ORIGINAL ARTICLE

Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital

Siva Gowri PATHMANATHAN[1], Nor Azura SAMAT[2], Ramelah MOHAMED[3]

[1] Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 55100 Kuala Lumpur, Malaysia
[2] Department of Microbiology, Hospital Kuala Lumpur, 50586 Malaysia
[3] UKM Medical Molecular Biology Institute (UMBI), Kuala Lumpur, 5600 Malaysia

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Abstract

Ongoing surveillance of Pseudomonas aeruginosa resistance against antimicrobial agents is fundamental to monitor trends in susceptibility patterns and to appropriately guide clinicians in choosing empirical or directed therapy. The in vitro activity level of eight antimicrobial drugs was assessed against 97 clinical isolates of *P. aeruginosa* collected consecutively for three months in 2007 from a Malaysian hospital. Antimicrobial susceptibility was determined using the E-test method in addition to the hospital's routine diagnostic testing by the disk diffusion method. Respiratory and wound swab isolates were the most frequently encountered isolates. The E-test and disk diffusion methods showed high concordance in determining the in vitro activity of the antimicrobial agents against the *E* isolates. Piperacillin-tazobactam was the most active antimicrobial agent with 91.8% susceptibility, followed by the aminoglycosides (amikacin, 86.6% and gentamicin, 84.5%), the quinolone (ciprofloxacin, 83.5%) and the beta-lactams (cefepime, 80.4%, ceftazidime, 80.4%, imipenem, 79.4% and meropenem, 77.3%). Incidence of multidrug resistance was 19.6% (19 out of 97 isolates). Periodic antibiotic resistance surveillance is fundamental to monitor changes in susceptibility patterns in a hospital setting.

Keywords: *antibacterial agents, bacterial drug resistance, Pseudomonas aeruginosa, medical sciences*

Introduction

Pseudomonas aeruginosa is an aerobic, motile, nutritionally versatile, gram-negative rod exhibiting intrinsic resistance to several antimicrobial agents (1,2). The rapid increase of drug resistance in clinical isolates of this opportunistic human pathogen is of worldwide concern (3,4,5,6,7).

Ongoing surveillance of *P. aeruginosa* resistance against antimicrobial agents is fundamental to monitor trends in susceptibility patterns and to appropriately guide the clinician in choosing empirical or directed therapy, especially when new antimicrobial agents may not be readily available in the near future (8,9). However, there are few recent surveillance studies reporting antimicrobial resistance patterns of *P. aeruginosa* in Malaysia (10,11). Thus, in this study, we assessed the current in vitro activity level of eight antimicrobial drugs against clinical isolates of *P. aeruginosa* obtained from the Kuala Lumpur Hospital. The concordance between the E-test and disk diffusion aeruginosamethods in antimicrobial susceptibility testing was also evaluated.

Materials and Methods

Clinical isolates

A total of 97 consecutive clinical isolates of *P. aeruginosa* were collected between October 2007 and December 2007 at the Kuala Lumpur Hospital, Malaysia a government tertiary referral hospital with 81 wards and 2,502 beds. Of the 97 specimens, 21 were obtained from general paediatric wards, 20 from general medicine wards, 14 from neurology wards, 11 from intensive care units, 9 from orthopaedic wards, 7 from general surgery wards, 5 from respiratory medicine, 4 from urology wards, 2 from uronephrology and 1 each from dermatology,

ENT (ear, nose and throat), burn and nephrology wards. The isolates were identified by standard laboratory methods (1).

Antibiotic susceptibility test

Minimal inhibitory concentrations (MICs) of piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin and ciprofloxacin were determined by E-test (AB Biodisk, Solna, Sweden) in addition to the hospital's routine antimicrobial susceptibility testing by the disk diffusion method. Results of E-test and disk diffusion methods were interpreted in accordance to the Clinical and Laboratory Standards Institute (CLSI) (12). Control strains included *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

Multidrug-resistant (MDR) isolates were defined as isolates demonstrating resistance to antimicrobials from at least two of the five antipseudomonal classes of antimicrobial drugs tested in this study: piperacillin-tazobactam, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones.

Statistical Analysis

Statistical analysis was done using SPSS software, version 15. Statistical analysis by Spearman's rank correlation was carried out to assess the correlation in susceptibility between two drugs. Cross-tab analysis was performed to obtain a Kappa value to measure the concordance between E-test and disk diffusion methods. The percent concordance of the two methods was calculated as follows: $[(a + d)/(a + b + c + d)]*100$, where *a* is the number of isolates sensitive by both tests, *b* is the number of isolates sensitive by E-test and resistant by disk diffusion, *c* is the number of isolates resistant by E-test and sensitive by disk diffusion, and *d* is the number of isolates resistant by both tests (13). The Spearman's rank correlation was also performed to evaluate the association between occurrence of drug resistance and i) ward of patient origin and ii) specimen of isolates. In all cases, a *P* value of < 0.05 was considered indicative of significance.

Results

The results of the antimicrobial susceptibility testing are shown in Table 1. Piperacillin-tazobactam was the most active antimicrobial agent in vitro with 91.8% susceptibility, followed by the aminoglycosides (amikacin and gentamicin), quinolone (ciprofloxacin), the cephalosporins (ceftazidime and cefepime) and the carbapenems (meropenem and imipenem).

Twenty-five isolates were resistant to at least one of the five antipseudomonal classes of antimicrobial agents and revealed a total of 12 antimicrobial resistance patterns (Table 2). The most prevalent pattern, P2, displaying resistance to all antimicrobial drugs except piperacillin-tazobactam was observed in 9 (36%) of the 25 isolates. The MIC of piperacillin-tazobactam on these isolates was between 3 and 16 ìg/mL. Pattern P7 was the second most common with resistance to piperacillin-tazobactam, the cephalosporins and the carbapenems. Pattern P9 exhibited resistance to the carbapenems in 3 isolates. Two isolates were resistant to all antimicrobial agents tested. Resistance to both carbapenems was observed in 20 of the 25 isolates. The overall incidence

Table 1: Antimicrobial susceptibility of *P. aeruginosa* isolates to eight antimicrobial agent

Antimicrobial agent	% susceptible	MIC (µg/mL)			No. of isolates [MIC (µg/mL) breakpoint]		
		50%	90%	Range	S	I	R
PT	92.8	4	24	1 - >256	90 [<64]	0	7 [>128]
CAZ	80.4	2	>256	0.5 - >256	78 [<8]	0	19 [>32]
CPE	80.4	2	>256	0.09 - >256	78 [<8]	0	19 [>32]
IMP	79.4	1	>32	0.25 - >32	77 [<4]	1	19 [>16]
MER	77.3	0.25	>32	0.032 - >32	75 [<4]	1	21 [>16]
GN	84.5	3	96	1 - >256	82 [<4]	0	15 [>8]
AK	86.6	4	32	2 - >256	84 [<16]	4	9 [>32]
CIP	83.5	0.19	>32	0.064 - >32	81 [<1]	1	15 [>4]

Note: S, sensitive; I, intermediate; R, resistant
PT=piperacilin-tazobactam, CAZ=ceftazidime, CPE=cefepime, IMP=imipenem, MER=meropenem, GN=gentamicin, AK=amikacin, CIP=ciprofloxacin

Table 2: Antibiotype patterns of the P. aeruginosa strains exhibiting resistance to at least one antimicrobial agent

Pattern type	Antimicrobial class*								Number of strains with pattern (%)
	PT	Cephalosporin		Carbapenem		Aminoglycoside		Quinolone	
	PT	CAZ	CPE	IMP	MER	GN	AK	CIP	
P1	R	R	R	R	R	R	R	R	2 (8)
P2		R	R	R	R	R	R	R	9 (36)
P3	R		R	R	R	R		R	1 (4)
P4		R	R	R	R	R		R	1 (4)
P5		R	R		R	R	R	R	1 (4)
P6		R		R	R	R	R	R	1 (4)
P7	R	R	R	R	R				3 (12)
P8	R	R	R						1 (4)
P9		R	R						1 (4)
P10				R	R				3 (12)
P11					R				1 (4)
P12								R	1 (4)
Total									25 (100)

Note: Isolates above broken lines are MDR
*See Footnote Table 1
R=resistant

of multidrug resistance was 19.6% (19 out of 97 isolates).

Table 3 shows the distribution of the 97 *P.* aeruginosa isolates according to the specimen type and its correlation to multidrug resistance. The E-test and disk diffusion methods showed high percentage of concordance (>96%) and an excellent Kappa measure of agreement (0.8 to 1) (Table 4).

Discussion

Periodic antimicrobial resistance monitoring in *P. aeruginosa* is fundamental to updating the current activity level of commonly used antipseudomonal drugs. In the present study, the carbapenems were the least active agents evaluated with only 77.3% and 79.4% of isolates being susceptible to meropenem and imipenem, respectively. Imipenem has been reported to be very active against *P. aeruginosa* in a number of recent studies, (3,10,14) while others have reported otherwise (6,15). A study done in another tertiary care hospital in Malaysia (10) involving isolates collected in 2005 reported a low incidence of imipenem resistance (9.90%) compared to the present (20.6%). Another Malaysia/Singapore study in 1999 that did not include our hospital found imipenem to be the most active â-lactam (14.7% resistance), but cefepime and piperacillin-tazobactam had higher resistance rates than the 31present study (11). Varying drug resistance levels in different hospitals in the same country have been reported in the past and is attributed to the differential usage of antibiotics in the respective hospitals. An Indian study (4) noted that the low incidence of imipenem resistance (7.2%) at their hospital compared to a higher resistance rate detected in another setting in the same country (16) was due to the fact that imipenem is still used as a reserve drug in the former. In general, when compared to previous Malaysian studies (10,11), our study showed higher resistance rates to all drugs tested except cefepime, meropenem and piperacillin-tazobactam. However, the difference in MDR rates between the present and other studies could not be compared due to varying definitions of multidrug resistance.

A number of studies found piperacillin-tazobactam to be either the most active antimicrobial agent against *P. aeruginosa* or the second most active after amikacin (3, 4,7,10,17). However, a recent report has questioned the appropriateness of the current CLSI resistance breakpoint of piperacillin-tazobactam since the study discovered an increased mortality rate

Table 3: Distribution of the P. aeruginosa isolates according to the specimen type and its correlation to multidrug resistance

Type of specimen	No (%) isolates studied (n=97)	No MDR isolates (n=19)	Spearma's rho[a]
respiratory tract	40 (41.2)	11	0.167
wound swab	52 (33.0)	1	-0.291[b]
urine	15 (15.5)	5	0.148
blood	5 (5.2)	2	0.120
tissue	4 (4.1)	0	-0.102
CSF	1 (1.0)	0	-0.50

n= total number
[a]Values are Spearman correlation coefficient. The sign of the correlation coefficient indicates the direction of the relationship (positive or negative)
[b]Highly signifivant correlation (*P*<0.001)

Table 3: Agreement between E-test and disk diffusion methods

Antimicrobial agent	% agreement	Measure of agreement, Kappa[a]
Piperacillin-tazobactam	98	0.823
Ceftazidime	99	0.967
Cefefime	97	0.896
Imipenem	98	0.937
Meropenem	98	0.939
Gentamicin	99	0.959
Amikacin	98	0.905
Cirofloxacin	99	0.962

[a]poor agreement = <0.20; fair agreement = 0.200.40; moderate agreement = 0.400.60; good agreement = 0.600.80; very good agreement = 0.801.00

associated with empiric piperacillin-tazobactam therapy given to patients with *P. aeruginosa* bacteraemia; the isolates had reduced piperacillin-tazobactam susceptibility (18).

Although amikacin was the second most potent drug in vitro, the resistance rate was higher compared to other studies (5,6,7,10). In the other studies, the resistance rate of amikacin was far lower than its aminoglycoside counterpart, gentamicin. In the present study, however, there was a significant correlation between the two aminoglycosides (rho > 0.9, $P < 0.01$), although the MIC_{90} value of amikacin (32) was lower than that of gentamicin (96). A significant correlation between class members was also observed among the cephalosporins and carbapenems (rho > 0.9, $P < 0.01$) with equal MIC_{90} values (i.e., > 250 and > 32, respectively).

The high percentage of concordance and an excellent Kappa measure of agreement showed that both methods have high agreement in determining the in vitro activity of the antimicrobial agents on *P. aeruginosa* isolates, which corroborates similar studies (19,20) that reported an excellent and acceptable correlation, respectively, between the disk diffusion and E-test methods. Therefore, although the E-test is rapid, easy to perform and has an added ability to determine MIC value, the disk diffusion method is equally reliable and more cost-effective for routine hospital use.

There was no significant correlation between drug resistance and the wards from which isolates originated (data not shown). The distribution rank of the isolates according to the types of specimens (respiratory > wound swab > urine > blood) was similar to that described by a worldwide SENTRY antimicrobial surveillance study (8), even though the total number of isolates included in the present study is incomparably small. Respiratory isolates (41.2%), including tracheal and nasopharyngeal aspirates as well as sputum, were the most frequently encountered. *P. aeruginosa* isolates

from respiratory tract also showed the highest rate of multidrug resistance, as observed in a similar study of inpatient isolates done in a Saudi Arabian hospital (21). Wound swab isolates (33.0%) were the second most frequently encountered. However, the incidence of resistance was statistically less likely to be observed in these isolates ($P < 0.001$). Of the 32 pus isolates, 31 were fully susceptible to all the antimicrobial agents tested, suggesting that wound swab isolates are less likely to be multidrug resistant. Nevertheless, the correlation between specimen type and multidrug resistance would have been more noteworthy if supported by data on patients' clinical conditions, which is a limitation of our study.

In conclusion, the higher resistance rate, when compared to previous studies, calls for prudent use of antibiotics in order to limit further increases in resistance. Antimicrobial surveillance should be done periodically to monitor the current susceptibility patterns in local hospitals. A standard definition of P. aeruginosa multidrug resistance will allow better comparisons between studies.

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Correspondence

Siva Gowri Pathmanathan,
BSc (Malaya), MPhil (Malaya) Faculty of Medicine and Health Sciences,
Universiti Sains Islam Malaysia,
Tingkat 13, Menara B, Persiaran MPAJ,
Jalan Pandan Utama, Pandan Indah,
55100, Kuala Lumpur, Malaysia.
Tel: 03-42892400
Fax: 03-42892477
Email: gowri@usim.edu.my

Author contributions

Conception and design: SGP, RM
Collection and assembly of data: NAS, SGP,
Analysis and interpretation of data: RM, SGP
Drafting of the manuscript: SGP
Critical revision and final approval of the manuscript: RM
Administrative, technical, or logistic support: NAS

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