

Original Article



Antibiotic resistance pattern in *Pseudomonas aeruginosa* isolated from a private Medical College Hospital

Mahmuda Siddiqua¹, Ahmed Nawsher Alam², Sonia Akter³, Reena Saad Ferdousi⁴

Abstract

Background: *Pseudomonas aeruginosa* is an aerobic, motile, gram negative rod that belongs to the family, Pseudomonadaceae. They are often multidrug resistant due to intrinsic and acquired determinants. Continued emergence of resistance among *P. aeruginosa* to common antimicrobial drugs has been reported world-wide. **Objectives:** This study investigated the antimicrobial resistance as well as susceptibility patterns of isolates of *P. aeruginosa* in clinical specimens. **Materials & Methods:** One hundred and thirty-eight isolates of *Pseudomonas aeruginosa* were obtained from 4489 different clinical specimens. Antimicrobial susceptibility pattern of each isolate was carried out by the Kirby- Bauer disk diffusion method as per guidelines of Clinical Laboratory Standard Institute (CLSI). **Results:** Majority of isolates of *P. aeruginosa* were obtained from specimens of wound swab 89 (64.5%), pus 18 (13.05%), and urine 17 (13.1%). The isolated pathogens showed high resistance (91% to 96%) to cotrimoxazole and cefuroxime. Resistance rates to cefepime, ceftriaxone, cefotaxime, and gentamicin varied from 47% to 88%. All the isolates were comparatively better susceptible to meropenem, ciprofloxacin, amikacin and imipenem ranges from 76% to 87%. **Conclusion:** The results confirmed the occurrence of drug resistance of *P. aeruginosa* to anti-pseudomonal drugs. Imipenem, amikacin, ciprofloxacin and meropenem were found to be the most effective antimicrobial drugs. Therefore, judicious and rational treatment prescription is needed by the physicians to limit the further spread of antimicrobial resistance among the *P. aeruginosa*.

Keywords: Antimicrobial resistance, *Pseudomonas aeruginosa*.

Date of received: 21. 11. 2017

Date of acceptance: 05. 01. 2018

Introduction

Pseudomonas aeruginosa is an aerobic, motile, gram negative rod that belongs to the family, Pseudomonadaceae.¹ They are often multidrug resistant due to intrinsic and acquired determinants.² It can survive with low levels of nutrients and grow in temperatures ranging from 4 to 42°C.^{3,4} It can cause urinary tract infections, respiratory infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections and gastrointestinal infections. It is responsible for a variety of systemic infections, particularly in patients with severe burns, and bed sore.^{5,6} *P. aeruginosa* also causes severe infections in patients with diseases including cancer, diabetes, cystic fibrosis, immune-suppression, and major surgery.⁷ The bacteria can colonize implanted devices, catheters, heart valves, ventilators or dental implants resulting in device-associated hospital acquired infections which are of major concern globally.⁸ The resistance rates of *P. aeruginosa* are

increasing globally creating a serious public health threat.⁹ *P. aeruginosa* is characterized by increased resistance to anti-pseudomonal agents.¹⁰ In vitro sensitivity tests are used as a guide for appropriate antimicrobial therapy prior to antibiotic treatments. Globally, the differences in the resistance rates of *P. aeruginosa* usually correlate with the prescription patterns of antibiotics by physicians, overuse of antibiotics, and the selective pressure of certain antibiotics.¹¹ Recent, studies showed that susceptibility of *P. aeruginosa* to currently used anti-pseudomonal agents, including β -lactams, aminoglycosides, and fluoro-quinolones is decreasing.¹² Since resistance of *P. aeruginosa* to carbapenems, piperacillin, and other highly active antibiotics has emerged and is increasing, it makes treatment of *P. aeruginosa* infections troublesome.¹⁰ The current study further examined the susceptibilities and resistances of *P. aeruginosa* to anti-pseudomonal drugs, isolated from various clinical specimens.

1. Associate Professor, Department of Microbiology, Ibn Sina Medical College, Dhaka, Bangladesh.

2. Principal Scientific Officer, Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh.

3. Assistant Professor, Department of Microbiology, Ibn Sina Medical College, Dhaka, Bangladesh.

4. Professor & Head, Department of Microbiology, Ibn Sina Medical College, Dhaka, Bangladesh.

Correspondent: Dr. Mahmuda Siddiqua, Associate Professor, Department of Microbiology, Ibn Sina Medical College, Dhaka, Bangladesh. Phone: +8801711300433, e-mail: mahmuda99@yahoo.com

Materials & Methods

This study was conducted at the Microbiology Department, Ibn Sina Medical College Hospital, Dhaka. Approximately, 4489 clinical samples were included from period of January 2015 to October 2016 having various samples like wound swab, pus, urine, sputum, ear swab, throat swab and catheter tips. The samples were received for routine culture and sensitivity test from both sexes with different ages having clinical infections. The samples were cultured onto MacConkey's, nutrient and blood agar plates, and then incubated at 37°C for overnight. After obtaining the pure culture, the growths were subjected to biochemical tests to identify the isolate. *P. aeruginosa* was identified by colonial morphology, gram stain, a positive oxidase, catalase, citrate, production of characteristic pigments and motility test. Finally, 138 samples were found positive for *P. aeruginosa*.

Antimicrobial susceptibility

Antimicrobial susceptibility was performed on Mueller-Hinton agar by standard disc diffusion method recommended by Clinical and Laboratory Standards Institute (CLSI).¹³ This was done by soaking a sterile swab in MacFarlands solution and then carefully swabbing the entire surface of Mueller-Hinton agar plates. The antibiotic discs were placed on the surface of the inoculated plates and gently pressed. The antibiotics used against the *P. aeruginosa* were as follows: amikacin (30µg), cefepime (30µg), cefotaxime (30µg), ceftazidime (30µg), cefuroxime (30µg) ceftriaxone (30µg), cotrimoxazole (25µg), ciprofloxacin (5µg), imipenem (10µg), meropenem (10µg), and gentamicin (10µg). The plates were incubated at 37°C for 18-24 hours. The diameter of inhibition zone was measured in millimeters and isolates were scored as sensitive or resistant by comparing with values recommended on standard.¹³

Results

Out of 4489 various clinical samples 138 *P. aeruginosa* were isolated. The rate of isolation of *P. aeruginosa* was 3.07%. Of these 138 isolates of *P. aeruginosa*, 75.36% were from males and 24.64% were from females (Table I). Among the positive isolates, 89 (64.49%) were from wound sample, which were the predominant source of specimens of *P. aeruginosa*, and followed by pus 18 (13.04%), urine 17 (12.31%). Isolates from sputum, ear swab, throat swab and catheter tips were very small quantity (Table II). In this study, *P. aeruginosa* showed high resistance to cotrimoxazole and cefuroxime 91 to 96%. Resistance rates to cefepime, ceftriaxone, cefotaxime, and gentamicin varied from 47% to 88%. All the isolates were comparatively better susceptible to meropenem, ciprofloxacin, amikacin and imipenem which range from 76 to 87%.

Table I: Gender-wise distribution of clinical isolates of *P. aeruginosa* (n = 138)

Gender	Number	Percent (%)
Male	104	75.36%
Female	34	24.64%

Table II: Distribution of *P. aeruginosa* among different clinical specimens

Ample	Frequency	Percent
Wound swab	89	64.49 %
Pus	18	13.04 %
Urine	17	12.31 %
Sputum	11	7.97 %
Ear swab	1	0.73 %
Throat swab	1	0.73 %
Cather tips	1	0.73 %

Discussion

Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains has been reported worldwide^{14,15} and this is a serious therapeutic problem in the management of disease due to these organisms. The resistance profiles of *P. aeruginosa* to the anti-microbial agents tested varied among the isolates investigated. Male preponderance (75.36%) was noted in this study. Similar observations were made by Andhale et. al., (76.66%)¹⁶ and Ahmed et. al., (77.7%).¹⁷ Outdoor activity, personal habits, nature of work and exposure to soil, water and other areas which are inhabited by organism could be the reason for male preponderance. But Chander et. al. found higher female patients (55.17%)¹⁸ in another study. In this study, the commonest sample was wound swab 64.49%, followed by pus 13.04%, urine 12.31% and sputum 7.97%. The distribution of specimens of *P. aeruginosa* may vary with each hospital as each hospital facility has a different environment associated with it. About 90% of the *P. aeruginosa* isolates of this study were obtained from only three important specimens e.g. wound swab, pus, and urine. Similar results had been obtained in India reported by Andhale et. al.,¹⁶ and Pathi et. al.,¹⁹ in different studies.

In our study, percentage of *P. aeruginosa* isolated was 3.07% which was close to the result reported in a study as 3.27%.²⁰ In Nepal, *P. aeruginosa* accounted for 1.20% of the total cultures.²¹ Study from Palestine showed prevalence of *P. aeruginosa* to be 9%.²² According to our study, *P. aeruginosa* can be best treated with imipenem with minimum resistance (13.24%), followed by amikacin (16.06%). Similar kind of results was seen in studies from countries like India,^{16,20,23} Nepal,¹⁹ Pakistan²⁴ and Poland²⁵ where highest sensitivity was observed against imipenem followed by amikacin. But the sensitivity in Poland showed almost 100%, where for South East Asian region it was < 90% and lower sensitivity seen in a study done in Iran²⁶ which is ranged from 55-57%. Sensitivity of gentamicin in this study was found 53.3% against *P. aeruginosa* while similar observation were found in two studies from India²³ and Nigeria²⁷ had sensitivity of 49% and 54.5% respectively. Higher sensitivity to gentamicin was also observed from India¹⁶ (70%) and Pakistan²⁴ (64.7%) and low sensitivity was observed in a European study from Poland²⁵ (12.3%). In this study, among the cephalosporins, cefotaxime was found most effective against *P. aeruginosa* with 41.9% sensitivity level, followed by ceftriaxone (36.5%), ceftazidime (31.16%) and cefepime (19.57%).

A study from Pakistan in 2015 also found similar kind of result about cephalosporin's sensitivity where cefotaxime was found most effective against *P. aeruginosa* with 54.5% sensitivity, followed by ceftazidime (43.9%), ceftriaxone (38.3%) and cefepime (36.1%).²⁴ *P. aeruginosa* strains in this study exhibited a high rate of resistance to the third generation cephalosporin drug-ceftriaxone (63.5%). A much higher resistance to ceftriaxone of 75%, 86% and 93.9% had been reported in studies done in India²⁸, Bangladesh²⁹ and Nepal.³⁰ Lower rate of resistance to ceftriaxone (40%) had been reported in another study from Andhra Pradesh, India.³¹ Another cephalosporin drug- cefepime showed higher resistance rate of 80.43% in this study. But low resistance observed from studies from Pakistan,²⁴ India,²³ and Iran.²⁶ A study from Palestine,²² showed resistance of ceftazidime to be 13.3% and that of Cefuroxime to be 53.3% while in our study it was 68.84% and 96.27%, respectively. In contrast, resistance level of ceftazidime and ceftriaxone found by Paryani et. al.,²⁰ were 3% and 13% respectively, which differs greatly with our study. It does indicate the relative efficacy of these drugs amongst the cephalosporins against *P. aeruginosa*. The rate of resistance for the anti-folate drug co-trimoxazole in the present study was 91.24%. Similarly, a previous study in Bangladesh³² showed rate of resistance for co-trimoxazole to be 92% in ICU patients while studies from Nigeria³³ and Pakistan²⁴ showed resistance of 100% and 98.25% respectively to co-trimoxazole. Report from Nepal showed lower resistance 51.72% by Chander et. al.¹⁸ Meropenem was found 76.09% sensitive in this study, whereas Profulla, et. al. found 73% sensitive in a study at Indore (M.P.), India.²³ Variation in susceptibilities among different studies indicate difference in prescription pattern and usage of the drug in different locality.

Ciprofloxacin (78.26% sensitive) proved to be one of the most effective drugs for routine use among the *P. aeruginosa* strains investigated in this study. A study from Kathmandu, Nepal³⁴ showed ciprofloxacin 70.3% sensitive among *P. aeruginosa* examined. But other studies have showed varying degrees of sensitivity in recent years which ranged from 37 to 66.6%.^{16,18,23-26,35} Irrational and inappropriate use of antibiotics is responsible for the development of resistance of many microorganisms including *Pseudomonas* species to antibiotic. So, emphasize should be given to the rational use of antimicrobials. Some drugs should kept reserve to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential for monitoring of the antibiogram patterns for better patient management.

Conclusion

P. aeruginosa is one of the most frequently isolated pathogen from the clinical specimens. In this study, imipenem, and amikacin proved to be the most sensitive drug against *P. aeruginosa*. Variation in sensitivity among these drugs in different studies poses importance of keeping those drugs as reserve drugs. This study found that ciprofloxacin can be used for routine treatment against *P. aeruginosa*. Use of cephalosporins and cotrimoxazole should be restricted as it found to be least effective against *P. aeruginosa*.

Acknowledgements

We want to acknowledge the authority of Ibn Sina Medical College Hospital for providing samples to microbiology department for culture and sensitivity tests. We also acknowledged the continuous support of laboratory personnel of the Microbiology department.

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