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RECENT TREND OF ACQUISITION OF MULTI-DRUG RESISTANCE IN PSEUDOMONAS AERUGINOSA

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ABSTRACT

Continues emergence of resistance among Pseudomonas (P.) aeruginosa strains to common antimicrobial drugs have been documented world-wide. This study investigated the antimicrobial resistance patterns of P.aeruginosa among the patients in mid & far western region of Nepal.

KEYWORDS

spinal cord injury; transplantation; olfactory ensheathing cells; efficacy evaluation.

MATERIALS AND METHODS

The study was conducted on 917 patients with suspected P.aeruginosa infections, attending outpatient and inpatient departments of Nepalgunj Medical College and teaching Hospital, Banke, Nepal from September 2011 to September 2013. Specimens were collected from Pus/wound, sputum, urine, tracheal aspirates, central venous catheter tip, broncho-alveolar lavage fluid, catheters and vaginal swabs and processed for isolation and identification of P.aeruginosa following the standard microbiological methods while the disc diffusion test was used to determine antimicrobial resistance patterns of the recovered isolates at the central Laboratory of Microbiology.

RESULTS

One hundred ninety four isolates were identified as P.aeruginosa. Resistance to chloramphenicol (74.23%), ceftriaxone (69.56%), Cefepime,(57.22%), Cefoperazone-Salbactum (54.12%) and co-trimoxazole (53.02%) was observed. All the isolates were susceptible to imipenem. 48 (24.74%) of P.aeruginosa isolates were multi-drug resistant to ≥3 classes of antibiotics. Among 194 isolates, 88 (45.36%) were from 21-40 years age group and this group was statistically significant (P<0.05), compared to the other age groups.

CONCLUSIONS

The study revealed the occurrence of drug resistant strains of P.aeruginosa. Many isolates showed appreciable levels of antibiotic resistance apparently due to antibiotic abuse. It therefore calls for a very judicious, rational treatment regimens prescription by the physicians to curb the increasing multi drug resistant of P.aeruginosa strains in this region of Nepal.

KEYWORDS

Clinical isolates, pseudomonas aeruginosa, antimicrobial resistance, Nepal

1. INTRODUCTION

Biological Infectious diseases are an important cause of morbidity and mortality throughout life and in this regard, opportunistic pathogens play an important role. Based on a study, Pseudomonas (P.) aeruginosa is an aerobic gram-negative rod-shaped bacterium that belongs to the Pseudomonadaceae family [1]. Study showed P. aeruginosa is a ubiquitous and versatile human opportunistic pathogen and has implications on morbidity, mortality and healthcare costs both in hospitals and in the community [2]. According to a study, the development of resistance to all available antibiotics in some organisms may preclude the effectiveness of any antibiotic regimen [3,4]. Infections caused by P.aeruginosa are frequently life- threatening and difficult to treat as it exhibits intrinsically high resistance to many antimicrobials and the development of increased particularly multi-drug resistance in health care settings [5,6]. 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 and amino-glycoside modifying enzymes) via horizontal gene transfer and mutation of chromosomal genes (target site, efflux mutations) are the target of the

fluoroquinolones particularly ciprofloxacin [7-9]. This pathogen is intrinsically resistant to most antibiotics such as, chloramphenicol, tetracycline, macrolides, trimethoprim–sulfamethoxazole, and rifampin [10]. Resistance in P. aeruginosa may be due to outer membrane modifications, production of extended-spectrum beta- lactamase and efflux pumps, which confers various levels of resistance to expanded spectrum cephalosporins [11,12].Biofilm formation in P.aeruginosa, particularly in the case of pulmonary infections in patients with cystic fibrosis, contribute to its resistance to antimicrobial agents [13]. Hypermutable strains of P.aeruginosa exhibiting increased mutation rates are common in chronic infections such as those that occur in the lungs of cystic fibrosis patients [14]. 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. Based on a study, data on antimicrobial susceptibility profiles of P. aeruginosa is limited in Nepal [15,16]. To our best knowledge, no study has been done regarding drug resistance of Pseudomonas aeruginosa in mid & far western region of Nepal. Therefore this study was thus designed to find out the current antimicrobial resistance patterns of P.aeruginosa strains in Nepalese patients at mid and far western region of Nepal.

2. MATERIAL AND METHODS

2.1 Study Background and Subjects

This was a prospective study conducted on 917 Nepalese patients, attending outpatients and inpatients departments of Nepalgunj Medical College and teaching Hospital, Banke, Nepal, between September 2011 and September 2013.

2.2 Sample Collection and Processing

Specimens were collected from various sources like pus/wound, sputum, urine, tracheal aspirates, central venous (CV) catheter tip, broncho-alveolar lavage (BAL) fluid, catheters and vaginal swabs and processed following the standard Microbiological methods at the central Laboratory of Microbiology of Nepalgunj Medical College and teaching Hospital, Banke, Nepal and were inoculated on routine culture media like cetrimide agar, Mac-Conkey agar, blood agar, nutrient agar and eosin-methylene blue agar [17]. According to a researcher, a battery of tests was 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 [17].

2.3 Susceptibility Tests

Anti-microbial susceptibility tests were done by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Himedia Lab. Pvt Ltd.) as per the recommendations of National Committee for Clinical Laboratory Standards (NCCLS), USA against a panel of anti-pseudomonal antimicrobials of standard strengths as follows: Gentamicin (30µg), Ceftazidime (30µg), Amikacin 30 µg, Ciprofloxacin (5µg), Aztreonam (50µg), Cefepime (50µg), Cefoperazone - Salbactum (75/30µg), Piperacillin - Tazobactum (100/10µg), Ticarcillin - Clavulanic acid (75/10µg), Imipenem (10µg), Meropenem (10µg), piperacillin(100µg), co-trimoxazole (25µg), ceftriaxone (30µg), chloramphenicol (25µg), (Hi Media Laboratories Pvt. Ltd., India). P. aeruginosa ATCC 27853 was used as the control strain [18].

2.4 Statistical Analysis

Data obtained were analyzed using the SPSS (v. 16.0) Chicago, U.S.A. Association of gender and age-groups of P. aeruginosa was assessed using chi-square test. P values < 0.05 were considered to be statistically significant.

3. RESULTS

194 strains of P. aeruginosa were isolated and identified by standard microbiological procedures, out of a total of 917 clinical specimens investigated. Sputum, Wound/pus, urine, tracheal aspirates and vaginal Swab (173, 89.18%) were the predominant sources of specimens of P.aeruginosa clinical isolates as depicted in Table 1. The rate of isolation of P. aeruginosa was 21.16 %. Of these 194 strains of P. aeruginosa, 112 (57.73%) were from female and 82 (42.27%) were from male. P. aeruginosa were isolated from patients aged between 1 and > 60 years. A high prevalence (45.36%) was identified in subjects aged 21-40 years and this age group was statistically significant (P<0.05), compared to the other age groups. However, there was no significant difference in the overall prevalence of isolates according to sex as shown in Table 2.

Table 1: Distribution of specimens of pseudomonas aeruginosa clinical isolates

S.N.	Source of Specimen	Number (%)
1	Sputum	57 (29.38)
2	Pus / wound	52 (26.80)
3	Urine	34 (17.53)
4	Tracheal aspirate	19 (9.79)
5	Vaginal Swab	11 (5.67)
6	BAL fluid	7 (3.61)
7	Catheter	6 (3.09)
8	Bile	4 (2.06)
9	CV Catheter tip	4 (2.06)
10	Total	194 (100)

Table 2: Age and gender wise distribution of clinical isolates of pseudomonas aeruginosa

Age group (in years)	Gender		Total No. (%)
	Male	Female	
<20	4	11	15(7.73)
21- 40	31	57	88(45.36)
41- 60	19	21	40(20.62)
>60	28	23	51(26.29)
Total	82(42.27%)	112(57.73%)	194(100)

Table 3: Resistance of pseudomonas aeruginosa to a panel of fifteen antibiotics

S.N.	Antimicrobial agent (Concentration)	P. aeruginosa N=194 No. (%)

1	chloramphenicol(25µg)	144(74.23)
2	ceftriaxone (30µg)	135(69.56)
3	Cefepime (50µg)	111(57.22)
4	Cefoperazone - Salbactum (75/30µg)	105 (54.12)
5	co-trimoxazole (25µg)	103(53.02)
6	Gentamicin (30µg)	91 (46.91)
7	piperacillin(100µg)	88 (45.36)
8	Ticarcillin - Clavulanic acid (75/10µg)	76 (39.18)
9	Aztreonam (50µg)	62 (31.96)
10	Ceftazidime(30µg)	61 (31.44)
11	Ciprofloxacin (5µg)	57 (29.38)
12	Amikacin (30µg)	37 (19.07)
13	Piperacillin - Tazobactum (100/10µg)	35 (18.04)
14	Meropenem(10µg)	15 (7.73)
15	imipenem (10µg)	0(0)

Table 4: Multi-drug resistance of p. aeruginosa isolates in different classes of antibiotics

S.N.	P.aeruginosa isolates, No.(%)	Resistance to no. of classes of antibiotics
1	31(15.98)	0
2	52(26.80)	1
3	63(32.47)	2
4	48(24.74)	≥3
	Total 194	

3.1 Antimicrobial Susceptibility Patterns

Antimicrobial susceptibility patterns of P.aeruginosa varied markedly with the antibiotic tested. P.aeruginosa isolates showed maximum resistance to chloramphenicol (74.23%) and the least resistance to Meropenem (7.73%). All isolates were sensitive to imipenem. The resistance pattern of the P.aeruginosa to a panel of fifteen antibiotics is in shown in Table 3. Multi-drug resistance of P.aeruginosa isolates in different classes of antibiotics is shown in Table 4.

4. DISCUSSION

In this study, a total of 194 isolates of P.aeruginosa were isolated and identified from various clinical sources, from the outpatients, inpatients and their antimicrobial susceptibility patterns were determined. 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 Sputum, Wound/pus, urine, tracheal aspirates and Vaginal Swab. Similar results have been obtained in different studies in Nepal by a group of researchers in India [19-21]. Most of them belonged to older age group of 21-40 years (88, 45.36%) and elderly age group of > 60 years (51, 26.29%). This could be explained as due to decreased immunity, prolonged hospitalization and other associated co-morbidities in these age groups. Similarly, a high prevalence of pseudomonas infection was found in the age group of 21-40 years (60, 41.40%) in Nepal. A study done in Ahmadabad, India shown (29, 29.00%) of patients were aged between 31-45 years [22]. Sex-wise, female patients (112, 57.73%) constituted a larger group in our study. Similarly, in other study of Nepal larger female group was also found [19]. A researcher reported an increased incidence in male (77.7%) as well as a higher prevalence rate among elderly 61-80 years (43.92%) [23]. Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains has been reported world-wide [24-27] and this is a serious therapeutic problem in the management of disease due to these organisms. The resistance profiles of P. aeruginosa to a panel of fifteen anti-microbial agents tested varied among the isolates investigated. In the present study, an overall high rate of resistance was observed to chloramphenicol, ceftriaxone, Cefepime, Cefoperazone-Salbactum and co-trimoxazole. The maximum resistant isolates were observed in age group 21 -40 and >60.One striking feature in this study was that all the P. aeruginosa isolates were found to be sensitive to imipenem whereas meropenem showed 7.73% resistance. This may be due to the restricted use of imipenem in this hospital. Based on a study, this is consistent with a report published in 2013 in Nepal [19] and in 2002 in Mangalore, India but other studies have showed varying degrees of resistance to imipenem in recent years [27-31]. Gentamicin and Amikacin shows 46.91%, 19.07% resistance respectively.

High resistance to aminoglycosides have been reported in studies done in Nepal, India, Bangladesh, Turkey and Malaysia [32]. In our study fluoroquinolones such as ciprofloxacin was 29.38% resistance observed. Similarly rates of resistance to fluoroquinolones such as ciprofloxacin (33%) have been reported in Nepal,other report from Nepal showed 51.72% resistance of ciprofloxacin, the higher rates of resistance to ciprofloxacin (40.5%) have been reported in a study done in North Kerala, India, and 92% resistance was shown in a study from Malaysia [33]. Piperacillin alone tested showed a resistance rate of 45.36% in this study. Based on research, similar resistance rates for piperacillin 44% and 55.17% have been shown from Nepal reported 54.66% resistance for piperacillin [34]. Relatively low piperacillin resistance (11.5%) have been reported in a study from Saudi Arabia [35].

In this study the beta-lactam / beta-lactamase inhibitor drug Piperacillin–Tazobactum, Aztreonam, Ticarcillin- Clavulanic acid, cefoperazone-sulbactum showed resistance of 18.04%, 31.96%, 39.18% and 54.12% respectively, indicating beta-lactamase inhibitor markedly expands the spectrum of activity of beta-lactams, 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 [36]. In a study done in Kathmandu, Nepal, 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 [37]. In two study of Nepal cefoperazone-sulbactum showed resistance of 16.05%.

The rate of resistance for the anti-folate drug co-trimoxazole in the present study was 53.02%.similar resistance 51.72% reported from Nepal. In contrast, a study done in Bangladesh showed rate of resistance for co-trimoxazole to be 93.5% in wound swab and pus isolates of P. aeruginosa while a Nigerian study 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 69.56%, cefepime57.22% and ceftazidime 31.44%. which is more or less similar to studies done in Nepal, India and Bangladesh [38]. This study revealed that chloramphenicol have the highest rate of

resistance 74.23% 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. In other study of Nepal 72.41% of resistance rate of chloramphenicol was also reported. A study done in Kano, Nigeria demonstrated a much higher rate of resistance (97.7%) of P. aeruginosa isolates to chloramphenicol [39]. Another significant finding in this study was the rate of multi-drug resistance to be 24.74%.A MDR rate of 19.6% ,20.69%, 89.4%, and100% among P.aeruginosa isolates have been reported from studies conducted in Malaysia, Nepal and Iran respectively.

5. CONCLUSION

Results of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the P. aeruginosa isolates. The emergence of resistance to many drugs, such as flouroquinolones and third generation cephalosoprins, semi-synthetic penicillin with beta-lactamase inhibitors, in P.aeruginosa strains is a cause of great concern not only at local and regional level, but also in a national and international scale. The culture of antimicrobial abuse needs to be soon stopped. Continuous surveillance of multidrug resistant strains is very important to know the changing antibiotic susceptibility patterns from time to time. A network of laboratories for real time monitoring of antibiotic resistance of P. aeruginosa and timely dissemination of such information to the clinicians for modification of treatment strategy are urgently necessary to prevent the emergence of multi-drug resistant strains of P. aeruginosa.

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