	0	86	100	>1024
Cefotaxime	0	0	36	42	50	58	256
Ceftraixone	0	0	61	71	28	29	256
Ceftazidime	50	58	25	29	11	13	32
Cefepime	34	40	24	28	28	32	64
Streptomycin	0	0	0	0	86	100	>1024
Kanamycin	0	0	0	0	86	100	>1024
Gentamicin	8	9	7	8	71	83	512
Amikacin	86	100	0	0	0	0	8
Tetracycline	0	0	0	0	86	100	>1024
Doxyciline	0	0	0	0	86	100	>1024
Erythromycin	0	0	0	0	86	100	>1024
Azithromycin	0	0	0	0	86	100	>1024
Nalidixic acid	0	0	0	0	86	100	>1024
Norfloxacin	4	5	5	6	77	89	128
Ciprofloxacin	51	59	6	7	29	34	128
Chloramphenicol	0	0	0	0	86	100	>1024
Polymyxin B	86	100	0	0	0	0	2

[a]Percentage of the number with respect to the total number of 86 clinical *P. aeruginosa* isolates

Table 4. Effect of adding efflux pump inhibitors on antibiotic resistance pattern of *P. aeruginosa* isolates

Isolate mumber	Cefotaxime MIC in the presence of			Ticarcillin MIC in the presence of			Aztreonam MIC in the presence of			Meropenem MIC in the presence of			Norfloxacin MIC in the presence of			Streptomycin MIC in the presence of		
	MIC (µg/ml)	DNP (µg/ml)	DCCD	MIC (µg/ml)	DNP (µg/ml)	DCCD	MIC (µg/ml)	DNP (µg/ml)	DCCD	MIC (µg/ml)	DNP (µg/ml)	DCCD	MIC (µg/ml)	DNP (µg/ml)	DCCD	MIC (µg/ml)	DNP (µg/ml)	DCCD
1	32	32	32	64	4	4	64	8	8	2	0.25	0.25	64	4	4	256	256	256
2	32	8	8	32	32	32	32	4	4	2	0.25	0.25	4	4	4	128	128	128
3	32	8	8	32	32	32	32	4	4	2	0.25	0.25	4	4	4	128	128	128
4	32	32	32	64	64	64	32	8	8	2	0.25	0.25	64	64	64	64	64	64
5	32	32	32	64	8	16	64	8	8	2	0.25	0.25	64	64	64	256	256	256
6	128	32	32	64	4	4	128	8	8	2	0.25	0.25	64	8	4	64	64	64
7	32	8	8	64	64	64	32	8	8	2	0.25	0.25	64	64	64	64	64	64
8	128	128	128	64	4	8	128	16	16	2	0.25	0.25	4	4	4	32	32	32
9	32	32	32	64	64	64	64	16	16	2	0.25	0.25	64	64	64	32	32	32
10	≤512	64	64	128	128	128	64	16	16	2	0.50	0.50	64	64	64	64	64	64
11	≤512	≤512	≤512	128	128	128	64	16	16	2	0.25	0.25	64	8	8	256	128	64
12	≤512	32	32	64	4	4	128	8	8	2	0.25	0.25	128	4	4	256	128	128
13	≤512	64	64	128	128	128	128	16	16	2	2	2	64	64	64	512	512	512
14	64	64	64	64	16	16	64	8	16	2	0.50	0.50	64	8	8	256	256	256
15	32	32	32	64	8	4	128	16	16	2	0.25	0.25	128	4	4	128	128	128
16	64	64	32	64	64	64	128	8	8	2	1	1	128	16	8	32	32	32
17	32	32	32	128	8	4	128	16	16	2	0.25	0.25	128	4	4	64	32	32
18	64	32	32	128	128	128	128	16	16	2	0.50	0.50	128	128	128	512	512	512
19	32	32	32	128	4	4	32	8	8	2	0.25	0.25	64	4	4	256	256	256
20	32	32	32	16	4	4	64	8	16	2	0.25	0.25	4	4	4	64	64	64
21	64	64	64	128	128	128	128	16	16	2	0.25	0.25	256	256	256	512	512	512
22	32	32	32	128	4	4	128	16	16	2	0.25	0.25	128	8	4	512	512	512
23	32	32	32	64	64	64	64	8	8	2	1	1	128	128	128	512	512	512
24	32	32	32	128	128	128	64	16	16	2	2	2	4	4	4	64	64	64
25	32	32	32	128	4	4	64	8	8	2	0.25	0.25	128	128	128	256	256	256
26	32	32	32	64	4	4	32	16	8	2	0.25	0.25	128	4	4	256	256	256
27	32	32	32	128	128	128	32	16	8	2	0.25	0.25	8	8	8	512	512	512
28	32	32	32	64	64	64	64	16	8	2	0.25	0.25	64	8	8	256	256	256
29	128	64	32	128	128	128	128	8	16	2	0.50	0.50	128	128	128	512	512	512
30	128	32	16	64	8	8	64	8	4	2	0.25	0.25	64	4	4	64	64	64
31	128	32	32	64	64	64	128	32	16	2	0.25	0.25	128	128	128	512	512	512
32	128	32	32	64	64	64	128	32	16	2	2	2	128	128	128	512	512	512
33	64	64	64	64	64	64	16	8	8	2	0.50	0.50	8	8	8	32	32	32
34	256	64	64	128	128	128	128	8	16	2	0.25	0.25	256	256	256	512	512	512
35	≤512	64	32	128	4	8	32	16	16	2	0.25	0.25	8	8	8	256	256	256
36	256	64	64	64	16	16	16	8	8	2	0.25	0.25	8	8	8	256	256	256
37	≤512	64	32	128	128	128	64	16	16	2	0.25	0.25	128	128	128	256	256	256

of efflux in the emergence of resistance in *P. aeruginosa*.[1, 9, 11, 20, 38, 44] Therefore, the possible predominant existing mechanisms of resistance of the current *P. aeruginosa* isolates are β-lactamase production and the use of multiple drug resistance efflux pumps.

CONCLUSION

Although data presented in this study showed that resistance of clinical isolates of *P. aeruginosa* to commonly used antibiotics is high, the importance of the results is indicating that escalating rates of MDR among isolates still pose a clinical problem for patients and health officials. The low resistance rates of piperacillin and carbapenems reflect the limited use of these categories of antibiotics in Zagazig. By contrast, other high resistance rates could be attributed to extensive usage of these antibiotics and the contribution of different mechanisms to the current resistance levels of *P. aeruginosa*. It is suggested that, to minimize the impact of resistance and its spread, an implementation of a regional and nationwide surveillance program be instituted to monitor antimicrobial resistance trends among *P. aeruginosa*.

For practicing physicians, clinical microbiologists, and public health officials, knowledge of antibiotic resistance patterns is essential to guide empirical therapy. In addition, preventive strategies such as continuous surveillance of *P. aeruginosa* resistance against antimicrobial agents, prudent antimicrobial use and infection control policy in healthcare settings should be advocated to delay emergence of clinically significant MDR *P. aeruginosa*. Also, emphasis should be made on the importance of legislation of usage of antibiotics. This requires an urgent need for control policy of prescription and use of antibiotics in hospitals for the prevention of the steady increase in *P. aeruginosa* resistance. In addition, this alarming trend of resistance deserves attention and concern among health care providers and requires continuation of surveillance studies worldwide to control antibiotic resistance. Furthermore, search for new antimicrobial agents is a requirement to bypass the steady resistance of *P. aeruginosa* to currently used antibiotics.

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Conflict of interest

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[#]*Note: This article has been reprinted due to omittance of tables in the last published issue of JPAM Vol. 5 No. 2 Sept. 2011.*