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Original Article

Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital

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Background and Purpose: *Pseudomonas aeruginosa* is an important cause of morbidity and mortality in hospitalized, critically ill patients and patients with underlying medical conditions such as cystic fibrosis, neutropenia, and iatrogenic immunosuppression. The prevalence of multiresistant *P. aeruginosa* isolates has been increasing. The aim of this study was to determine the antimicrobial susceptibility patterns in *P. aeruginosa* strains isolated at a university teaching hospital in Kuala Lumpur, Malaysia.

Methods: The Laboratory Information System of the microbiology department was retrospectively reviewed to determine the susceptibility patterns of *P. aeruginosa* isolates to anti-pseudomonal antibiotics, from January to June 2005. Disk diffusion methods were employed and results were interpreted according to National Committee for Clinical Laboratory Standards guidelines.

Results: 505 clinical isolates of *P. aeruginosa* were tested. Major sources of these isolates included respiratory tract, wound, urine and blood. The rates of antimicrobial resistance of isolates were 6.73% to amikacin, 12.9% to gentamicin, 10.1% to netilmicin, 10.9% to ceftazidime, 11.3% to ciprofloxacin, 9.9% to imipenem, 10.8% to piperacillin, 9.4% to piperacillin-tazobactam and 0% to polymyxin B. Of the 505 isolates, 29 (5.74%) were found to be multidrug-resistant; these were most commonly isolated from respiratory tract specimens of patients in surgical units, followed by respiratory tract specimens in patients in medical units.

Conclusions: The data in this study showed low rates of antibiotic resistance among *P. aeruginosa* isolates. Combinations of aminoglycosides plus beta-lactams or quinolones should be the appropriate choice for empirical therapy in *P. aeruginosa* infections. Active antibiotic susceptibility testing and surveillance should be continued in order to curtail the problem of antibiotic resistance.

Key words: Disk diffusion antimicrobial tests; Drug resistance, bacterial; Infection control; *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is one of the most common and complex Gram-negative aerobic bacilli isolated from soil, water, plants, animals and humans. It is occasionally a pathogen in plants and animals. *P. aeruginosa* requires nominal nutrition in order to survive on a wide variety of surfaces and in aqueous environments. It rarely causes serious infections in otherwise healthy individuals and is infrequently identified as normal microbial flora in healthy persons [1]. *P. aeruginosa* infections can cause significant morbidity and mortality especially in susceptible hosts such as those who have disruption in the integrity of physical barrier to bacterial invasion due to insertion of intravenous line, urinary catheter, endotracheal tube, etc. The organism causes infections in the critically ill and in patients with underlying illness such as cystic fibrosis, neutropenia, iatrogenic immunosuppression [1,2]. An important nosocomial pathogen, *P. aeruginosa* colonizes the gastrointestinal tract of postoperative patients and is associated with urinary tract infections, ventilator-associated pneumonia, surgical skin infections, biliary tract infections and severe sepsis [1].

Beta (β)-lactam antimicrobial agents such as cephalosporins, carbapenems, anti-pseudomonal penicillins and penicillin/β-lactamase inhibitors are

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frequently employed as an empirical therapy for serious *P. aeruginosa* infections. These agents may be given as monotherapy or combination therapy with aminoglycosides or quinolones. The emergence of antibiotic resistance has caused great concern among health professionals and the prevalence of multidrug-resistant *P. aeruginosa* isolates has been increasing [3]. Desirable clinical outcome of patients with severe infections caused by *P. aeruginosa* depends on the prompt administration of adequate antimicrobial therapy. The choice of appropriate antimicrobial treatment, however, requires active surveillance of antimicrobial resistance patterns within the institution [4].

The aim of this study was to evaluate the antibiotic susceptibility patterns of *P. aeruginosa* clinical isolates in the University of Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia in order to facilitate more effective empirical antimicrobial therapy for *P. aeruginosa* infections.

Methods

The UMMC is a 900-bed well-equipped modern, urban tertiary care university teaching hospital of international standards and provides services to all of the surgical and medical subspecialities, including orthopedics, obstetrics and gynecology, pediatrics and oncology. UMMC also has two intensive care units (adults and pediatrics). A retrospective study was carried out at UMMC, in order to evaluate antimicrobial susceptibility of clinical isolates of *P. aeruginosa* from bloodstream, respiratory tract, wound and urinary tract specimens and others (including ear, nose and throat). The Laboratory Information System of medical microbiology was reviewed to identify all positive *P. aeruginosa* clinical cultures from patients from January to June 2005. No duplicate isolates were included in this study.

Identification of *P. aeruginosa*

All specimens sent to the microbiology laboratory were cultured on blood agar, MacConkey agar and chocolate agar plates, incubated in an aerobic chamber and 5-10% carbon dioxide at 37°C for two days. *P. aeruginosa* was identified by Gram stain, positive oxidase reaction, color appearance, conventional biochemical methods and API (bioMérieux, Marcy l'Etoile, France) if needed.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing by the disc susceptibility method was in accordance with the National Committee for Clinical Laboratory Standards [5]. Antimicrobial susceptibility tests were done by using disc diffusion methods on Muller Hinton agar plates. *P. aeruginosa* was declared multidrug-resistant if it was found to be resistant to 2 or more classes of antimicrobials, such as β-lactam antimicrobials (including cephalosporins, carbapenems, monobactams, antipseudomonal penicillins, etc), aminoglycosides, quinolones, and polypeptides. The following antibiotic discs were employed: gentamicin (10 μg/mL), netilmicin (30 μg/mL), amikacin (30 μg/mL), cefoperazone (75 μg/mL), ceftazidime (30 μg/mL), ciprofloxacin (5 μg/mL), imipenem (10 μg/mL), meropenem (10 μg/mL), polymyxin B (300 μg/mL), cefoperazone-sulbactam (10 μg/mL), cefepime (10 μg/mL), piperacillin (100 μg/mL) and piperacillin-tazobactam (110 μg/mL).

Results

In total, 505 non-repetitive *P. aeruginosa* isolates from various clinical specimens were tested. There were 311 males and 194 females, a male-to-female ratio of 1.6:1. The ages ranged from two days to 94 years. Of the 505 isolates, 168 specimens (33.3%) were received from surgical patients and 138 (27.3%) were from patients in medical wards. Intensive care units (adult and pediatric) and pediatric wards accounted for 30 patients (5.9%) and 32 patients (6.3%), respectively. The outpatient department contributed 9.1% of the specimens (Table 1). The most common source of *P. aeruginosa* isolates was the respiratory tract and the least common source was blood (Table 2). Respiratory tract specimens were

Table 1. Location of patients with *Pseudomonas aeruginosa* infections

Location	No. of infections (%)
Surgical wards[a]	168 (33.3)
Medical wards	138 (27.3)
Outpatient department	46 (9.12)
Pediatric wards	32 (6.34)
Intensive care units[b]	30 (5.94)
Renal wards	12 (2.38)
Hematology[b]	11 (2.18)
Miscellaneous[c]	68 (13.5)
Total	505 (100)

[a]All units of surgery such as general surgery, orthopedic, neurosurgery, and urology.
[b]Adult and pediatric units.
[c]Miscellaneous includes gynecology, ear nose and throat, ophthalmology, operating theatre and emergency department settings.

Table 2. Types of specimens obtained from patients with *Pseudomonas aeruginosa* infections

Source	No. of specimens (%)
Respiratory tract specimens (n= 216)	
Tracheal secretions	86 (17.03)
Sputum	85 (16.83)
Broncheoalveolar lavage[a]	14 (2.77)
Others[b]	31 (6.14)
Swab specimens (n = 168)	
Wound site swabs	106 (21.0)
Bones	3 (0.64)
Others[c]	59 (11.7)
Other specimens (n = 118)	
Urine	53 (10.5)
Blood	38 (7.52)
Ear nose and throat	11 (2.18)
Eye	7 (1.46)
Surveillance	12 (2.38)

[a]Broncheoalveolar lavage.

[b]Includes nasopharyngeal swabs, tonsils and throat swabs.

[c]Includes bed sores and pus.

Table 3. Antibiotic resistance of *Pseudomaonas aeruginosa* isolates

Antimicrobial	Resistant No. (%)	Organisms tested No.
Gentamicin	65 (12.8)	505
Netilmicin	50 (10.1)	495
Amikacin	34 (6.73)	505
Cefoperazone	73 (14.5)	505
Ceftazidime	55 (10.9)	505
Piperacillin	54 (10.8)	498
Piperacillin-tazobactam	47 (9.43)	498
Ciprofloxacin	57 (11.3)	505
Imipenem	50 (9.90)	505
Meropenem[a]	25 (36.8)	68
Polymyxin B[a]	0 (0)	35
Cefoperazone-sulbactam[a]	29 (40.3)	72
Cefepime[a]	28 (38.9)	72

[a]These antimicrobials were not used in routine testing in our laboratory at the time of the study, and were only used with multidrug-resistant isolates or if specifically requested by physicians.

received most commonly from patients in medical wards, followed by those in surgical wards. The most common respiratory tract specimens from patients in medical wards were tracheal secretions followed by sputum. Among surgical patients, wound, respiratory tract and urine were the most common sources of specimens. Of the 30 specimens from intensive care units, 24 specimens were from the respiratory tract.

Table 3 illustrates the frequency of antibiotic resistance among *P. aeruginosa* isolates from clinical specimens. The antimicrobial susceptibility results indicated low levels of resistance to amikacin (6.7%) and piperacillin-tazobactam (9.4%). Approximately 10% of clinical isolates of *P. aeruginosa* were resistant to imipenem, while 11% showed resistance to ciprofloxacin, piperacillin and ceftazidime. *P. aeruginosa* showed varying degrees of relatively low-level resistance to other antimicrobials. Only multidrug-resistant strains were tested against polymyxin B and all tested strains were found to be sensitive. Of the third-generation cephalosporins, 55 strains (11%) were resistant to ceftazidime and 73 (15%) to cefoperazone. Twenty nine ceftazidime-resistant isolates (53%) were sensitive to imipenem and 17 each (31%) were sensitive to ciprofloxacin and piperacillin-tazobactam. More than 41% of gentamicin-resistant strains were susceptible to amikacin. More than half of the ciprofloxacin-resistant isolates (57) displayed resistance to imipenem and 20 each (35%) were susceptible to ceftazidime and piperacillin-tazobactam. Thirty (60%) and twenty three (46%) imipenem-resistant isolates were susceptible to piperacillin-tazobactam and ciprofloxacin, respectively. Ceftazidime also inhibited growth of 30 imipenem-resistant clinical isolates (60%). Imipenem showed activity against 27 piperacillin-tazobactam-resistant isolates (57%). In this study, 29 *P. aeruginosa* isolates (5.7%) were found to be multidrug-resistant organisms. Of these, 14 pathogens were resistant to all antimicrobials except polymyxin B, while 14 strains showed resistance to ciprofloxacin and aminoglycosides. Only one strain was resistant to ciprofloxacin and β-lactams. These multidrug-resistant pathogens were most commonly isolated from respiratory tract specimens from surgical units, followed by respiratory specimens from medical units.

Discussion

The data in this study indicate that the prevalence of resistance of *P. aeruginosa* isolates to the antimicrobials tested was low. This low resistance can be attributed to the implementation of the antibiotic prescribing guidelines, that restrict antibiotic use to specific situations, and implementation of infection control measures in our health facility. No local or regional (southeast Asia) data of antimicrobial resistance rate among *P. aeruginosa* could be found in the English literature to compare with the findings of this study. However, increasing resistance to the various anti-pseudomonal agents has been reported worldwide and

this poses a serious problem in therapeutic management of *P. aeruginosa* infections [6,7].

Reports from the United States have shown dramatic increases in antimicrobial resistance to ciprofloxacin (from 15% to 32%), imipenem (from 15% to 23%), piperacillin (from 11% to 15%), amikacin (from 7% to 10%) and ceftazidime (from 15% to 19%) over the ten-year period to 2002 [7]. In Russia, 79% and 75% of *P. aeruginosa* strains exhibited resistance to piperacillin and gentamicin, respectively [8]. In our study, amikacin was the most effective drug tested among the aminoglycosides, while gentamicin was the least effective. Despite the increased use of ceftazidime, it retains good activity against *P. aeruginosa*. The high susceptibility to ceftazidime in our data was consistent with previous studies [9,10]. On the other hand, we found a higher ceftazidime resistance rate (10.9%) than a study from Japan in which only 4.6% of the 372 *P. aeruginosa* isolates were resistant to ceftazidime [11].

Piperacillin is hydrolyzed by plasmid-mediated β-lactamases and tazobactam inhibits most β-lactamases produced by most Gram-negative bacteria. A study revealed that the combination of piperacillin and tazobactam exhibited in vitro activity against Gram-negative bacteria harboring Temoniera or sulphydryl variable β-lactamases and their derivatives, such as extended-spectrum β-lactamases, but the combination of piperacillin and tazobactam did not enhance the activity of piperacillin against *P. aeruginosa*, possibly due to the involvement of mechanisms of resistance other than plasmid-mediated β-lactamases [12]. The results for piperacillin and tazobactam in combination in this study were consistent with a previous study [12]. Although carbapenems are the most potent agents against several microbes and remain one of the last therapeutic options against life-threatening *P. aeruginosa* infections, carbapenem resistance in *P. aureuginosa* has recently been reported [13]. A study suggested that the high intrinsic carbapenem resistance of *P. aeruginosa* is generated via the interplay between the active efflux system, the outer membrane and AmpC β-lactamase [14]. Carbapenem resistance is certainly not due to the lower affinity of penicillin-binding proteins.

This study reports a lower resistance to imipenem (9.9%) than previously reported [9,15]. Ciprofloxacin, a 4-fluoroquinolone, inhibits the bacterial enzyme DNA gyrase (topoisomerase that manipulates the topological state of DNA), and resistance to ciprofloxacin in *P. aeruginosa* remains problematic worldwide. The incidence of ciprofloxacin resistance among *P. aeruginosa* has been reported to range between 30% and 40% [3,16]. On the contrary, the situation in our health facility regarding ciprofloxacin susceptibility (89.8%) is thus far satisfactory. The increasing number of episodes of infections caused by multidrug-resistant *P. aeruginosa* isolates susceptible to polymyxin B is troubling; therefore, polymyxin susceptibility testing is recommended if the drug is considered for systemic therapy [17]. Approximately 6% of *P. aeruginosa* isolates in our study were multidrug-resistant and were susceptible to polymyxin B.

Choices of antimicrobial therapy for possible *P. aeruginosa* infection must be guided by local and regional information as well as local antimicrobial susceptibility patterns of *P. aeruginosa* isolates. In addition, clinicians should consider the relative risk of resistance emerging during treatment in each patient. Empirical therapy for *P. aeruginosa* may require the initial use of two or more anti-pseudomonal agents until susceptibility testing results are known, in order to curtail antimicrobial resistance in this pathogen [18]. Aminoglycoside plus β-lactams or ciprofloxacin should be appropriate for the widest coverage of empirical therapy in our health facility.

In conclusion, the present study is important from a practical point of view. The clinical and therapeutic implications of antimicrobial resistance in *P. aeruginosa* confirm the need to continue surveillance in local settings and for comparison of findings with regional reports. The emergence of resistance in microbes can be prevented by implication of strict guidelines for antibiotic prescribing and appropriate infection control measures.

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