Original Research Article

Pseudomonas aeruginosa: distribution and antibiotic profile of one of the ESKAPE pathogen

Karthick M.[1]

[1]Dr. Mowna Karthick, Lab Specialist, Dr. Sulaiman Al Habib Hospital, Riyadh, Saudi Arabia.

Corresponding Author: Dr. Mowna Karthick, Lab Specialist, Dr. Sulaiman Al Habib Hospital, Riyadh, Saudi Arabia. Email: drmowna@gmail.com

Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic gram-negative pathogen known for its ingenious mode of infection. The management of infections with Pseudomonas has been quite a challenge. The bacteria have intrinsic resistance against most of the routine antibiotics. Such a scenario places the health care delivery system at a challenging end point with very minimal options of care and increased rates of morbidity and mortality. This study was done to assess the pattern of presentation of Pseudomonas aeruginosa in hospital settings. **Methods:** This cross-sectional study was carried out among 280 specimens which were isolated for a period of 17 months in our tertiary care hospital. The blood culture bottles were placed in Bac T/ Alert 3 D and the positive culture bottle was processed by Grams stain and in routine bacteriological media for inoculation and incubated. Pseudomonas aeruginosa organisms isolated from all the clinical samples were subjected for determining the identification and antibiotic susceptibility profile by VITEK 2 and manual methods. **Results:** A total of 280 samples were analyzed in this study. The background characteristics of the specimens analyzed is given in table 1. In this study, majority of the samples were from inpatients (71.4%). Among the total samples, most specimens were urine samples (37.5%), followed by pus (23.1%). The organisms showed high sensitivity to Amikacin, Ceftazidime and Gentamycin (98%). **Conclusion:** From the present findings, we understand that Amikacin and Gentamycin can be the proposed drug of choice for severe infections with Pseudomonas aeruginosa.

Keywords: Antimicrobial resistance, Carbepenems, Fluoroquinolones, Pseudomonas aeruginosa

Introduction

Pseudomonas aeruginosa is an opportunistic gram-negative pathogen known for its ingenious mode of infection. The mechanism of action of the bacteria is by production of exotoxins inside the host body which blocks protein synthesis by binding to the coenzyme Nicotinamide Adenine Dinucleotide (NAD) followed by the release of nicotinamide, which significantly damages oxidation reduction reactions and protein synthesis. It also invades the immune system by inactivating the complement cascade [1]. Pseudomonas is capable of surviving in a variety of environmental conditions as it requires minimum oxygen and nutrition.

It has been strongly associated with severe life-threatening infections in immunocompromised individuals, apart from wound infections in burns patients and other secondary infections in patients with diabetes mellitus, etc [2]. The management of infections with *Pseudomonas* has been quite a challenge.

Manuscript received: 20th August 2019
Reviewed: 30th August 2019
Author Corrected: 7th September 2019
Accepted for Publication: 11th September 2019

The bacteria have intrinsic resistance against most of the routine antibiotics. For many years, Carbapenem was considered as the drug of choice, however inappropriate use and misuse of the drug has resulted in drug resistance in clinical settings [3]. The mechanism of resistance is either plasmid mediated or chromosome mediated. Plasmid mediated resistance act by hydrolyzing imipenem and meropenem [4]. Chromosome mediated resistance is achieved by the loss of porin and overexpression of efflux pumps [5]. In such scenario Carbepenem resistant bacteria are generally resistant to most antimicrobials except polymyxins and tigecyclines [6]. Considering the high rate of toxicity and suboptimal pharmacokinetics, the use of polymyxins and tigecyclines are restricted [7].

The existing resistance mechanisms has resulted in the formulation of multidrug resistance *P.aeruginosa* phenotype which is defined as a bacterium which is resistant to antimicrobial agents which are included in three or more antipseudomonal anti-microbial classes namely carbepenem, fluoroquinolones, penicillins/

cephalosporines aminoglycosides [8]. Such a scenario places the health care delivery system at a challenging end point with very minimal options of care and increased rates of morbidity and mortality.

Objectives

This study was carried out to-

1. Observe the pattern of distribution of *P. aeruginosa* among the clinical specimens
2. Analyze the antibiotic sensitivity pattern in the isolates.

Methodology

Study setting: This study was carried out in the Microbiology laboratory of our tertiary care hospital in Riyadh, Saudi Arabia.

Duration of study: The study was carried out for a period of 17 months from January 2017 to May 2018.

Type of study: Cross sectional study

Sampling methods: The study samples were selected by convenient sampling

Sample size calculation: Based on intensive literature review, the lowest prevalence of *Pseudomonas* infections was observed to be 7-10% in urinary tract infections. [9] Therefore, assuming 7% as the lowest prevalence, at 95% confidence limits and 3% absolute precision, the sample size was calculated as 277.76 and was rounded off to 280.

Inclusion criteria: All the microbiological samples received during this period from both outpatients and inpatients were examined. Cultures positive for *Pseudomonas aeruginosa* were included in the study.

Exclusion criteria: All Samples which were repeated from the same patient and same site of collection were excluded.

Data collection procedure: The blood culture bottles were placed in Bac T/ Alert 3 D and the positive culture bottle was processed by Grams stain and in routine bacteriological media for inoculation and incubated. All other samples were inoculated in the respective media and methods as per standard guidelines and incubated.

Pseudomonas aeruginosa organisms isolated from all the clinical samples were subjected for determining the identification and antibiotic susceptibility profile by VITEK 2 and manual methods.

Data analysis: Data was entered and analyzed using Microsoft Excel spreadsheet 2010. The distribution and pattern of sensitivity of *P. aeruginosa* was expressed as percentages.

Ethical consideration and permission: Approval was obtained from the Institutional Ethics Committee prior to the commencement of the study. Each participant was explained in detail about the study and informed consent was obtained prior to the sample collection.

Results

A total of 280 samples were analyzed in this study. The background characteristics of the specimens analyzed is given in table 1. In this study, majority of the samples were from inpatients (71.4%). Among the total samples, most specimens were urine samples (37.5%), followed by pus (23.1%).

Table-1: Background characteristics of the specimens analyzed:

S. No.	Characteristics	Frequency (N=280)	Percentage (%)
1	**Source of specimens**		
	Outpatients	80	28.6
	Inpatients	200	71.4
2	**Type of specimen**		
	Pus	65	23.1
	Urine	105	37.5
	Blood	15	5.4
	Tracheal aspiration	17	6.1
	Ear discharge	11	3.9
	Sputum	61	21.8
	Pleural fluid	3	1.1
	Conjunctival smear	3	1.1

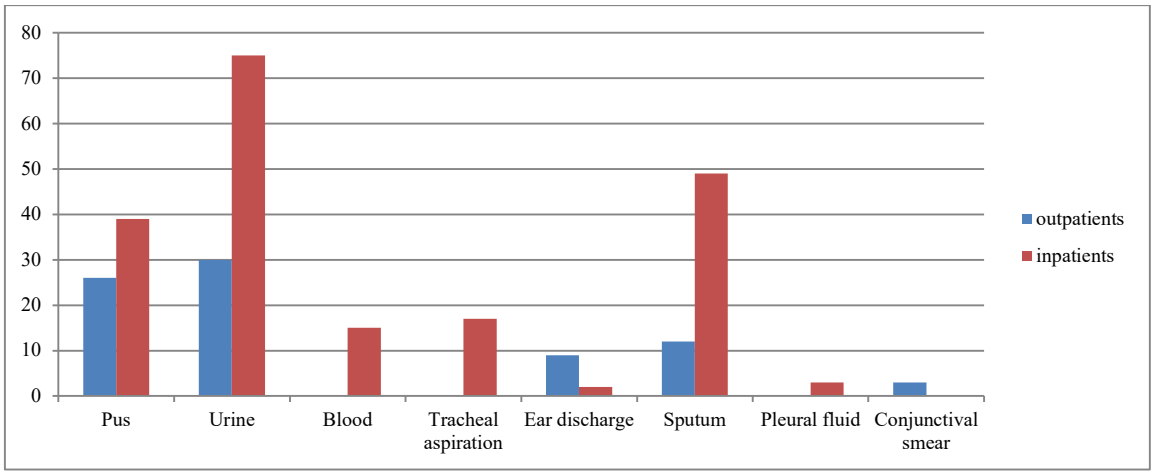

Figure-1: Comparison of isolates between outpatients and inpatients

Table-2: Antibiotic sensitivity pattern among the isolates:

S. No	Antibiotic	Outpatients (%)	Inpatients (%)
1	Amikacin	98	95
2	Gentamycin	97	92
3	Ceftazidime	98	85
4	Cefepime	98	87
5	Ciprofloxacin	83	77
6	Levofloxacin	83	77
7	Piperacillin+ Tazobactam	94	87
8	Imipenem	93	84
9	Meropenem	94	84
10	Aztreonam	88	78

The comparison of the isolates between outpatients and inpatients is given in figure 1. Among the inpatients, the majority of the samples were urine, followed by pus and sputum. Among the inpatients, the highest samples were from urine and pus.

The results of antibiotic sensitivity testing are given in table 2. As far as the outpatient samples were analyzed 98% sensitivity was found with Amikacin, Ceftazidime and Cefipime. With inpatient samples, sensitivity was found to be highest (95%) with Amikacin, followed by Gentamycin (92%).

Discussion

Pseudomonas aeruginosa is one of the most common gram-negative infections resulting in multidrug resistance and hospital acquired infections. Apart from urinary tract infections, several practices including prolonged hospital stay, ICU admissions, tracheal intubations, etc are considered as proven risk factors for *P.aeruginosa* infections [10]. Our study was done to evaluate the characteristic profile of *P.aeruginosa* infections in hospital settings. We observed that majority of the isolates were from inpatients, especially with urine samples. Djordjevic Z et al in his study reported that 7-10% of all urinary tract infections are caused by *P.aeruginosa* [9]. *Psuedomonas* causing urinary tract infections are often a resultant of cathether associated urinary tract infections (CAUTI) which accounts for 40% of all nosocomial infections [11]. These infections are associated with a high 30-day mortality of 17.7%. Moreover, the sensitivity of these bacteria towards antibiotics were as low as 69% for piperacillin and tazobactam [12].

Our study also demonstrated a higher antibiotic sensitivity to Amikacin for both outpatients and inpatients and Gentamycin for inpatient infections. A

study done by Golia S et al also reported similar findings [13]. Another study done by Javiya VA et al demonstrated high rates of sensitivity to Cefipime, which correlated with our findings in outpatient samples [14]. The antibiotic resistance observed in Pseudomonas could be due to synergy between type 1AmpC beta-lactamase and low outer membrane permeability [15].

In our study, high level of resistance was observed with fluoroquinolones namely ciprofloxacins and levofloxacin. Moreover, the presence of MDR rate (resistance to three antipseudomonals) was quite low (18%). A study done by Golia et al also reported similar finding of 10% [13].

The mechanism of resistance to quinolones proposed were due to point mutations in DNA gyrase (gyrA and gyrB) and topoisomerase IV(parC and pare genes), presence of transferable plasmid mediated quinolone resistance and mutations in genes regulating efflux pumps [16]. Another study by Lee YJ et al also reported increased resistance to parenteral use of quinolones for the treatment of *Pseudomonas* infections [17].

The resistance mechanism exhibited by *Pseudomonas* is through decreased permeability, expression of efflux systems, increased production of antibiotic inactivating enzymes and target modifications. Multidrug resistance is said to occur when a combination of more than one such mechanisms happen [18]. Although our study demonstrated resistance to fluoroquinolones only, MDR resistance is significant when there is resistance to more than one antimicrobial agent in more than three categories out of eight categories including amino-glycosides, carbapenems, cephalosporins, penicillin with beta lactamase inhibitors, monobactams, fluoro-quinolones, fosfomycins and polymyxins [19].

The results of our study were further substantiated by molecular studies done by Lila G et al [20]. Although their study demonstrated further resistance to more than one category of antibiotics, genotypic analysis identified significant clusters of Pulse Field Gel Electrophoresis (PFGE) patterns, which were identifiable with cross contamination of specimens isolated from Intensive Care Units (ICU), post-ICU units, neurosurgery and plastic surgery units. Certain other PFGE strains were also isolated from pulmonology, abdomen surgery and orthopedics unit.

This significantly correlates with the impact of hospital acquired infections on *Pseudomonas* resistance. Further analysis at the molecular level showed the presence of point mutations in the MDR phenotype resulting in multidrug resistance in *Pseudomonas* infections [21].

Considering the fact that hospital acquired infections contribute majorly to the antibiotic resistance in Pseudomonas infections, it is highly pertinent that stringent infection control measures are in place in order to combat these infections. There is an imminent need to develop standard protocols for case management, especially in ICU settings, so as to minimize the vulnerability towards infections. In addition, the judicious and optimized use of antibiotics is crucial for preventing antibiotic resistance. Periodic audit of prescriptions and capacity strengthening for primary care physicians should be in place to combat the menace of antibiotic resistance, so as to minimize morbidity and mortality of *P. aeruginosa* infections in the future.

Conclusion

The present study emphasized on the increased prevalence of urinary tract infections, predominantly nosocomial, resulting in Pseudomonas infections. Though our study reported a high sensitivity to Amikacin, we observed a high level of resistance to standard antipseudomonal agents like quinolones and carbepenems. There is a need for further research in exploring the mechanism of antibiotic resistance at the genetic and molecular level to give a better under-standing on the prudency of antibiotic use. From the present findings, we understand that Amikacin and Gentamycin can be the proposed drug of choice for severe infections with Pseudomonas aeruginosa.

Limitation: Our study has highlighted the profile of presentation of bacteria with respect to specimen isolates and antibiotic resistance. An in-depth analysis of the clinicopathological conditions and the infection profile of the study participants could have helped substantiate the magnitude of the infectious nature of the bacteria.

What the study adds to the existing knowledge?

Although it is an established fact that urinary tract infections are predominantly caused by gram negative organisms, the present study has elicited the veracity of the infection spread through hospital sources. Further, this study has provided the basis for evaluating the antibiotic resistance to standard antipseudomonal agents, thereby focusing the need for molecular studies to evaluate antibiotic susceptibility for Pseudomonas.

Declaration

Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes
Ethical approval – Obtained

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How to cite this article?

Karthick M. *Pseudomonas aeruginosa*: distribution and antibiotic profile of one of the ESKAPE pathogen. Trop J Path Micro 2019;5(9):678-683.doi:10.17511/jopm.2019.i09.10.

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