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Antibiotic susceptibility profiles of clinical isolates of *Pseudomonas aeruginosa* from Selayang Hospital, Malaysia

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Abstract

Fifty four clinical isolates of *Pseudomonas aeruginosa (P. aeruginosa)* were collected from Selayang hospital, Selangor, Malaysia. Thirty six percent of the isolates were identified from pus followed by respiratory tract (22%) and urine (18.51%). All the isolates were re-identified and confirmed as *P. aeruginosa* in our laboratory. The antibiotic susceptibility profiles of all the isolates were determined using Kirby-Bauer disk diffusion method as recommended by CLSI. Quinolone (ciprofloxacin) was found to be the most active antimicrobial agent with 83.34% susceptibility followed by imipenem (81.49%), aminoglycosides (amikacin, 74.08% and gentamicin, 72.23%), and the beta-lactams (cefepime 62.97%, ceftazidime, 35.19%). Piperacillin showed the maximum resistance (50%) followed by ceftazidime (29.63%). It was also found that, 29% of the *P. aeruginosa* strains were resistant to one antibiotic, 20% strains were resistant to two antibiotics and 51% were multidrug resistant. *P. aeruginosa* isolated from blood, urine and sputum showed the highest rate of multidrug resistance.

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

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Introduction

Widespread occurrences of *Pseudomonas* in nature were observed early in the history of microbiology. Members of the genus *Pseudomonas* bacterium are widely distributed in nature, but the most common human pathogen is *Pseudomonas aeruginosa. (P. aeruginosa). P. aeruginosa* is an important pathogen causing severe and life threatening infections in immunocompromised hosts, such as patients suffering from respiratory disease, cancer patients undergoing chemotherapy, and children and young adults with cystic fibrosis. Moreover, it is a leading cause of nosocomial infections and is associated with a high mortality rate. One reason for this high mortality is its notable resistance to many currently available antibiotics. Yet, comparative analyses of the emergence of resistance associated with different classes of antipseudomonal drugs are lacking, even though knowledge about the relative risks of resistance with different antibiotics could be useful in helping to guide therapeutic choices [1].

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 [2]. However, there are few recent surveillance studies reporting antimicrobial resistance patterns of *P. aeruginosa* in few locations in Malaysia [3].

Over the past few years, a notable increase in antibiotic resistance among gram negative bacteria recovered from hospitalized patients has been reported, especially for critically ill patients [4]. Infections caused by multidrug resistant (MDR) gram negative bacteria, especially MDR *P. aeruginosa* have been associated with increased morbidity, mortality and costs [5]. Multidrug-resistant strains of *P. aeruginosa* are often isolated among patients suffering from nosocomial infections particularly those receiving intensive care treatments [6].

The aim of this study was to assess the current levels of antimicrobial susceptibility and to evaluate the resistance mechanisms to antipseudomonal antimicrobial agents among the clinical isolates of *P. aeruginosa* isolated from patients admitted to Selayang hospital, Malaysia.

Materials and Methods

Fifty four clinical isolates of *P. aeruginosa* strains were collected from different patients who were admitted to Selayang hospital, Selangor, Malaysia between January 2010 and June 2010. The isolates were obtained from different clinical specimens, including pus, urine, respiratory fluids, blood, tissue, and genitalia. All the clinically isolated samples were identified as *P. aeruginosa* by the hospital personnel. We have re-identified all the isolates at our Laboratory by the conventional biochemical tests [7] *i.e.,* gram staining, catalase test, oxidase test , motility test, Triple Sugar Iron Assay**,** citrate test, urease test and indole test etc.

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method [8] was performed to determine the antibiotic susceptibility. The antibiotics tested were Gentamicin (10 µg), Impenem (10 µg), Amikacin (30 µg), Piperacillin (100 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Cefoperazone (75 µg), Piperacillin / Tazobactam (110 µg), Meropenem (10 µg), and Cefepime (30 µg). Results of disk diffusion method were interpreted in accordance to the Clinical and Laboratory Standards Institute (CLSI, 2009)

Results

The sources of clinical specimens from patients of Selayang hospital are shown in Figure1. The antimicrobial susceptibility testing revealed that *P. aeruginosa* strains were highly sensitive to most of the antibiotics tested, which are shown in Figure 2.

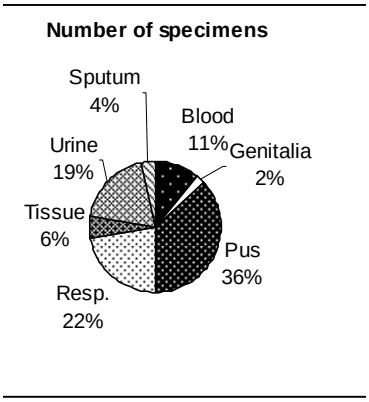

Figure1. *Percentage of P. aeruginosa isolated from various clinical specimens.*

The percentage of sensitivities were ciprofloxacin (83.34%), imipenem (81.49%), amikacin (74.08%), gentamicin (72.23%), cefepime (62.97%), ceftazidime (35.19%), meropenem (79.63%), piperacillin (35.19), cefoperazone (22.23%), tazobactam 10/piperacillin 75 (51.86%) and the percentage of resistance were ciprofloxacin (9.25%), imipenem (16.67%), amikacin

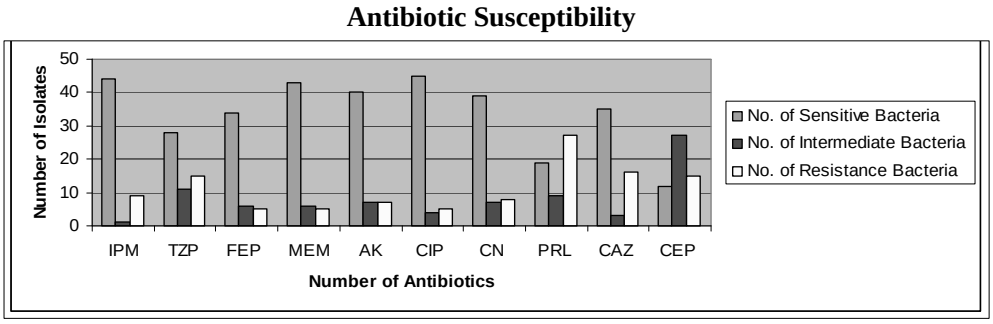

Figure 2*. Number of bacterial strains based on antibiotic susceptibility.*

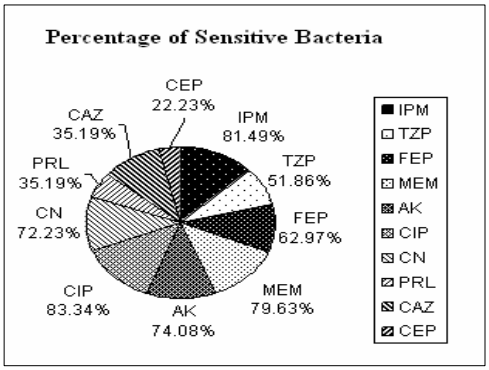

Figure 3. *Percentage of P. aeruginosa isolates sensitive to different antibiotics.*

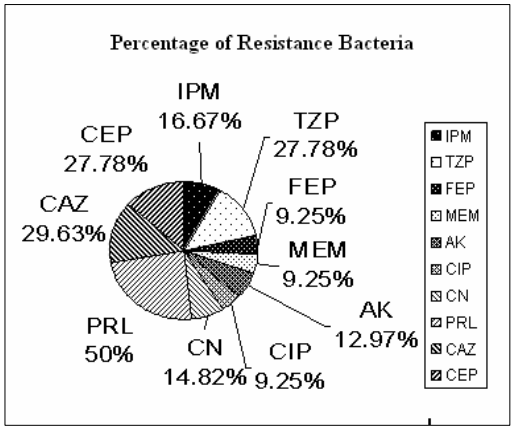

Figure 4. *Percentage of P. aeruginosa isolates resistant to different antibiotics.*

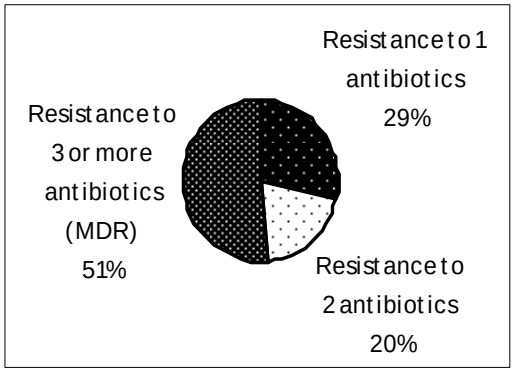

Figure 5. *Percentage of P. aeruginosa resistant to various numbers of antibiotics.*

(12.97%), gentamicin (14.82%), cefepime (9.25%), ceftazidime (29.63%), meropenem (9.25%), piperacillin (50%), cefoperazone (27.78%), tazobactam/piperacillin (27.78%) (analysed from Figure 2).

Distribution of the *P. aeruginosa* isolates according to the specimen type and its correlation to Multidrug Resistance (MDR) which are shown in Table 1.

Table 1. *Presence of multidrug resistant isolates (MDR) based on specimen type.*

Specimen	Number of specimens	No. MDR isolates
Blood	6	4
Genitalia	1	0
Pus	20	7
Respiratory tract	12	1
Tissue	3	1
Urine	10	4
Sputum	2	2

Discussion

P. aeruginosa infection is a serious cause of nosocomial infections. With the widespread use of antibiotics and increase in the number of immunosuppressed hosts, *P. aeruginosa* has become a leading cause of gram-negative bacterial infections especially in immunosuppressed patients who need prolonged hospitalization [9]. The increasing rate of *P. aeruginosa* strains in a wide spectrum of clinical steerings determine them as emerging pathogens, especially in intensive care units (ICUs) and justifies the necessity for antimicrobial-resistance surveillance. Periodic antimicrobial resistance monitoring in *P. aeruginosa* infection is fundamental to updating the current activity level of commonly used antipseudomonal drugs [2].

In this present study, it was found that the out of 54 clinically *P. aeruginosa* isolates, 22 strains (40.74%) were identified from pus followed by urine, 10 cases (18.51%). Ciprofloxacin was found to be the most effective agents (83.34% sensitivity) followed by imipenem and meropenem (81.49% and 79.63%, respectively). Another study in Malaysia also showed 83.5% of the *P. aeruginosa* isolates were found to be sensitive to ciprofloxacin followed by imipenem (79.4%) and meropenem (77.3%) [2]. It was reported that the majority of meropenem-resistant *P. aeruginosa* showed resistance to imipenem, but almost half the imipenem resistant strains were susceptible to meropenem. Moreover, the strains resistant to meropenem were also resistant to ciprofloxacin and carbenicillin [10]. Imipenem has been reported to be very active against *P. aeruginosa* in a number of recent studies [11] while other has reported otherwise [12].

In our studies the rates of antimicrobial resistance of the isolates were 9.25 % to ciprofloxacin, meropenem and cefepime, 12.97% to amikacin, 27.78% to Tazobactam, 16.67% to imipenem, 27.78% to cefoperazone, 29.63% to ceftazidime, 14.82% to gentamicin and 50% to piperacillin. A study done in another tertiary care hospital in Malaysia [3] showed 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 while 5.74% of the strains were found to be multidrug-resistant. Raja *et al.*, reported a low incidence of piperacillin resistance (10.8%) compared to our findings (50%) but ciprofloxacin had higher resistance rate than the present study [3]. Similarly, another study showed the resistance of piperacillin was 54.66% [13]. Drug resistance levels in different hospitals in Malaysia

and others countries too have been reported in the past and antibiotics in the respective hospitals are recognized to the differential usage. When we compared to previous Malaysian studies [3], our studies showed higher resistance rates to all drugs tested except ciprofloxacin and imipenem. Among the 54 clinical isolates of *P. aeruginosa* tested in our study, many strains were found to be multidrug-resistant (MRD). In this study it was found that 29% of the *P. aeruginosa* strains were resistant to one antibiotic, 20% strains were resistant to two antibiotics and 51% were multidrug- resistant. The resistance to antibiotics of the investigated problematic strains of *P. aeruginosa* was higher than the mean P. *aeruginosa* resistance found in Malaysia [3]. In this study, *P. aeruginosa* isolated from blood, urine and sputum showed the highest rate of multidrug resistance. But the correlation between the multidrug resistance and the site of infection is not known.

In summary, ciprofloxacin was found to be the most active antimicrobial agent with 83.34% susceptibility followed by imipenem (81.49%). Piperacillin showed the maximum resistance (50%) followed by ceftazidime (29.63%). Fifty-one percent of the isolates were multidrug resistant. *P. aeruginosa* isolated from blood, urine and sputum showed the highest rate of multidrug resistance.

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