Original Article

Antibiotic Susceptibility Pattern of Isolates of Pseudomonas Aeruginosa in Respiratory Tract Infection

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Abstract

Background: *Pseudomonas aeruginosa is one of the most common gram-negative bacteria, identified in the clinical samples of Sylhet region . A major problem in P. aeruginosa infection may be that this pathogen exhibits a high degree of resistance to a broad spectrum of antibiotics. The study aimed to isolate and determine the antimicrobial susceptibility patterns of the P. aeruginosa to commonly used antimicrobial agents.*

Methodology: *Forty one clinical isolates of Pseudomonas aeruginosa (P. aeruginosa) were isolated from sputum specimens of the patients suspected of having respiratory tract infection. The antibiotic susceptibility profiles of all the isolates were determined using disk diffusion method as recommended by Clinical Laboratory Standards Institute.*

Results: *Ciprofloxacin was found to be the most effective antimicrobial agent with 85.4% susceptibility followed by imipenem (75.6%), aminoglycosides (amikacin, 95.1% and gentamicin, 90.3%) and the beta-lactams (cefepime 65.8%, ceftazidime,51.2%). Piperacillin showed the maximum resistance (46.3%) followed by Aztreconam (36.6%). Regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. An effective national level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management.*

Key Words: *Antibiotic susceptibility patterns, P. aeruginosa, respiratory tract infection, multiple antibiotic resistance.*

Introduction

Widespread occurrences of Pseudomonas bacteria in nature were observed early in the history of microbiology. The bacterial strains of Pseudomonas genus are widely distributed in nature, but the most common human pathogen is pseudomonas aeruginosa[1]. This bacterium is an important pathogen causing severe and life threatening infections in immunocompromised hosts, such as patients suffering from respiratory disease, cancer patients undergoing chemotherapy, and children and young adults with cystic fibrosis. Moreover, it is a leading cause of nosocomial infections and is associated with a high mortality rate. One of the remarkable reasons for this high mortality is its notable resistance to many currently available antibiotics. Yet, comparative analyses of the emergence of resistance associated with different classes of antipseudomonal drugs are lacking, even though knowledge about the relative risks of resistance with different antibiotics could be useful in helping to guide therapeutic choices[2].

Ongoing surveillance of P. aeruginosa resistance against antimicrobial agents is fundamental to monitor trends in susceptibility patterns and to appropriately guide the clinician in choosing empirical or directed therapy, especially when new antimicrobial agents may not be readily available in the near future[3]. However, there are a few recent surveillance studies reporting antimicrobial resistance patterns of P. aeruginosa in Sylhet region.

Over the past few years, a notable increase in antibiotic resistance among gram negative bacteria recovered from hospitalized patients has been reported, especially for critically ill patients[6]. Infections caused by multidrug resistant (MDR) gram negative bacteria, especially MDR P. aeruginosa are associated with significant morbidity and mortality[7]. Additionally, Hospital Acquired Infections increase hospital lengths of stay and health care expenditures[8]. Multidrug-resistant strains of *P. aeruginosa* are often isolated among patients suffering from nosocomial infections particularly those receiving intensive care treatments [9].

The aim of this study was to assess the current levels of antimicrobial susceptibility and to evaluate the resistance mechanisms to antipseudomonal antimicrobial agents among the clinical isolates of *P. aeruginosa* isolated from patients suffering from respiratory tract infection in Sylhet region.

Materials and methods

A total of 147 sputum specimens of the patients suspected of having respiratory tract infection across a diverse range of clinical samples of Sylhet region were collected of these 98 were males and 49 females spanning from June 2018 to July 2019. The mean age of the patients was 22.2 years and the male-female ratio was 2:1. The specimens were collected in sterile bottles and brought to the Microbiology Laboratory and then processed within one or two hours. The specimens were cultured on blood agar plates (Oxoid,UK) and MacConkey agar (Oxoid,UK) plates . All plates were incubated at 37º C aerobically in an incubator for the isolation of the probable pathogenic bacteria . The plates were read after 24 hours. Bacterial colonies on blood agar plates were later gram stained. A total of forty one isolates of pseudomonas strains were identified as *P. aeruginosa* by using colonial morphology and a positive oxidase reaction.

Oxidase test was done by soaking a few drops of oxidase reagent (tetramethyl para-phenylenediamine hydrochloride) on a piece of filter paper. A colony of the test organism was then smeared on that paper. Appearance of deep purple color within 10 seconds indicates that the bacteria were oxidase positive.

Colonies which displayed a positive oxidase reaction were subcultured on nutrient agar. The growth was then stabbed into Triple Sugar Iron (TSI) agar media and incubated aerobically overnight .There was no acid or gas formation [10].

Antibiotic Susceptibility Testing:

The Kirby-Bauer disk diffusion method[11] according to the National Committee for Clinical and Laboratory Standards criteria (NCCLS) was performed to determine the antibiotic susceptibility. Mueller-Hinton agar was used as the growth medium. The Zone of inhibition was compared with standard value as recommended by NCCLS. The antibiotics tested were Gentamicin (10 ∞g), Impenem (10 ∞g), Amikacin (30 ∞g) , Piperacillin (100 ∞g), Ciprofloxacin (5 ∞g), Ceftazidime (30 ∞g) , Aztreonam (30 ∞g), Meropenem (10 ∞g), and Cefepime (30 ∞g). Results of the disk diffusion method were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2000)[12].

Results

Of the 147 samples subjected to culture and sensitivity, 41 reported the presence of *Pseudomonas aeruginosa,* thereby suggesting 27.9% as the occurrence level, of which 63.4% (i.e. 26 samples) and 36.6% (i.e. 15 samples) were reported from males and females respectively. The antimicrobial susceptibility testing revealed that *P. aeruginosa* stains were highly sensitive to most of the antibiotics tested which are shown in Table I and Figure 3. The percentage of sensitivities were ciprofloxacin(85.4%), imipenem (75.6%), amikacin (95.1%), gentamicin (90.3%), cefepime (65.8%), ceftazidime (51.2%), meropenem (70.7%), piperacillin (46.3%), Aztreonam (36.6%) and the percentage of resistance were ciprofloxacin (14.6)%, imipenem (24.4%), amikacin (4.9%), gentamicin (9.7%), cefepime (43.2%), ceftazidime (48.8%), meropenem (29.3%), piperacillin (53.7%), Aztreonam (63.4%).

Table I : Antibiotic sensitivity Pattern of Pseudomonas aeruginosa.

Percentage (%)	Amikacin	Gentamicin	Ciprofloxacin	Impenem	Meropenem	Cefepime	Ceftazidime	Piperacillin	Aztreonam
Sensitive (S)	95.1	90.3	85.4	75.6	70.7	65.8	51.2	46.3	36.6
Resistant (R)	4.9	9.7	14.6	24.4	29.3	34.2	48.8	53.7	63.4

Figure 1: Presence of P.aeruginosa in a media

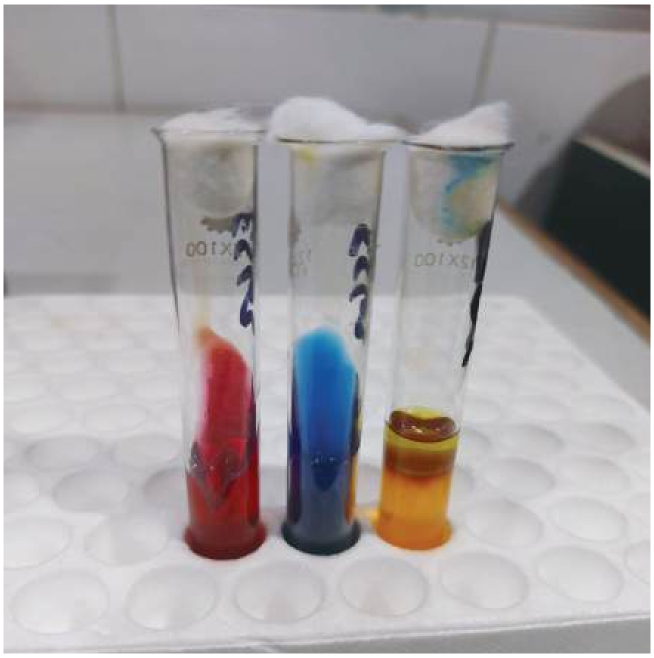

Figure 2: Biochemical test for diagnosis of P.aeruginosa

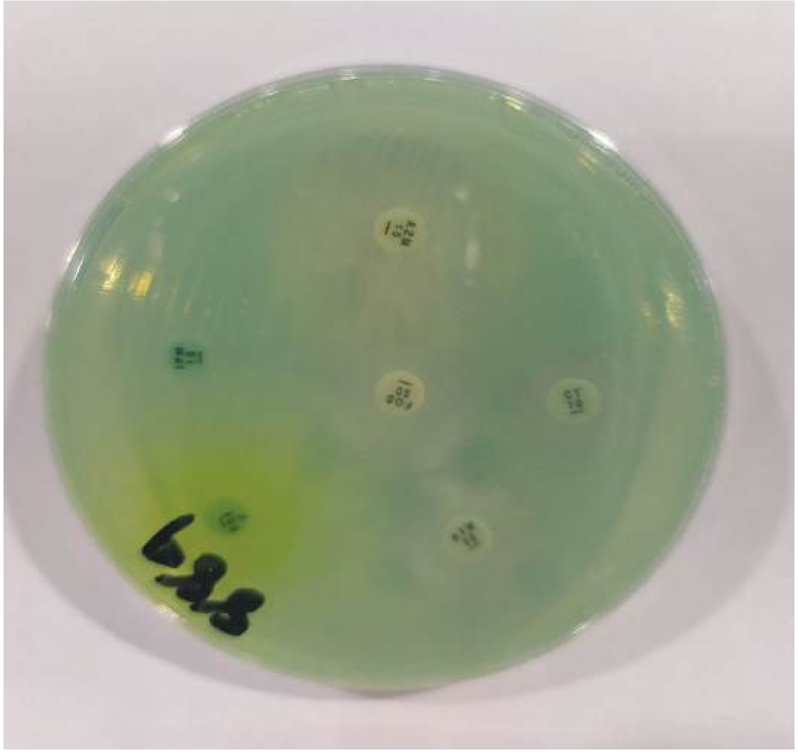

Figure 3: Antibiotic sensitivity testing for diagnosis of P. aeruginosa

Discussion

P. aeruginosa infection is a serious cause of nosocomial infections. With the widespread use of antibiotics and increase in the number of immunosuppressed hosts, *P. aeruginosa* has become a leading cause of gram-negative bacterial infections especially in immunosuppressed patients who need prolonged hospitalization[13]. The increasing rate of *P. aeruginosa* strains in a wide spectrum of clinical steering determine them as emerging pathogens, especially in intensive care units (ICUs) and justifies the necessity for antimicrobial-resistance surveillance. Periodic antimicrobial resistance monitoring in *P. aeruginosa* infection is fundamental to updating the current activity level of commonly used antipseudomonal drugs[3].

Ciprofloxacin was found to be the most effective agent (85.4% sensitivity) followed by imipenem and meropenem (75.6% and 70.7%. respectively). A study in Saudi Arabia also showed 85% of the *P. aeruginosa* isolates sensitive to ciprofloxacin[3]. In our study we found a high resistance to Piperacillin. Aztreonam Ceftazidime. Similar results have been reported in a study from Saudi Arabia [14-15].

It was reported that the majority of meropenem resistant *P. aeruginosa* showed resistance to imipenem, but almost half the imipenem resistant strains were susceptible to meropenem. Moreover,the strains resistant to meropenem were also resistant to ciprofloxacin and carbenicillin[16]. Imipenem has been reported to be very active against *P. aeruginosa* in a number of recent studies[17] while others have reported otherwise[18]. Resistance to 3 or more antibiotics (MDR) was about 20%. In our study the rates of antimicrobial resistance of the isolates were 14.6% to ciprofloxacin, 34.2% to cefepime, 4.9% to amikacin, 24.4% to imipenem, 48.8% to ceftazidime, 9.7% to gentamicin and 53.7% to piperacillin. Among the aminoglycosides, amikacin has the highest sensitivity against *P. aeruginosa*, which is in correlation with an earlier report published from Saudi Arabia[14]. Amikacin was designed as a poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation, but some organisms have developed enzymes that inactivate this agent as well. Amikacin seems to be a promising therapy for Pseudomonas infection. Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains[19]. The problem of increasing resistance to *P. aeruginosa* has limited the use of other classes of antibiotics like the fluoroquinolones, tetracyclines, macrolides and chloramphenicol[20].

Our study showed higher resistance rates to all drugs tested except ciprofloxacin and imipenem. Among the 41 clinical isolates of *P. aeruginosa* tested in our study. twenty percent (20%) of the isolates were found to be multidrug-resistant (MDR).

In summary, Ciprofloxacin was found to be the most active antimicrobial agent followed by imipenem. Aztreonam showed the maximum resistance followed by Piperacillin. Among aminoglycosides amikacin was found to be highly sensitive. Periodic susceptibility testing should be carried out over a period of two to three years, to detect the resistance trends. Also, a rational strategy on the limited and prudent use of antipseudomonal agents is urgently required.

The threat of *P. aeruginosa* remained alive as before and combating the infection offered ever increasing challenges to the microbiologists, pharmacologists and physicians alike. Better understanding of pseudomonas infection dynamics and epidemiology in the present day hospital environment remained an important research option to counter the decreasing number of effective antipseudomonal drugs coupled with expensive treatment modalities.

References

1. Fazlul MKK, Zaini MZ, Rashid MA, Nazmul MHM. Antibiotic susceptibility profiles of clinical isolates of Pseudomonas aeruginosa from selayang Hospital, Malaysia. Biomedical Research. 2011; 22 (3):07-09.
2. Camell Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa:* comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother. 1999;43: 1379-1382.
3. Gowri SP, Azura NS, and Ramelah M. Antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa from a Malaysian Hospital, Malaysian *J Med Sci* 2009; 16: 2-9.
4. Jaflur A, Al-Tawfiq, Occurrence and antimicrobial resistance pattern of inpatient and outpatient isolates of pseudomonas aeruginosa in a Saudi Arabian hospital 1998-2003. International journal of infectious Diseases. 2007; 11: 109-114. http//dx.doi.org/10.1016/j,ijid.2005.11.004 PMiD: 16750928.
5. Mohanasundaram KM. The Antimicrobial Resistance pattern in the clinical isolates of *Pseudomonas aeruginosa* in a Tertiary Care Hospital 2008-2010 (A 3 year study) Journal of Clinical and Diagnostic research. 2011; 5 (3): 491-494.
6. Fridkin SK, Gaynes RP. Antimicrobial resistance in intensive care units. Clin Chest Med. 1999; 20: 303-316. http://dx.doi.org/10.1016/S0272-5231 (05)70143-X.
7. Paladino JA, Sunderlin JL, Price CS, Schestag J. Economic consequences of antimicrobial resistance. Surg infect (Larchmont). 2002; 3: 259-267. http://dx.doi.org/10.1089/ 109629602761624225. PMiD:12542927.
8. Perencevich EN. Raising standards while watching the bottom line: making a business case for infection control. Infect. Control Hosp. Epidemiol. 2002:28: 1121-1133. http://dx.doi.org/ 10.1086,521852 PMiD:-17933084.
9. Tassous PT, Gennimata V, Spaliara-Kalogeropoulou L, Katris D, Koutsia C, Vatopoulos AC et al. Multiresistant Pseudomonas aeruginosa serogroup O: 11 outbreaks in an intensive care unit. Clin Microbiol infect. 1997; 3: 621-628. PMiD: 11864203 http://dx.doi.org /10.1111/j.1469-0691. 1997. tb00468. x.
10. Murray PR. Manual of Clinical Microbiology. 9th ed. Asm Press, Washington DC., USA. 2007.
11. Bauer AW, Kirby WM, Sherris JC , Turek M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45(4) : 493-496. PMiD: 5325707.
12. Performance standards for antimicrobial susceptibility by testing. Tenth informational

supplement. National Committee for Clinical Laboratory Standards (NCCLS). January 2000; M100-S10 (M2): 14-21.

13. Korvick JA, Marsh JW, Starzl TE, Ya VL. Pseudomonas aeruginosa bacteremia in patients undergoing liver transplantation : An emerging problem Surgery. 1991; 109 : 62-68, PMid: 1984637 PMCid: PMC2981790.

14. Shamweel A, Ahmad F, Shawky M, Gugni HC. Antibiotic sensitivity of isolates of Pseudomonas aeruginosa in Buraidah, Saudi Arabia. J Common Dis. 1995 Sep; 27 (3) : 151-4.

15. Ahamad H, Babay H. Antimicrobial resistance among clinical isolates of Pseudomonas aeruginosa from patients in a teaching Hospital, Riayadh , Soudi Arabia. 2001-2005. Jpn J Infect, Dis. 2007; 60: 123-125.

16. Bonfiglio C, Carciotto V, Russo G. Antibiotic resistance in Pseudomonas aeruginosa, an ltalian surveys. J Antimicrob Chemother. 1998; 41: 307-310.http://dx.doi.org/10.1093/jac/41.2.307. PMID:9533479

17. Ling TKW, Xiong J, Yu Y, Lee CC, Ye H, Hawkey PM. The MK0826 China Study Group. Multicenter Antimicrobial Susceptibility Survey of Gram-Negative Bacteria Isolated from Patients with Community-Acquired Infection in the People's Republic of China. Antimicrob Agents Chemother. 2006;50:374-378 http://dx,doi,org/10.1128/AAC.50.1.374-378.2006 PMID:16377716PMCid: PMC1346789.

18. Patzer JA, Dzierżanowska D. Increase of imipenem resistance among Pseudomonas aeruginosa isolates from a polish paediatric hospital. Int J Antimicrob Agents. 2007; 29: 153-158. http://dx.doi.org/10.1016/j.ijantimicag.2006.08.044 PMid:17157481.

19. Poole K. Aminoglycosides resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chem. 2005;49:479-87. http://dx.doi.org/10.1128/AAC.49.2.479-487.2005 PMid:15673721 PMCid: PMC547279

20. Chambers HF. General Principles of antimicrobial therapy. In: Brunton LL, Lazo JS, Parker KL editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed.Mcgraw Hill: Medical Publishing Division. 2006;1095-110.