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Full Length Research Paper

Antimicrobial resistance pattern of clinical isolate of *Pseudomonas aeruginosa* in the University of Malaya Medical Center, Malaysia

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Pseudomonas aeruginosa **is considered as one of the leading causes of nosocomial infections. The start of antimicrobial therapy is often empirical and selective pressure on panel of antibiotics; therefore, it is important to know the susceptibility pattern of pathogens in order to select the most appropriate antibiotic. The aim of the current study is to update the rational empirical antimicrobial therapy recommendations. Antimicrobial resistance was done using the E-test method. Urine and wound swab samples were the highest encountered isolates; results were interpreted according to National Committee for Clinical Laboratory Standards guidelines. A total of 88 clinical isolates of *P. aeruginosa* were collected randomly from April 2009 to March 2010 from the University of Malaya Medical Center. *P. aeruginosa* isolated from various clinical samples has lost susceptibility and showed increasing resistance to Gentamicin with 94.3%, followed by (ciprofloxacin) 92%, (ceftazidime) 89.8%, (imipenem) 73.9%, Pipracilline/tazobactam 61.4%, (aztreonam) 52.3%, and (amikacin) 50% and only susceptible to colistin with 92%. In conclusion, most of the isolates showed high levels of resistance to examined antibiotics except colistin and this may indicate the importance of antibiotic susceptibility testing and optimal treatment by combination of drugs.**

Key words: *Pseudomonas aeruginosa*, antimicrobial resistance, nosocomial infections, Malaysia.

INTRODUCTION

Pseudomonas aeruginosa is considered as one of the leading nosocomial pathogens worldwide (Strateva and Yordanov, 2009). It is mostly the causes of morbidity and mortality cases. A problem is made worse when nosocomial pathogens acquire antibiotic resistance (Lagamayo, 2008). The emergence of this organism has a significant impact on treatment outcomes and poses a challenge to the provision of health care and cost-effectiveness.

Unfortunately, the resistance to antipseudomonal agents is on the rise (Rubin et al., 2008). *P. aeruginosa* is known to readily develop multi-drug resistance to various classes of antimicrobial agents, the extensive use of antimicrobial agents and the evolutionary antimicrobial resistance strategies of bacteria have resulted in the emergence of pan-drug resistant bacteriathat is, bacteria with evidence resistance against antipseudomonal penicillin's, cephalosporin's, carbapenem, monobactam, aminoglycosides, fluroquinolone and polymyxins (Babic et al., 2006). In this regard, the current study aims to update the rational empirical antimicrobial therapy recommendation. *P. aeruginosa* isolates were considered to be multidrug resistant if the isolate was resistant to at least three of the following eight drugs: Pipracilline/ tazobactam, ceftazidime, aztreonam, amikacin, gentamicin, ciprofloxacin, imipenem and colistin. These agents were selected as representatives of the primary

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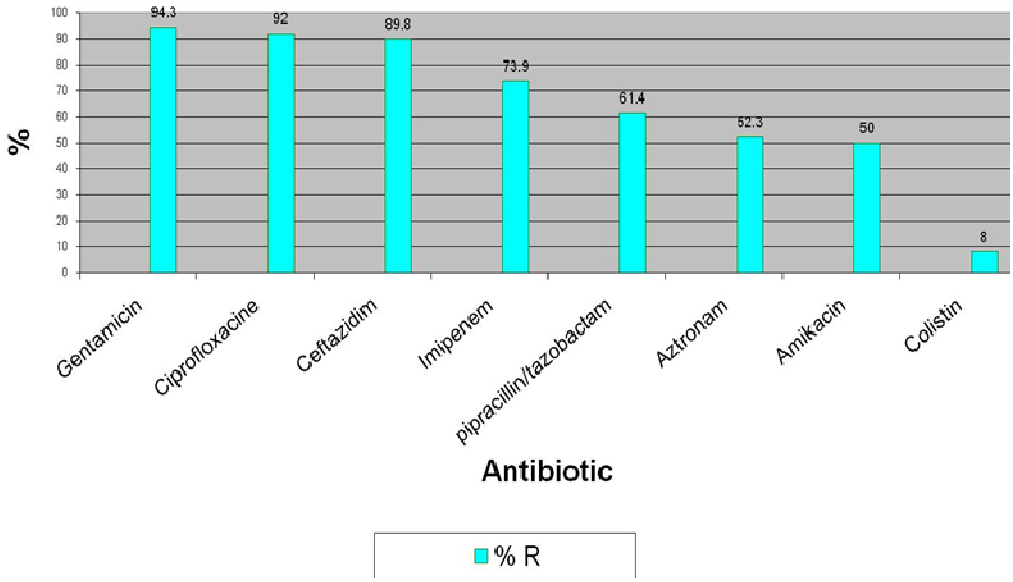

Figure 1. Antibiotic resistance of *P. aeruginosa*.

antibiotic classes used to treat *P. aeruginosa* infections.

MATERIALS AND METHODS

Study location

P. aeruginosa were collected randomly from April 2009 to March 2010 from the University of Malaya Medical Center.

Bacterial isolate

A total of 88 *P. aeruginosa* clinical isolates isolated from various samples collected from different wards of University of Malaya Medical Center. *P. aeruginosa* colonies were identified based on morphology, grams staining, pyocyanin production and biochemical test by API20NE test (bioMérieux, Marcy-l'Etoile, France). According to the manufacturer's instructions using standard laboratory procedures (Osterhout et al., 1991).

Antibiotic susceptibility testing

The susceptibility of various antibiotics against clinical isolates of *P. aeruginosa* was determined using E-test (BIOMERUX) in accordance with the guidelines of the Clinical and Laboratory Standards Institute (Tholen, 2006). *P. aeruginosa* ATCC 27853 were utilized as quality control strains. The antimicrobial tested in this study were pipracilline/tazobactam, ceftazidime, aztreonam, amikacin, gentamicin, ciprofloxacin, imipenem and colistin.

The minimum inhibitory concentration (MIC) for each antibiotic was determined on Mueller- Hinton agar by the E-test® method according to 2006 CLSI, guidelines. Overnight cultures of *P. aeruginosa* on Mueller-Hinton broth were diluted to an initial cell density of 10^7 cfu/ml with fresh Mueller-Hinton broth. Inoculums of 10^5 cfu to achieve a bacterial suspension equivalent to a 0.5 McFarland turbidity standard. Two different E-test® antimicrobial strips placed in opposite gradient directions on Mueller-Hinton agar plate by sterile forceps, printed MIC values are faced upward, [that is, that the bottom surface of the strip containing the antimicrobial gradient is in contact with the agar.

The plates incubated in an inverted position at 37℃ for 20 to 24 h and after incubation. The MICs were read in the intersection point of inhibitory eclipse according to the manufacturer's recommendation.

RESULTS

P. aeruginosa isolated from various clinical samples showed increasing resistance to Gentamicin with 94.3%, followed by (ciprofloxacin) 92%, the (ceftazidime) 89.8%, the (imipenem) 73.9%, pipracilline/tazobactam 61.4%, (aztreonam) 52.3%, and (amikacin) 50% and only susceptible to colistin with 92 % (Figure 1).

Most clinical isolates of *P. aeruginosa* were isolated from urine samples (53.4%), followed by wound (21.5 %), sputum 5.6%, blood 5.6%, tissue 2.2% and 1.13% for other samples (Table 1) Specimens were isolated from different hospital wards. 31 were obtained from surgery ward, 20 from general medicine wards, 13 from orthopedics wards, 7 from paediatric wards, 7 from Neurosurgery wards, 4 from intensive care units, 1 from Cardiac intensive care units, 3 from ENT and 2 from Gynecology wards (Table 2). In the present studies the highest resistant rate of *P. aeruginosa* infections were observed in the surgical department for all antibiotics except colistin that showed the least of resistance followed by the Department of Medicine and Orthopedic (Table 3). The MICs of different antibiotics against *P. aeruginosa* based on the site of the specimen appear highly resistant with urine and wound specimen to be most of the antibiotic except colistin (Table 4).

Table 1. The distribution of *P. aeruginosa* from various clinical specimens.

Source/Site	No of isolates	%
Urine	47	53.4
Wound	19	21.6
Sputum	5	5.6
T/suction	5	5.6
Blood	5	5.7
Tissue	2	2.3
Pus	1	1.1
Lumen tip	1	1.1
Catheter tip	1	1.1
Peritoneal fluid	1	1.1
CSF	1	1.1
Total	88	100

Table 2. Distribution of specimens based on wards.

Wards	Specimen type											
	wound	T/suction	Urine	CSF	sputum	Tissue	Pus	Lumen tip	Catheter	Peritoneal fluid	Blood	Total
Orthopedic	8	0	3	0	0	1	0	0	0	0	1	13
Neurosurgery	1	1	4	0	1	0	0	0	0	0	0	7
Surgery	5	1	20	0	2	1	0	0	1	0	1	31
Paeditric	1	0	3	1	0	0	0	0	0	1	1	7
Gynaecology	0	0	2	0	0	0	0	0	0	0	0	2
Medicine	2	2	12	0	2	0	0	0	0	0	2	20
Cicu	0	1	0	0	0	0	0	0	0	0	0	1
Ent	1	0	1	0	0	0	1	0	0	0	0	3
ICU	1	0	2	0	0	0	0	1	0	0	0	4
Total	19	5	47	1	5	2	1	1	1	1	5	88

DISCUSSION

P. aeruginosa emerged as an important pathogen and responsible for nosocomial infections that is one of the important causes of morbidity and mortality among hospital patients.

In this study, 88 isolates of *P. aeruginosa* from clinical samples received from University of Malaya Medical Centre were studied. These numbers represent the multidrug resistance isolates. The two similar studies, show low rate of resistance in the last three years however this study confirm the increasing rate of resistance rate of colistin (Raja and Singh, 2007; Pathmanathan et al., 2009).

Most of the isolates in current study were collected from urine samples, accounted for 53.4% of the total isolates; this is not surprising as the fact that almost all patients going in for major surgery would be get catheterized. Another study has been shown that the use of indwelling

Table 3. Antibiotic resistance of *P. aeruginosa* based on wards.

Antibiotics		ORTH.	NEURO.	SURG.	PAED.	GYNAE.	MEDIC.	CICU	ENT	ICU	Total
Imipenem	S	4 30.8%	1 14.3%	7 22.6%	2 28.6%	0 0%	6 30 %	0 0.0%	2 66.7%	0 0.00%	22 25.0%
	M	0 0.0%	1 14.3%	0 0.0%	0 0.0%	0 0%	0 0%	0 0.0%	0 0.00%	0 0.0%	1 1.1%
	R	9 69.2%	5 71.4%	24 77.4%	5 71.4%	2 100%	14 70 %	1 100.0%	1 33.3%	4 100.0%	65 73.9%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%
Ceftazidime	S	0 0.0%	0 0.0%	0 0.0%	1 14.3%	0 0.0%	2 10.0%	0 0.0%	1 33.3%	0 0.0%	4 4.5%
	M	1 7.7%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 1.1%
	R	12 92.3%	7 100.0%	31 100.0%	6 85.7%	2 100.0%	18 90.0%	1 100.0%	2 66.7%	4 100.0%	83 94.3%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%
Amikacin	S	0 0.0%	1 14.3%	6 19.4%	1 14.3%	1 50.0%	8 40.0%	1 100.0%	1 33.3%	1 25.0%	20 22.7%
	M	3 23.1%	5 71.4%	11 35.5%	0 0.0%	0 0.0%	3 15.0%	0 0.0%	1 33.3%	1 25.0%	24 27.3%
	R	10 76.9%	1 14.3%	14 45.2%	6 85.7%	1 50.0%	9 45.0%	0 0.0%	1 33.3%	2 50.0%	44 50.0%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%
Pipracilline/ tazobactam	S	4 30.8%	6 85.7%	11 35.5%	0 0.0%	0 0.0%	6 30.0%	0 0.0%	2 66.7%	2 50.0%	31 35.2%
	M	2 15.4%	0 0.0%	1 3.2%	0 0.0%	1 50.0%	1 5.0%	0 0.0%	1 33.3%	0 0.0%	6 6.8%
	R	7 53.8%	1 14.3%	19 61.3%	7 100.0%	1 50.0%	13 65.0%	1 100.0%	0 0.0%	2 50.0%	51 58.0%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%

Table 3. Cont.

Antibiotics		ORTH.	NEURO.	SURG.	PAED.	GYNAE.	MEDIC.	CICU	ENT	ICU	Total
Ciprofloxacin	S	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 5.0%	1 100.0%	0 0.0%	1 25.0%	3 3.4%
	M	1 7.7%	0 0.0%	1 3.2%	1 14.3%	0 0.0%	1 5.0%	0 0.0%	1 33.3%	0 0.0%	5 5.7%
	R	12 92.3%	7 100.0%	30 96.8%	6 85.7%	2 100.0%	18 90.0%	0 0.0%	2 66.7	3 75.0%	80 90.9%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%
Colistin	S	11 84.6%	7 100.0%	28 90.3%	5 71.4%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	81 92.0%
	M	1 7.7%	0 0.0%	2 6.5%	1 14.3%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	4 4.5%
	R	1 7.7%	0 0.0%	1 3.2%	1 14.3%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	3 3.4%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%
Gentamicin	S	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 5.0%	1 100.0%	0 0.0%	0 0.0%	2 2.3%
	M	0 0.0%	0 0.0%	0 0.0%	1 14.3%	0 0.0%	1 5.0%	0 0.0%	0 0.0%	1 25.0%	3 3.4%
	R	13 100.0%	7 100.0%	31 100.0%	6 85.7%	2 100.0%	18 90.0%	0 0.0%	3 100.0%	3 75.0%	83 94.3%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%
Aztreonam	S	4 30.8%	1 14.3%	3 9.7%	0 0.0%	0 0.0%	2 10.0%	0 0.0%	2 66.7%	0 0.0%	12 13.6%
	M	2 15.4%	2 28.6%	7 22.6%	5 71.4%	1 50.0%	9 45.0%	0 0.0%	1 33.3%	0 0.0%	27 30.7%
	R	7 53.8%	4 57.1%	21 67.7%	2 28.6%	1 50.0%	9 45.0%	1 100.0%	0 0.0%	4 100.0%	49 55.7%
Total		13 100.0%	7 100.0%	31 100.0%	7 100.0%	2 100.0%	20 100.0%	1 100.0%	3 100.0%	4 100.0%	88 100.0%

The abbreviations ORTH, NEURO, SURG, PAED, GYNAE, MEDIC, CICU, ENT, ICU designated for orthopedic , neurosurgery, surgery ,pediatrics, gynaecologyl, medicinal, cardic intensive care unit, ear nose throat, intensive care unit, respectively.

Table 4. Antibiotic susceptibility pattern of pseudomonas aeruginosa isolates based on site of specimens.

Source	Imipenm	Ceftazidim	Amikacin	Aztronam	Pipracillin/tazobactam	Ciprofloxacin	Colistin	Gentamicin
Urine	S	M	R	S	M	R	S	M
Wound	12	1	34	1	0	46	10	17
Sputum	6	0	13	0	1	18	5	4
T/suction	0	0	5	1	0	4	2	0
Blood	0	0	5	0	0	5	1	2
Tissue	2	0	3	0	0	5	0	0
Pus	1	0	1	0	0	2	0	1
Lumen tip	1	0	0	1	0	0	1	0
Catheter tip	0	0	1	0	0	1	0	0
Peritoneal fluid	0	0	1	0	0	1	0	0
CSF	0	0	1	1	0	0	0	0

catheters lead to an inherent risk for infection (Olayinka et al., 2004).

Isolates collected from wound infections re-present (21.59 %), the wound infections different from patient to patient, in addition from one hospital to another, from one surgical procedure to other (Humphreys, 2009), in many cases hospital patients used broad-spectrum antibiotics as prophylaxis are mostly colonized by *P. aeruginosa* in the lower intestinal tract (Zuanazzi et al., 2010).

Blood and sputum represent 5.6% of the isolates isolated in the present study. This may be referred due to the empirical therapy of *P. aeruginosa* infection and high pressure of this drugs choice, there is a concordance in the highly susceptibility to as the low number of this samples sent in during the study period. Furthermore, *P. aeruginosa* is the main cause for pneumonia and septicemia with attributable deaths reaching 30% in immunocompromised patients (Scarffand and Goldberg, 2008).

The incidence of multidrug resistant *P. aeruginosa* often changeable dramatically between communities, hospitals in the same area and among many patient, populations in the same hospital (Wroblewska et al., 2006; Marcel et al., 2008). This variation faced by physician in the clinical treatment to be responsible for madding clinical management on the antibiotic choice to be effectively and correctly use according to the update data on the prevalence and resistance pattern.

The antibiotic policy guidelines preparation is the need to avoid any bitter experience of misuse of this class of drug therapy, the team efforts of the members comprising consultants, prescribing physicians, and members of health services team should participate in producing an effective plan to make better decisions in this regards (Masood, 2010). In addition, surveillance programs should be conducted periodically to evaluate the sensitivity and susceptibility of these bacteria against prescribed antibiotics. Since the emergence of resistance remains silent for some periods and takes time to become apparent, these antibiotics should be prescribed with cautions so that the bitter experience of antibiotic resistance can be avoided (Jombo et al., 2010). These *in vitro* sensitivity studies also help in cost-effective; prescribing different brands of the antibiotic to the different patients with varying socioeconomic back-grounds (Masood, 2010). Overall, resistance patterns in different regions should be conducted so that the profiles of the specific region are maintained and monitored accordingly. This will therefore act as a guide towards the formulation of a comprehensive monitoring program of chemotherapy.

In conclusion, most of the clinical isolates had a high level of resistance to examined antibiotics except colistin. Inappropriate and incorrect administration of antimicrobial agents in empiric therapies could be one of the reasons of this alarming phenomenon. This problem indicates importance of performing antibiotic susceptibility testing. What is most worrying is the fact that there is a prevalence of a multiresistant phenotype that was only sensitive to colistin. The emergence and rapid spread of multidrug-resistant isolates of *P. aeruginosa* are of great concern worldwide. It is necessary to limit the overuse of antibiotics and to implement a new antibiotic policy and continuous efforts of clinician, microbiologist, pharmacist and community to

promote greater understanding of this problem, better hygiene, postoperative care and management.

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