e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(1); 179-185

Original Research Article

Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Isolates in Clinical Samples with Special Reference to Metallo Beta Lactamase Detection at a Tertiary Care Hospital

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Received: 15-11-2022 / Revised: 18-12-2022 / Accepted: 10-01-2023

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Conflict of interest: Nil

Abstract

Introduction: In recent years, a significant rise in the incidence of *P. aeruginosa* as well as multidrug resistance (MDR) has been seen, which has been accompanied by an increase in morbidity and death. The purpose of this study was to ascertain the current status of antimicrobial susceptibility to anti-Pseudomonal drugs and to identify the presence of metallo- beta lactamase (MBL) in these isolates.

Material and Methods: A prospective study was carried out to obtain *P. aeruginosa* isolates from various clinical samples and to detect MBL production in them. *Pseudomonas aeruginosa* was isolated from a total of one hundred various clinical samples for the purpose of this study. The organisms were identified based on the cultural characteristics and results of biochemical reactions. The modified Kirby-Bauer disc-diffusion method was used, as recommended by CLSI 2018, to determine the antimicrobial susceptibility to various antipseudomonal drugs. Antimicrobial susceptibility to colistin was detected by Epsilometric test. The isolates which showed resistance to imipenem were further identified as MBL producers by the phenotypic approach known as the IPM-EDTA combined disc synergy test.

Results : Colistin was shown to be the most effective treatment against *P. aeruginosa* in this study. This was followed by Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Imipenem, Meropenem, Ciprofloxacin, Amikacin, Gentamicin, Ceftazidime, Tobramycin, and Aztreonam. There were 33 isolates that showed resistance to imipenem, and out of those 33 isolates, 17 were detected as MBL producers when tested using the IPM-EDTA combined disc synergy method.

Conclusions: In order to treat multidrug-resistant *P. aeruginosa*, colistin is the most effective for treatment. The early identification of MBL producing *P. aeruginosa* may be helpful in determining

the right anti-pseudomonal medication to inhibit the formation and transmission of these multidrug resistant strains.

Keywords: Antimicrobial Susceptibility, *Pseudomonas aeruginosa*, Metallo Beta Lactamase, Combined Disc Synergy Test

Introduction

Pseudomonas aeruginosa is a gram-negative, opportunistic bacterium that is responsible for 9–10% of all nosocomial infections. It is a bacterium that thrives under adverse conditions. [1] Despite the advances that have been made in medical and surgical treatment, as well as the introduction of a wide array of antimicrobial drugs with anti-pseudomonal activity, life-threatening infections caused by *Pseudomonas aeruginosa* continue to create complications.

These infections can lead to a variety of health problems, including death. [2] Bacterial resistance may be caused by a variety of causes, including the intrinsic low permeability of a bacterium's cell wall, chromosomal mutation, plasmids, and transposons; these sources all have the potential to spread resistance determinants across different species of microorganisms. [3,4] The discovery of multidrug-resistant (MDR) and metallo beta lactamase (MBL) positive isolates in a range of clinical samples is a significant reason for concern, and the main objective of this study is to determine the extent to which these types of bacteria are prevalent.

Material and Methods

A prospective cross-sectional study was carried out in the Department of Microbiology of a tertiary care hospital over a period of one year after the institutional ethic committee approval. A total of 100 *Pseudomonas aeruginosa* isolates were obtained from various clinical samples like pus, ear swabs, blood, urine, sputum, pleural fluids. from both in patient and out patient departments.

Exclusion criteria: Repeat isolates from the same patient; Doubtful isolates were excluded.

The specimens were processed according to the standard procedures that were provided. [5] The organisms were identified based on the cultural characteristics and results of biochemical reactions. The antimicrobial susceptibility test was performed by modified Kirby Bauer disc diffusion method on Mueller-Hinton agar plates by making use of discs that were available for purchase from a company called Hi Media in Mumbai against various anti- pseudomonal drugs. Antimicrobial Susceptibility to colistin was detected by Epsilometric test. The results were interpreted in accordance with the CLSI 2018 standards. [6] The ß- lactamase negative strain of *P. aeruginosa* ATCC 27853 was used to as a control for this study.

Colistin (Ezy Mic strips 0.016-256µg), Aminoglycosides [amikacin (30 µg), Gentamicin (10 µg), and Tobramycin (10 µg)], Cephalosporins, ceftazidime (30 µg), Floroquinolones ciprofloxacin (5 µg), Carbapenems [imipenem (10 µg), meropenem (10 µg)], and Metallo—lactamase detection test with an imipenem-EDTA combination disc was carried out in order to adhere to the guidelines that were supplied by CLSI 2018.

Result

The present study included a total of one hundred different *P. aeruginosa* isolates, all of which were derived from a range of different locations. There was no repetition in these isolated samples. These *P. aeruginosa* strains were recovered from pus 26 times, isolated from urine 25 times, sputum 24 times, ear swabs 21 times, and miscellaneous materials 4 times. Infections brought on by *Pseudomonas aeruginosa* were shown to be much more common in males, with a rate of 72%, compared to females, who had a rate of 29%. . According to Table-1, the age group of 11-20 years old had the largest number of occurrences of *Pseudomonas aeruginosa*, whilst those above the age of 70 had the lowest number of instances.

Table 1: Age distribution of patients

Age	Percentage (%) (n=100)
<10 years	08%
11-20 years	28%
21-30 years	17%
31-40 years	07%
41-50 years	04%
51-60 years	22%
61-70 years	12%
>70 years	02%

Table 2: Sensitivity pattern of *Pseudomonas aeruginosa* isolated from different clinical samples.

Antibiotic	Sensitivity (%) (n=100)
Ceftazidime	21%
Piperacillin/tazobactam	75%
Meropenem	61%
Amikacin	31%
Tobramycin	22%
Colistin	100%
Aztreonam	14%
Cefoperazone/sulbactum	69 %
Imipenem	67%
Gentamicin	28%
Ciprofloxacin	40%

Table 3: No. of MBL producer *Pseudomonas aeruginosa* detected by phenotypic methods

Phenotypic Methods	MBL producing strains
CDST	17

According to the susceptibility pattern of *P. aeruginosa* strains isolated from a variety of clinical samples, the highest level of sensitivity was observed for Colistin, while the highest level of resistance was observed for Aztreonam, Tobramycin, and Ceftazidime. This was the case despite the fact that Aztreonam, Tobramycin, and Ceftazidime exhibited the broadest spectrum of activity (see Table-2 for further explanation).

According to Table-3, out of a total of one hundred bacteria, thirty-three were resistant to imipenem, and seventeen of those strains

developed MBL isolated from various clinical samples.

Discussion

According to the results of this study, when clinical isolates were analyzed depending on the gender of the patient, it was shown that infections caused by *P. aeruginosa* were more common in males than they were in women. Comparable findings were found in the studies carried out by Javiya *et al*. (2008)[7], Jamshaid Ali Khan *et al*. (2008)[8], and Rashid *et al*. (2007) [9]

According to the findings of our study, the age group spanning from 11 to 20 years had the greatest frequency of *Pseudomonas aeruginosa* clinical isolates.

Antibiotic susceptibility patterns can serve as a useful reference for determining which drugs will be most effective in treating a particular illness. Colistin had the highest level of sensitivity (100%) among clinical isolates of *P. aeruginosa*, as shown by the current study's susceptibility pattern of clinical isolates of *P. aeruginosa*. This was followed by Piperacillin/ Tazobactum (75%), Cefoperazone/ Sulbactam (69%), Imipenem (67%), Ciprofloxacin (40%).

Among the aminoglycosides, sensitivity to amikacin was observed in 31% of the isolates in our study; however, Sharma *et al*. (2010)[10], Picao *et al*. (2008)[11], and Behera *et al*. (2008) [12] reported a lower proportion of sensitivity (8.8-19%). This is in spite of the fact that higher rates of sensitivity to amikacin were reported by Murugan *et al*. 57. (2008). [13] The findings of the present study indicated that there was a sensitivity to Gentamicin of 28%, which is comparable to the result of 53% made by Kumar *et al*. (2010)[14]. On the other hand, several studies came to a different conclusion and discovered a lower sensitivity, such as 7.7% by Sharma *et al*. (2010)[10] and 4.35% by Prakash *et al* (2012). [17] In our analysis, tobramycin sensitivity was observed in 22% of the isolates; however, in the investigations done by Kumar *et al*. (2011) [14] , 39% of the isolates showed sensitivity to the antibiotic. The findings of the study that was carried out by Franco *et al*. (2010)[18], Jamasbi *et al*. (2008)[16], and Obritisch *et al*. (2004). [19] found an even higher percentage of sensitivity, which varied from 43.5 to 88.1%. The findings of our study revealed that sensitivity to the ceftazidime, which belongs to the third generation of cephalosporins, was 21%. Greater rates of sensitivity were reported between 30 and 90% by Sharma *et al*.(2008)[10], Javiya *et al*. (2008)[7], Obristich *et al*. (2004)[19], kumar *et al*. (2010)[14], and Hocquet *et al*. (2010)[15]. Similar rates were found by Franco *et al*. (14.5%) (2010)[18]. During the course of our investigation, we found that ciprofloxacin was effective in treating 40% of the isolates. Similar percentages were discovered in previous research by Sharma *et al*. 23.8% (2008)[10], Javiya *et al*. 26.79% (2008)[7], Gokale *et al*. 50.4% (2012)[20], and by Kumar *et al*. 63% (2010)[14]. Piperacillin and tazobactam, as well as cefoperazone and sulbactum, were the two distinct combinations of antibiotics that were used in the study. Among the 100 *P. aeruginosa* isolates, 75% of them were susceptible to the combination of piperacillin and tazobactam, which is comparable to the 64.29% success rate that Javiya *et al*. (2008)[7] found. On the other hand, Kumar *et al*. (2010) [14] and Hoc-quet *et al*. [15] revealed a higher degree of sensitivity to piperacillin and tazobactum (95% sensitivity). Also, 69% of the isolates were susceptible to the combination of cefoperazone and sulbactum, which is comparable to the study by Javiya *et al*. (2008)[7] (57.14%) and Kumar *et al* (2010) [14] (78%). In our study, 67% of the isolates were sensitivity to imipenem, however in other studies, the sensitivity to imipenem was Franco *et al*. 0% (2010)[18], Picao *et al*. 18.6% (2008)[11], Murugan *et al*. 28.6% (2010)[13], and Behera *et al*. 31% (2008) [12] thus, lower percentages of imipenem sensitivity were identified.

However, Javiya *et al*. reported a sensitivity rate of 78.57% (2008)[7] and Hocquet *et al*. reported a rate of 82.5% (2007) [15], both of which are higher than our study. In the present study, out of a total of 100 isolates of *P. aeruginosa*, 33 % of these isolates were resistant to imipenem and were further tested for MBL production. In our study, 17% of the strains out of a total of 100 isalates were detected as MBL producers by using the phenotypic technique of the IPM-EDTA paired disc synergy test. Whereas the vast majority of other studies have demonstrated a lower rate of MBL-producing strains of *P. aeruginosa*, such as Jay kumar *et al*. 2.4% (2007)[21], Agrawal *et al*. 8.04% (2008)[22], and Attal *et al*. 11.4% (2010)[23], while similar findings were observed by Saha *et al*. 21.83% (2010) [24], Fang *et al*. 24.1% (2008)[25]. Irfan *et al* (2008) [26] reported 100% imipenem-resistant Pseudomonas aeroginosa isolates were MBL producers.

It was also observed that all the MBL-producing *P. aeruginosa* strains were susceptible to colistin while they were resistant to the vast majority of other antibiotics. The sensitivity shown against cefoperazone/sulbactam was 69% in contrast to what was seen against third generation cephalosporins such ceftazidime and amikacin, which had a sensitivity of 21% and 31 respectively. The sensitivity of ciprofloxacin was 40% . This analysis came to the conclusion that clinical isolates of *Pseudomonas aeruginosa* are becoming resistant to antibiotics that are commonly used and are also acquiring resistance to medications that were created more recently. Antimicrobial drugs are losing their efficacy because of the widespread misuse of antibiotics, a lack of understanding, noncompliance on the part of patients, and unclean settings. These are all factors contribute to the growth of antibiotic-resistant organisms, which is causing antimicrobial drugs to lose their efficacy. Formulation of antibiotic policies and execution of a more rational approach to the use of antibiotics are both urgently needed in order to battle and get the upper hand on this emerging problem. Both are necessary in order to utilize antibiotics more effectively. It is vital that every precaution feasible be done in order to put a halt to the proliferation of germs that are resistant to treatment. [27]

Conclusion

Despite the fact that the rates of resistance significantly differ from region to region, it has been consistently documented all over the world that the prevalence of MBL producing clinical isolates of *Pseudomonas aeruginosa* is increasing day by day.

In our study, Colistin had shown the greatest degree of susceptibility against *Pseudomonas aeruginosa* isolates and also in MBL producing MDR isolates. Therefore, we recommend early detection of MBL producers & proper treatment of these infections in order to limit their spread. The findings from this study highlights the significance of anti-pseudomonal drugs susceptibility pattern which will help the clinicians to choose appropriate antibiotics in addition to proper implementation of suitable infection control practices therefore stopping spread of resistant strains in hospital.

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