Original article

Recent trend of multi-drug resistance in Pseudomonas aeruginosa

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Abstract:

Objective: Continuous emergence of resistance among Pseudomonas aeruginosa strains to common antimicrobial drugs have been documented world-wide. This study investigated the recent trend of antimicrobial resistance patterns of P. aeruginosa among the patients in mid & far western region of Nepal. ***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 January 2014. 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. The disc diffusion test was used to determined 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 the patients of 21-40 years age group, which was statistically significant (P<0.05) compared to the other age groups. ***Conclusions:*** The study revealed the presence of drug resistant strains of P. aeruginosa in Nepal. High levels of antibiotic resistance of many of the isolates might be due to antibiotic abuse. Therefore, we recommend judicious use of antibiotics by the physicians to curb the increasing multi-drug resistance of P. aeruginosa strains in Nepal.

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

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Introduction:

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[1]. 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 multi-drug resistance in health care settings[2,3]. 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[4] and amino-glycoside modifying enzymes[5] via horizontal gene transfer and mutation of chromosomal genes (target site, efflux mutations) are the target of the fluoro-

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quinolones particularly ciprofloxacin[6]. This pathogen is intrinsically resistant to most antibiotics such as, chloramphenicol, tetracycline, macrolides, trimethoprim–sulfamethoxazole, and rifampin[7]. 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[8,9].Biofilm formation in P.aeruginosa, particularly in the case of pulmonary infections in patients with cystic fibrosis, contribute to its resistance to antimicrobial agents[10]. 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[11]. Increase in the frequency of multi-drug resistant (MDR) strains of P.aeruginosa has severely limited the availability of therapeutic options. Data on antimicrobial susceptibility profiles of P. aeruginosa is limited in Nepal[12,13]. 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.

Material And Methods:

Study background and subjects

This was a Retrospective study conducted on 917 Nepalese patients, attending outpatients and inpatients departments of Nepalgunj Medical College and teaching Hospital, Banke, Nepal, between September 2011 and January 2014.

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[14]

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)[15], 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.

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.

Results:

194 strains of P. aeruginosa were isolated and identified 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%) of P. aeruginosa 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)

Abbreviations: - CV – central venous; BAL – bronchoalveolar lavage

Antimicrobial susceptibility patterns

Antimicrobial susceptibility patterns of P.aeruginosa varied markedly with the antibiotic tested. P.aeruginosa isolates showed maximum resistance to chlo-

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)

ramphenicol (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.

Table 3: Susceptibility 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 P. aeruginosa isolates

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	

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. 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 chander A.[16], in India reported by Mohanasoundaram[17] and Arora et al.[18]. Most of them in older age group of 21-40 years (88, 45.36%) and elderly age group of > 60 years (51, 26.29%). This could be 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[16] A study done in Ahmadabad, India, showing 29% prevalence of pseudomonas infection between the age groups 31-45 years[19] . 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 [16. Ahmed et al.[20] reported an increased incidence in male (77.7%) as well as a higher prevalence rate among elderly 61-80 years (43.92%). Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains has been reported world-wide[21-24] 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 antimicrobial 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 imipen-

em whereas meropenem showed 7.73% resistance. This may be due to the restricted use of imipenem in this hospital. This is consistent with a report published in 2013 in Nepal[16].

Results of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the P. aeruginosa isolates and the observed rate of multi-drug resistance was 24.74% which was more or less similar to the other recent study conducted in Nepal[13,16] and Malaysia[25]. 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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