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Research Article

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES AT A TERTIARY CARE HOSPITAL IN KATHMANDU, NEPAL

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ABSTRACT

Objective: Increasing number of reports had documented the continued emergence of resistance among *P. aeruginosa* strains to common antimicrobial drugs, world-wide. This study investigated the antimicrobial resistance patterns of *P. aeruginosa* clinical isolates obtained from hospitalized patients.

Methods: Between January 2012 and June 2012, one hundred and forty-five strains of P.aeruginosa were isolated from different clinical specimens and fully characterized by standard bacteriological procedures. Antimicrobial susceptibility patterns of each isolate was carried out by the Kirby-Bauer disk diffusion method as per guidelines of CLSI.

Results: Majority of isolates of P.aeruginosa (120, 83.75%) were obtained from specimens of pus, sputum, urine and tracheal aspirates. The isolated pathogens showed resistance to amikacin (17.25%), ciprofloxacin (27.59%) and cefoperazone -sulbactum (34.48%). Resistance rates to Co-trimoxazole, piperacillin, ceftriaxone and chloramphenicol varied from 51.00% to 73.00%. All the isolates were susceptible to imipenem. 30 (20.69%) of P.aeruginosa isolates were multi-drug resistant.

Conclusion: The results confirmed the occurrence of drug resistant strains of P.aeruginosa. Imipenem, amikacin, and ciprofloxacin were found to be the most effective antimicrobial drugs. It therefore calls for a very judicious, rational treatment regimens prescription by the physicians to limit the further spread of antimicrobial resistance among the P.aeruginosa strains.

Keywords: antimicrobial resistance, clinical isolates, Nepal, Pseudomonas aeruginosa

INTRODUCTION

Antimicrobial agents have been the only easily and widely used therapeutic option available to counter the infections caused by diverse microbial agents. However, microbial populations have developed various strategies to overcome these antimicrobial agents - a major contributing factor in the development of anti-microbial resistance world-wide. *Pseudomonas aeruginosa* is an ubiquitous and versatile human opportunistic pathogen and has implications on morbidity, mortality and healthcare costs both in hospitals and in the community [1]. The development of resistance to all available antibiotics in some organisms may preclude the effectiveness of any antibiotic regimen[2,3]. Infections caused by *P.aeruginosa* are frequently life-threatening and difficult to treat as it exhibits intrinsically high resistance to many antimicrobials [4] and the development of increased, particularly multi-drug resistance in health care settings [4,5]. Mechanisms that cause antimicrobial drug resistance and multi-drug resistance in *P.aeruginosa* are due to acquisition of resistance genes (e.g those encoding beta-lactamase [6] and amino-glycoside modifying enzymes [7] via horizontal gene transfer and mutation of chromosomal genes (target site, efflux mutations) are the target of the fluoroquinolones particularly ciprofloxacin [8] . Biofilm formation in *P.aeruginosa*, particularly in the case of pulmonary infections in patients with cystic fibrosis, contribute to its resistance to antimicrobial agents [9]. Hypermutable (or mutator) strains of *P.aeruginosa* exhibiting increased mutation rates are common in chronic infecions such as those that occur in the lungs of cystic fibrosis patients[10]. Increase in the frequency of multi-drug resistant (MDR) strains of *P.aeruginosa* has severely limited the availability of therapeutic options. Ongoing studies on current antimicrobial resistance profiles of *P.aeruginosa* are essential to find out the susceptibilities of this pathogen against commonly prescribed antibiotics in any health care facility. This would help the physicians to optimize the current therapeutic treatment options. Data on antimicrobial susceptibility profiles of *P. aeruginosa* is limited in Nepal [11,12] . This study was therefore designed to find out the current antimicrobial susceptibility patterns of *P.aeruginosa* strains in a centrally located urban tertiary care hospital in Kathmandu, Nepal.

MATERIALS AND METHODS

Setting

This investigation was carried out in the Department of Microbiology, Kathmandu Medical College Teaching Hospital, a centrally located urban tertiary care medical center in the Kathmandu valley, Nepal between the period January 2012 and June 2012.

Specimens

Specimens were collected from patients who were hospitalized for more than one week duration. A total of 850 clinical specimens were investigated for bacterial culture and identification. Only one isolate from each patient was considered in the study.

Laboratory Identification of Isolates

The specimens were collected from the hospitalized patients admitted in different wards of the hospital. These were processed for bacterial species identification by standard microbiological procedures. Specimens were taken from various sources like pus/wound, sputum, urine, tracheal aspirates, central venous (CV) catheter tip, broncho-alveolar lavage (BAL) fluid, catheters and high vaginal swabs and were inoculated on routine culture media like Mac-Conkey agar, blood agar and eosin-methylene blue agar. A battery of tests were performed that included gram's staining, colony morphology, motility tests, sugar fermentation tests and biochemical tests such as oxidase test, urease test and IMViC (indole, methyl red, Voges-Proskauer and citrate) tests for the confirmation of the isolates as *Pseudomonas aeruginosa* [13].

Susceptibility tests

Anti-microbial susceptibility tests were done by the Kirby-Bauer disk diffusion method as per the recommendations of National

Committee for Clinical Laboratory Standards (NCCLS)[14], USA against a panel of anti-pseudomonal antimicrobials of standard strengths as follows: amikacin 30 mcg, piperacillin 100 mcg, ceftriaxone 30 mcg, cefoperazone-sulbactum 75-10 mcg, ciprofloxacin 5 mcg, Co-trimoxazole 25 mcg, Chloramphenicol 30 mcg and imipenem 10 mcg (Hi Media Laboratories Pvt. Ltd., Mumbai, India). *P. aeruginosa* ATCC 27853 was used as the control strain.

RESULTS

Patients and specimens data

145 strains of *P. aeruginosa* were isolated and identified by standard microbiological procedures, out of a total of 850 clinical specimens investigated. The rate of isolation of *P. aeruginosa* was 17.05 % . Of these 145 strains of *P. aeruginosa* , 80 (55.17%) were from females and 65 (44.83%) were from males. Most of them belonged to the age group 21-40 years (60, 41.40%), followed by patients of > 60 years of age (45, 31.00%) as shown in Table 1. Wound/pus, sputum, urine and tracheal aspirates (120, 83.75%) were the predominant sources of specimens of *P.aeruginosa* clinical isolates as depicted in Table 2.

Table 1: Age and gender wise distribution of clinical isolates of *Pseudomonas aeruginosa*

Age group (years)	Male (no.)	Female (no.)	Total no. (%)
<20	0	10	10 (6.90)
21– 40	20	40	60 (41.40)
41 – 60	15	15	30 (20.70)
>60	30	15	45 (31.00)
Total	65 (44.83%)	80 (55.17%)	145 (100.00)

Table 2: Distribution of specimens of *Pseudomonas aeruginosa* clinical isolates

Source of Specimen	Number	Percentage (%)
Pus / wound	40	27.60
Sputum	35	24.10
Urine	30	20.70
Tracheal aspirate	15	10.35
CV Catheter tip	05	3.45
BAL fluid	05	3.45
Bile	05	3.45
Catheter	05	3.45
High Vaginal Swab	05	3.45
Total	145	100.00

Abbreviations: CV – central venous; BAL – bronchoalveolar lavage

Antimicrobial susceptibility pattterns

Antimicrobial susceptibility patterns of *P.aeruginosa* varied markedly with the antibiotic tested. *P.aeruginosa* isolates showed maximum resistance to chloramphenicol (72.41%) and the least resistance to amikacin (17.25%). All isolates were sensitive to the carbapenem drug- imipenem. The resistance pattern of the *P.aeruginosa* to various antibiotics tested was in the order: chloramphenicol (72.41%) > ceftriaxone (68.96%) > piperacillin (55.17%) > co-trimoxazole (51.72%), cefoperazone-sulbactum (34.48%) > ciprofloxacin (27.59%) > amikacin (17.25%) > imipenem (0.0%) as shown in Table 3. Multi-drug resistance (resistance to ≥ 3 different classes of antibiotics tested) was shown by 30 (20.69%) of *P.aeruginosa* isolates tested (Table 4).

Table 3: Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates

Antibiotic	Sensitive no.(%)	Resistant no. (%)
Amikacin	120 (82.75)	25 (17.25)
Piperacillin	65 (44.83)	80 (55.17)
Ceftriaxone	45 (31.04)	100 (68.96)
Cefoperazone-Sulbactum	95 (65.52)	50 (34.48)
Ciprofloxacin	105 (72.41)	40 (27.59)
Co-trimoxazole	70 (48.28)	75 (51.72)
Chloramphenicol	40 (27.59)	105 (72.41)
Imipenem	145 (100.00)	0.0

Table 4: Numerical antimicrobial resistance patterns of *Pseudomonas aeruginosa* clinical isolates

P.aeruginosa clinical isolates, n=145 (%)	Resistance to no. of classes of antibiotics tested
30 (20.69)	0
35 (24.14)	1
50 (34.48)	2
30 (20.69)	≥ 3

DISCUSSION

In this study, a total of 145 isolates of *P.aeruginosa* were isolated and identified from various clinical sources, from the hospitalized patients and their antimicrobial susceptibility patterns were determined. Most of them belonged to older age group of 21-40 years (60, 41.40%) and elderly age group of > 60 years (45, 31.00%). This could be explained as due to decreased immunity, prolonged hospitalization and other associated co-morbidities in these age groups. A study done in Ahmadabad, India [15] shown (29, 29.00%) of patients were aged between 31-45 years. Sex-wise, female patients (80, 55.17%) constituted a larger group in our study. In contrast, Ahmed etal.,[16] reported an increased incidence in male sex (77.7%) as well as a higher prevalence rate among elderly 61-80 years (43.92%). Similarly, a high prevalence of pseudomonas infection was found in the 35-50 years age group [17]. The distribution of specimens of *P.aeruginosa* may vary with each hospital as each hospital facility has a different environment associated with it. More than 80% of the *P. aeruginosa* isolates were obtained from wound / pus, sputum, urine and tracheal aspirates. Similar results had been obtained in different studies in India reported by Mohanasoundaram [17] and Arora etal.,[18] respectively.

Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains, has been reported world-wide [19,20] and this is a serious therapeutic problem in the management of disease due to these organisms. The resistance profiles of *P. aeruginosa* to the eight anti-microbial agents tested varied among the isolates investigated. One striking feature in this study was that all the *P. aeruginosa* isolates were found to be sensitive to imipenem. This may be due to the restricted use of imipenem in this hospital. This is consistent with a report published in 2002 in Mangalore, India [21] but other studies have showed varying degrees of resistance to imipenem in recent years [17,18,22,23]. Amikacin (82.75% sensitive), followed by ciprofloxacin (72.41% sensitive) proved to be the most effective drugs for routine use among the *P. aeruginosa* strains investigated in this study. An earlier study reported from Kathmandu, Nepal [11] , shown amikacin (81.4% sensitive) and ciprofloxacin (70.3% sensitive) among *P. aeruginosa* strains examined. High resistance to aminoglycosides had been reported in studies done in India [17,18], Bangladesh [24], Turkey [25] and Malaysia [26]. Similarly higher rates of resistance to fluoroquinolones such as ciprofloxacin (40.5%) had been reported in a study done in North Kerala, India [16] and ciprofloxacin resistance (92%) was shown in a study from Malaysia [23]. Piperacillin alone tested showed a resistance rate of 55.17% in this study whereas beta-lactam / beta-lactamase inhibitor drug cefoperazone-sulbactum showed a lower resistance of 34.48% only, indicating beta-lactamase inhibitor markedly expands the spectrum of activity of beta-lactams [22], which makes the combination drug the preferred choice against *P. aeruginosa* infections. Thus, emphasis should be given towards use of combined antibiotics in the treatment of pseudomonal infections [12]. Similar resistance rates for piperacillin (54.66%) had been reported in the study done by Shenoy etal.[21]. Relatively low piperacillin resistance (11.5%) had been reported in in-patients isolates of *P. aeruginosa* in a study from Saudi Arabia [27]. In a study done in Kathmandu, Nepal [12], *P. aeruginosa* isolates obtained from intensive care unit of a national heart centre showed a high cefoperazone-sulbactum sensitivity rate

of 84.8%. Low resistance rates for the cefoperazone-sulbactum (11.!%) had been shown in a study done in North Kerala, India [16]. The rate of resistance for the anti-folate drug co-trimoxazole in the present study was 51.72%. In contrast, a study done in Bangladesh [24] showed rate of resistance for co-trimoxazole to be 93.5% in wound swab and pus isolates of *P. aeruginosa* while a Nigerian study [28] showed *P. aeruginosa* isolates 100% resistant to co-trimoxazole. *P. aeruginosa* strains in this study exhibited a high rate of resistance to the third generation cephalosporin drug - ceftriaxone (68.96%). A much higher resistance to ceftriaxone of 75% , 86% and 93.9% had been reported in studies done in India [18], Bangladesh [24] and Nepal [12]. Lesser rate of resistance to ceftriaxone (40%) had been reported in another study from Andhra Pradesh, India [29]. This study revealed that chloramphenicol had the highest rate of resistance (72.41%) to *P. aeruginosa* strains suggesting that this drug should no longer be included in the treatment regimen for *P. aeruginosa* infections in this population group. A study done in Kano, Nigeria [28] demonstrated a much higher rate of resistance (97.7%) of *P. aeruginosa* isolates to chloramphenicol. Another significant finding in this study was the rate of multi-drug resistance to be 20.69%. A MDR rate of 19.6% , 89.4% and 100% among *P.aeruginosa* isolates had been reported from studies conducted in Malaysia [30], Nepal [12] and Iran [31] respectively.

This study has a few limitations. First, including the community acquired isolates of *P.aeruginosa* along with hospital isolates would have provided a much better picture of resistance patterns of strains in this geographical area. Second, it is essential to conduct a large scale study with newer anti-pseudomonal agents. Third, molecular typing and plasmid profile of the *P. aeruginosa* isolates would provide the much needed details about the strains and lastly extended spectrum beta-lactamse (ESBL) producing *P. aeruginosa* which have become a major cause of nosocomial infections with MDR strains should be analyzed [28,32].

CONCLUSION

Results of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the *P. aeruginosa* isolates. Imipenem was the only anti-pseudomonal drug against which all isolates of *P. aeruginosa* were fully sensitive. We suggest a more restricted and a more rational use of this drug in this hospital setting. Amikacin, ciprofloxacin and semi-synthetic penicillin with beta-lactamase inhibitors are the preferred drugs for optimal management of infections caused by *P. aeruginosa*. Regular anti-microbial susceptibility monitoring is essential for local, regional and national level isolates. This would help and guide the physicians in prescribing the right combinations of anti-microbials to limit and prevent the emergence of multi-drug resistant strains of *P. aeruginosa*.

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COMPETING INTERESTS

We have no conflict of interest

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