

Changing trends in antibiotic susceptibility pattern among clinical isolates of *Pseudomonas* species in a tertiary care hospital in Nepal

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ABSTRACT

Background

Pseudomonas spp is notoriously known to be important agent of hospital as well as community acquired infections. These organisms are resistant to many antibiotics by intrinsic and acquired mechanisms making infections difficult to treat. Regular surveillance of infection and antibiotic resistance patterns is necessary for selection of appropriate antibiotics for treatment.

Methods

Non repeating *Pseudomonas* spp isolated from all clinical samples during the year 2017-2019 were included in the study. Identification of Isolates were done by standard conventional tests and antibiotics sensitivity test was done by Kirby-Bauer disk diffusion method following the CLSI guidelines, 2017.

Results

A total of 42,545 specimens comprising of sputum, blood, pus, urine, body fluids and endotracheal tubes were processed during the entire 3 year period. There was significant increase (p-value <0.05) in yearly isolation rate of *Pseudomonas* spp i.e. 141(7.95%), 197(12.14%) and 303(15.69%) isolates in the year 2017, 2018, and 2019 respectively. Resistance rates of the isolates to cefoparazone sulbactam, Piperacillin Tazobactam, and Tobramycin was significantly decreasing (p-value <0.05). Overall resistance to ceftazidime (66.13%) was alarming. Decrease in MDR isolates were statistically significant over 3 years e.g. 32(22.69%), 31(15.73%) and 37(12.21%) isolates in the year 2017, 2018 and 2019 respectively (p-value <0.05).

Conclusion

The rate of infection of *Pseudomonas* spp is significantly increasing. There was gradual decrease in number of resistant isolates and MDR isolates over the period starting from 2017 to 2019 which is a favorable trend. The possible factors playing role have to be further studied, identified and promoted.

KeyWords

Antibiotic resistance, *Pseudomonas* spp

Introduction

Pseudomonas species, member of the family Pseudomonadaceae, is an aerobic, gram negative bacilli. It is saprophyte, commonly found in soil, and water, but have emerged as important human pathogen. *Pseudomonas spp* is a notorious nosocomial pathogen¹. It is gaining importance as a cause of community acquired pneumonia, blood stream infection, wound infections, including those of burn wounds. Pathogenicity of *Pseudomonas* is associated with numerous virulence factors like liposaccharide, type VI secretion system, biofilm production, exotoxin A, proteases and alginate production, etc.²

Antibiotic therapy of *Pseudomonas* infections is challenging as these are intrinsically resistant to a number of antibiotics, carry genes for antibiotic resistance and have adapted multiple mechanisms of antibiotic resistance³. Even, resistance among *Pseudomonas spp* to carbapenem, the last resort antibiobiotic, is on steady increase. This trait of intrinsic and acquired antibiotic resistance has narrowed down the choice of antibiotics and clinician has to rely on more toxic and more expensive antibiotics. In context to Nepal, authors like Baral et al, Yadav et al have reported MDR *Pseudomonas* as high as 60.1%, 73.3% respectively and are associated with prolonged hospital stay and increased mortality which is a serious situation^{4,5}. Such emergence of multidrug resistance (MDR), and carbapenem resistance are of primary concern globally, as these factors are importantly related to prolonged hospital stay, increased morbidity and mortality⁶.

The rate of infection by *Pseudomonas spp* vary in different clinical settings. There is a need of proper surveillance on occurrence of infection by *Pseudomonas* and their pattern of antibiotic susceptibility. This will guide clinicians to choose the best antibiotics for empirical therapy without delay. This study was, therefore, aimed to find out the infection rate due to *Pseudomonas spp* and determine the changing trend in antibiotic resistance pattern among the isolates.

Method

Setting and Design

This is an observational and descriptive study conducted at the tertiary care hospital located in

Nepal. The culture and sensitivity data of non-repeating isolates of *Pseudomonas spp* for 3 consecutive years (2017, 2018 and 2019) formed the material of the study.

Isolation and identification of Organism

All the clinical samples obtained in microbiological laboratory were processed by standard microbiological techniques. The specimens had been cultured on Chocolate agar, 5% Sheep Blood agar, MacConkey agar plates and biphasic media (Hi-Media, India) based on type of clinical samples. Isolates were identified as *Pseudomonas spp* by standard, conventional phenotypic methods using Triple sugar iron agar (TSI), Sulphur Indole Motility agar (SIM), Christensen's urease agar, Simmons citrate agar, oxidase strip and pigment production⁷.

Antibiotic sensitivity testing

Antibiotic sensitivity tests against Gentamicin (10 µg), Amikacin (30 µg), Tobramycin (10 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Piperacilin-Tazobactam (100/10 µg), Cefoperazone-sulbactam (75/10 µg), Colistin (10 µg), Polymixin B (300 units), and Imipenem (10 µg) (Hi-Media, India) were performed by Kirby-Bauer disk diffusion method and results were recorded and Interpreted in accordance with the criteria laid down by Clinical Laboratory Standard Institute (CLSI), 2017⁸. As, antibiotics like colistin, cefoperazone-sulbactam and polymixin B don't have CLSI interpretative criteria for disk diffusion antibiotic susceptibility test, guidelines given by Hi-Media laboratories was used for interpretation⁹. Multidrug resistance was defined according to the current guidelines given jointly by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC)¹⁰.

Statistical Analysis

Data were analyzed by SPSS version 20. Categorical variables were calculated as percentages. Chisquare test was used to compare two groups. All p values <0.05 were considered as statistically significant.

Results

A total of 42,545 specimens comprising of sputum, blood, pus, urine, body fluids and endotracheal tubes were processed during the entire three year

period. Of these, 13675 specimens were processed in the year 2017, 13780 specimens in 2018, and 15090 in 2019, which yielded 1772, 1622 and 1930 gram negative bacilli respectively. Out of these, *Pseudomonas spp* accounted for 141 (7.95%), isolates in the year 2017, 197 (12.14%) isolates in 2018, and 303 (15.69%) in 2019. The isolation rate of *Pseudomonas spp* was found to have significantly increased (p-value <0.05) over the years.

Table 1. Gender, age and season wise distribution of *Pseudomonas spp*.

	Year of isolation			
Gender	2017	2018	2019	Total
Male	90 (63.83%)	125 (63.45%)	170 (56.1%)	385 (60.06%)
Female	51 (36.17%)	72 (36.55%)	133 (43.89%)	256 (39.93%)
Total	141	197	303	641
Age				
0-14 year	24	44	75	143 (22.3%)
15-60 year	74	91	139	304 (47.42%)
>60 year	43	62	89	194 (30.26%)
Total	141	197	303	641
Season				
Rainy season	98	102	171	371 (57.87%)
Other season	43	95	132	270 (42.12%)
Total	141	197	303	641

Distribution of *Pseudomonas spp* according to gender, age and season is shown in table no 1. It was found that, in all 3 years, numbers of males suffering from *Pseudomonas* infection were significantly higher compared to females. *Pseudomonas spp* was isolated from subjects belonging to all age groups starting from neonates to individuals as old as or even older than 80 years. It was noticed that majority of *Pseudomonas* i.e.

47.42% (304/641) were isolated from adults (15-60 years of age), while 194 (30.26%) and 143 (22.30%) were from individuals of old age group (>60 years) and those of younger age group (0-14 years) respectively.

Pseudomonas spp isolation rate was more during the rainy season i.e. 371 (57.8%) when compared with other months 270 (42.12%) (p-value >0.05).

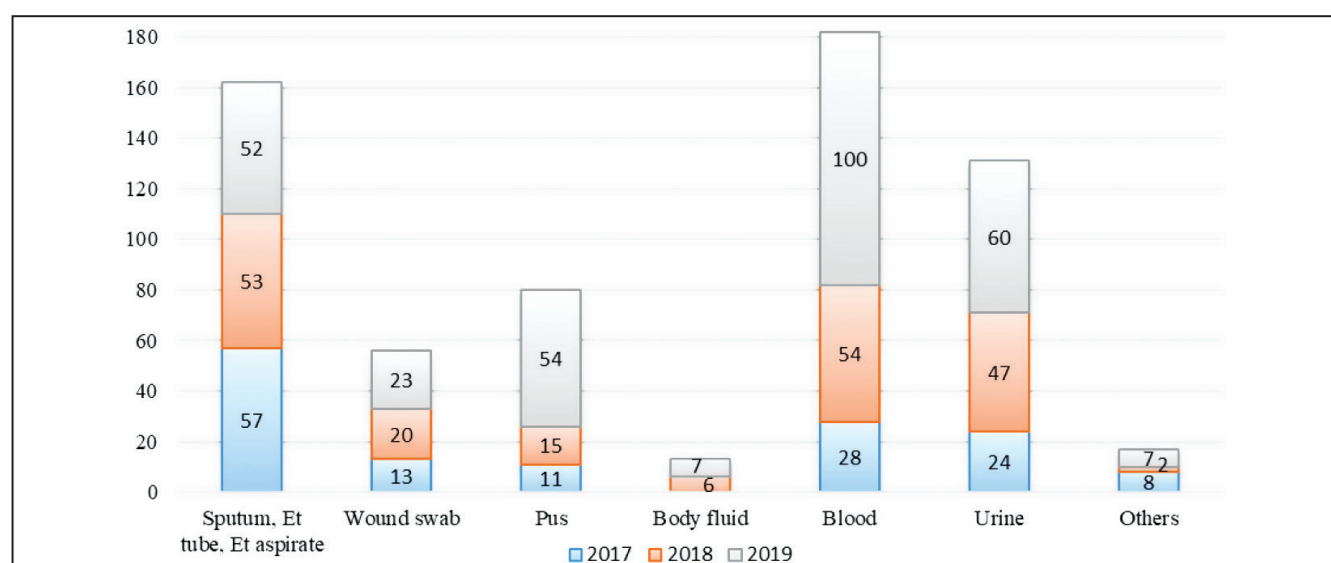


Figure 1. Isolation of *Pseudomonas spp* from different samples

Maximum number of isolates were from blood, followed by respiratory samples and urine samples (figure 1) though these differences were not significant statistically.

The antibiotic susceptibility profile revealed gradually decreasing number of resistant isolates over the period starting from 2017 to 2019 except for imipenem to which the organisms exhibited an increasing resistance trend. The percentage resistance of isolates against imipenem showed slow but gradual increase (6.20%, 8.05% and 8.40% respectively in the years 2017, 2018 and 2019) over the years. The number of MDR isolates were 32 (22.69%), 31 (15.73%) and 37 (12.21%) in the years 2017, 2018 and 2019 respectively (p-value <0.05).

Table 2. Antibiotic resistance pattern in 2017, 2018 and 2019

	2017 (R%)	2018 (R%)	2019 (R%)	p-value
Ciprofloxacin	25.20	11.63	15.85	>0.05
Cefoperazone sulbactam	43.18	24.28	13.26	<0.05
Ceftazidime	58.33	62.07	39.39	>0.05
Piperacillin-tazobactam	48.65	35.63	11.88	<0.05
Gentamicin	26.47	16.67	13.43	>0.05
Tobramycin	38.46	8.33	11.83	<0.05
Amikacin	16.24	13.14	6.25	>0.05
Imipenem	6.20	8.05	8.40	>0.05
Colistin	3.30	0.67	0.60	>0.05
Polymixin B	0.00	0.00	0.00	-
Multidrug resistant isolates (MDR)	32 (22.69%)	31 (15.73%)	37 (12.21%)	<0.05

Discussion

Pseudomonas spp is an important agent of hospital acquired as well as community acquired infections. It causes bacteremia, wound infection, respiratory tract infection, urinary tract infection etc. The outcome of infections is also associated with increased morbidity and mortality.

In this study, increase in number of *Pseudomonas spp* infection in consecutive years was observed. In all 3 years, total number of males affected were 19,754 and females were 21,228. Out of these, number of males whose specimens yielded *Pseudomonas* on culture were 385, a figure that was higher than the comparable figure among the females (n=256). Though this overall difference was not a significant one (p-value>0.05), yet, it was an interesting observation of male predominance of getting disease. Yadav et al have documented

similar observation of male patients getting more *Pseudomonas spp* infections when compared to females⁵. The reason could be multifactorial but the important factor could be males were found to be less compliant to hand hygiene practice in terms of timing, use of soap, and hand washing behavior as compared to females^{11,12}. Moreover, other factors like cultural, hormonal, immune status could also be related to male predominance of not only getting the disease, but also landing up with poor clinical outcome as well¹³.

In the present study, *Pseudomonas spp* was isolated starting from neonates to individuals as old as or >80 years of age. It was observed that majority of *Pseudomonas spp* (304; 47.42%) were obtained from patients of the adult age group, whereas there were comparatively lesser numbers

(194; 30.26%) from those belonging to the old age group. In spite of the fact that, the older age group category needs to be managed with optimum care as these individuals are often vulnerable to other co-morbid conditions and are prone to infections as a result of seasonal climatic variations¹⁴.

In Pokhara, rainy season extends from May to October¹⁵. As, *Pseudomonas spp* is a saprophytic organism, its dissemination is expected to be more in rainy season causing high number of infectious episodes¹⁶. In our study, 24,298 cultures were performed in rainy season and 17,355 during the non-rainy season. It was of note that *Pseudomonas spp* isolation rate was higher in the rainy months i.e. 371 (57.8%) when compared to the dry parts of the year (270; 42.12%). Though this difference was not statistically significant, (p-value >0.05), yet it was a notable difference which might have implications for the population in the community, especially for those in the old and much older age group.

Antibiotic resistance is the most feared aspect of bacterial infections. WHO warned it as a serious global situation. It was interesting and good to note that the dominance of antibiotic resistant *Pseudomonas spp* was in a decreasing trend (Table 2). Even the Multidrug resistant pseudomonas was on a decreasing trend (p-value <0.05), as shown by the present study. This declining trend in antibiotic resistance exhibited a statistically significant difference (p-value <0.05), especially with respect to antibiotics, such as cefoparazone sulbactam, piperacillin-tazobactam, tobramycin (table 2). This observation of ours was amply supported by the findings shown elsewhere¹⁷.

However, resistance rates seen in our study against ceftazidime, an antipseudomonal cephalosporin, was quite high varying between 58.33% in 2017 to 62.07% in 2018. This seems to be alarming. According to recent studies from Nepal as well as from other countries, cephalosporins were one of the most widely used antibiotics for many deep seated infections and other infections, as clinician prescribed and as selfused antibiotic^{18,19}.

Thus, our results could possibly implicate the rational use of cephalosporins in order to avoid rapid development of resistance to this drug by notorious bugs like *Pseudomonas*. Quinolone use and development of multidrug resistance

were previously found to be interrelated²⁰. In this context, it is important to note that ciprofloxacin is increasingly being used as a prescribed as well as a selfmedicated antibiotic^{19,21}. Beside these, resistance to β -lactam antibiotics is governed by common mechanism ie β -lactamase production which can be transferred within same or different bacterial species which might have contributed in higher resistance to β -lactam antibiotics.

Progressive increase in the resistance to imipenem, one of the last resort antibiotics, was noticed. However, it was seen in 7.55% of the isolates only, suggesting it could still be still a reliable antibiotic for treatment of multidrug resistant *Pseudomonas spp* infection. In contrast to our observation, authors within Nepal have mixed observation e.g. Gyawali et al in 2020 had observed 32.1% isolates resistant to Meropenem while Chander et al in 2018 had observed all the *Pseudomonas* isolates were sensitive to Imipenem^{22,23}. However, our isolates were mostly sensitive to WHO reserved antibiotics colistin and polymyxin B. The overall resistance to colistin was only 1.52% and the resistance rate was decreasing over the years. All the *Pseudomonas spp* isolated were sensitive to polymyxin B. It is a heartening observation. Colistin (polymyxin E) and polymyxin B belong to same group polymyxin with similar structure, mechanism of action and even cross resistance. Different authors from Nepal and from other countries had reported colistin resistance varying from as low as 2% to as high as 21.3%^{24,25}. Despite their systemic toxicity colistin and polymyxin B are regarded as the ultimate therapeutic option for critically ill patients due to gram negative bacterial infection²⁶.

As per the present data, less than 20% *Pseudomonas spp* were found to be resistant to ciprofloxacin and aminoglycosides like gentamicin, tobramycin, amikacin. Amikacin was found best choice with resistance rate 11.87%. The resistance rate of these antibiotics has de-creased in 2019 when compared with 2017 suggesting these WHO watch group of anti-biotics are still better option for management of infection by *Pseudomonas spp*.

Ourantibioticsusceptibilitypatternof*Pseudomonas* isolates shows increase in susceptibility in recent years to plenty of antibiotics tested. Such a trend is favorable. In our study, factors that might have played the role in the increase in susceptibility

pattern to certain antibiotics were not looked into. Even then the important observations we made certainly pave the way for further research.

Conclusion

The rate of infection of *Pseudomonas* spp is significantly increasing. Emergence of antibiotic resistance is multifactorial, similarly decrease in antibiotic resistance trait could be multifactorial. The resistance to cephalosporin was alarming. There was a significant decreasing trend in the resistance rates of the isolates to cefoparazone sulbactam, piperacillin-tazobactam, and tobramycin. In this era of antibiotic resistance, this is a favorable trend. The possible factors playing role have to be further studied, identified and promoted. Judicious use of these antibiotics should be continued.

Conflict Of Interest

None declared

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