ORIGNAL ARTICLE

DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATES IN VARIOUS CLINICAL SAMPLES WITH SPECIAL REFERENCE TO METALLO BETA LACTAMASE FROM A TERTIARY CARE HOSPITAL IN JAIPUR, INDIA

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ABSTRACT

Introduction: In recent years, considerable increase in the prevalence and multidrug resistance (MDR) in *P.aeruginosa* has been noticed with high morbidity and mortality. Aim of the present study was to determine the status of antimicrobial susceptibility to anti Pseudomonal agents and to detect Metallo Beta lactamase.

Material and Methods- This study was conducted on 100 isolates of *Pseudomonas aeruginosa.* The organisms were identified on the basis of their cultural characteristics and biochemical tests. Antimicrobial susceptibility of all the isolates was performed by the Kirby- bauer disc-diffusion method according to CLSI guidelines. MBL producing *P. aeruginosa* were detected by phenotypic method IPM-EDTA combined disc synergy test.

Results- In present study *P. aeruginosa* were most sensitive to Colistin followed by Polymyxin- B, Piperacilli/Tazobactam, Cefoperazone/Sulbactam, Imipenem, Meropenem, Ciprofloxacin, Amikacin, Gentamycin, Ceftazidime, Tobramycin and Aztreonam. 32 isolates were imipenem resistant and out of 32 isolates, 20 were MBL producers detected by IPM-EDTA combined disc synergy test.

Conclusions- Colistin and Polymyxin-B are more effective to treat multidrug resistant *P.aeruginosa.* The early detection of MBL producing *P. aeruginosa* may help in appropriate anti-Pseudomonal therapy to stop the development & dissemination of multidrug resistance strains.

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

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic gram-negative bacterium that causes 9-10% of nosocomial infections.[1] Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents with anti-pseudomonal activities, life threatening infection caused by *Pseudomonas aeruginosa* continue to cause complications.[2]

Bacterial resistance is caused by intrinsic low permeability of its cell wall, chromosomal mutation, plasmids and transposons that can transfer resistance determinants in diverse microbial species.[3,4] Our aim is to determine the prevalence of multi drug resistant (MDR) and metallobeta lactamase (MBL) positive isolates in various clinical samples which is a serious concern.

MATERIAL AND METHODS

One hundred non-repetitive isolates of *Pseudomonas aeruginosa* were obtained during one year period from August 2012 to September 2013 in the Department of Microbiology, NIMS Medical College, Jaipur, Rajasthan.. The specimens were processed according to established guidelines.[5] Identification of organisms was done by the standard laboratory techniques. Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available discs (Hi Media, Mumbai) by Modified Kirby Bauer disc diffusion method and interpreted as per CLSI recommendations.[6] *P. aeruginosa* ATCC 27853 (β-lactamase negative) strain was used as control.

The antibiotic sensitivity tests were put up for Aminoglycosides [amikacin (30μg), gentamicin (10 μg), tobramycin (10μg)], Cephalosporins [ceftazidime (30μg)], Floroquinolones [ciprofloxacin (5 μg)], Carbapenems [imipenem (10 μg), meropenem (10 μg)], Colistin (10 μg), Polymyxin-B (300 μg), Aztreonam (30 μg), Cefoperazone/Sulbactam (75/10μg) and Piperacillin/Tazobactum (100/10 μg). Detection of Metallo β-lactamases by Imipenem-EDTA combined disc synergy test was done as per CLSI guidelines.

RESULT

A total number of 100 non-repetitive *P. aeruginosa* isolates from various specimens were included in the present study. Out of these 100 strains of *P. aeruginosa*, 27 were isolated from pus, 26 from urine, 22 from sputum, 20 from ear swab and 5 from other specimens.

Pseudomonas aeruginosa infection were more common in males 71% (71) as compared to female 29% (29). Maximum number of *Pseudomonas aeruginosa* were found in age group of 11-20 years and least in >70 years as shown in Table-1.

Table 1: Age distribution of patients

Age	Percentage (%) (n=100)
<10 years	09%
11-20 years	27%
21-30 years	16%
31-40 years	08%
41-50 years	05%
51-60 years	21%
61-70 years	11%
>70 years	03%

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

Antibiotic	Sensitivity (%) (n=100)
Ceftazidime	22%
Aztreonam	13%
Piperacillin/tazobactam	74%
Cefoperazone/sulbactum	70 %
Meropenem	60%
Imipenem	68%
Amikacin	32%
Gentamycin	27%
Tobramycin	21%
Polymyxin B	94%
Colistin	97%
Ciprofloxacin	41%

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

Phenotypic Methods	MBL producing strains detect
CDST	18 (18%)

The susceptibility pattern of *P. aeruginosa* strains isolated from various clinical samples showed highest sensitivity for Colistin and Polymyxin-B, while high resistance was observed to Aztreonam, Tobramycin and Ceftazidime as shown in Table-2.

Out of 100 isolates, 30 strains were Imipenem resistant and 18 were MBL producer in various clinical samples as shown in Table-3.

DISCUSSION

In this study, sex wise distribution of clinical isolates showed that infections caused by *P. aeruginosa* were more common in males than females. This is comparable with study of Javiya et al. (2008)[7], Jamshaid Ali Khan et al. (2008)[8] and Rashid et al.(2007).[9]

In our study, age wise distribution of clinical isolates showed that *Pseudomonas aeruginosa* was common in the age group between 11-20 years. On comparison we found that little difference in results in other studies.

Antibiotic susceptibility patterns serve as a useful guideline for choosing the appropriate antibiotics. In the present study, the susceptibility pattern of clinical isolates of *P. aeruginosa* showed higher sensitivity to Colistin (97%) followed by Polymyxin-B (94%), Piperacillin/Tazobactum (74%), Cefoperazone/Sulbactam (70%), Imipenem (68%), Ciprofloxacin (41%), and Amikacin (32%) and lowest sensitivity was seen to Gentamycin (27%), Ceftazidime (22%), Tobramycin (21%) and Aztreonam (13%).

In our study, among the aminoglycosides, sensitivity to Amikacin was seen in 32% of the isolates, while lower rate of sensitivity (8.8-19%) was reported by Sharma et al. (2010)[10], Picao et al. (2008)[11] and Behera et al. (2008) [12]. While higher rates of sensitivity to Amikacin was reported by Murugan et al. 57.2% (2010)[13], Kumar et al. 68% (2010) [14], Hocquet et al. 93.3% (2007)[15] and Jamasbi et al. 97% (2008).[16] The present study showed 27% sensitivity to Gentamycin, comparable with Kumar et al. 53% (2010)[14], while in other studies lower sensitivity was observed such as Sharma et al. 7.7% (2010)[10] and Prakash et al. (4.35%) (2012).[17]

Sensitivity to Tobramycin was seen in 21% isolates in our study, while little higher rate of sensitivity was observed in the study of Kumar et al. 30% (2010) [14] and Javiya et al. 39% (2008).[7] Even higher rate of sensitivity (43.5-88.1%) was seen in study of Franco et al. (2010)[18], Jamasbi et al. (2008)[16] and Obritisch et al. (2004).[19]

In our study sensitivity to third generation Cephalosporins (ceftazidime) was seen to be 22 %. Similar rate was observed by Franco et al. (14.5%) (2010)[18] and higher rates of sensitivity were observed between 30-90% 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. (2007).[15]

We found that 41% isolates were sensitive to Ciprofloxacin in our study, similar to other studies 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] but lower sensitivity was reported by Franco et al. 14.5% (2010)[18] and Prakash et al. 8.69% (2012).[17]

In our study, two combination drugs Piperacillin/Tazobactam and Cefoperazone/Sulbactum were used. The Piperacillin/Tazobactam combination was effective in 74% of isolates which is comparable to that of Javiya et al. 64.29% (2008)[7], while higher sensitivity was reported by Kumar et al. 95% (2010)[14] and Hocquet et al. 95% (2007).[15] The Cefoperazone/Sulbactum combination was effective in 70% of isolates comparable with that of Javiya et al. 57.14% (2008)[7] and Kumar et al. 78 % (2010).[14]

Sensitivity to Imipenem was seen in 68% of isolates in our study, while lower rates were seen by Franco et al. 0% (2010)[18], Picao et al. 18.6% (2008)[11], Murugan et al. 28.6% (2010)[13], Behera et al. 31% (2008)[12] while higher rate of sensitivity were reported by Javiya et al. 78.57% (2008)[7] and by Hocquet et al. 82.5% (2007).[15]

In the present study out of 100 *P. aeruginosa* strains, 32% (n=100) isolates were seen to be imipenem resistant. We found 18% (n=100) strains were MBL producers, detected by phenotypic method of IPM-EDTA combined disc synergy test. Where many other studies have shown lower rate of MBL producing strains of *P. aeruginosa* i.e. Jay kumar et al. 2.4% (2007)[21], Agrawal et al. 8.04% (2008)[22], 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] and higher rates of MBL producers were reported by Behera et al 52% (2008)[12] and Irfan et. al. 59.5% (2008).[26]

A total of 18% isolates were found to be MBL positive by combined disc methods in our study, while in contrast, detection of MBL producing strains of Imipenem resistant isolates by combined disc synergy test, in other studies were found to be from 4 % to 100%. Berges et al. 4.4% (2007) [27], Behera et al. 10.53% (2008) [12], Deba et al. 11.66% (2011) [28], Hemalatha 14% (2005) [29] , Prakash et al. 67.85% (2012)[30], Picao et al. 80% (2008)[11], Kumar et al. 87.17% (2011)[14], Galani et al. 94.7% (2008) [31], Pandya et al. 96.30% (2011) [32] and Franklin et al. 100% (2006).[33]

In our study, MBL producing *P. aeruginosa* antimicrobial sensitivity pattern was little different from other studies, the MBL producing *P. aeruginosa* strains were found to be 95% to Colistin and 100% sensitive with Polymyxin-B. However, they were found resistant to most of the antibiotics. Very low sensitivity was observed against third generation Cephalosporins- ceftazidime and Amikacin, i.e., (5%) and (10%) Ciprofloxacin (20%) Piperacillin/Tazobactum and there was no sensitivity seen against Cefoperazone/Sulbactam (00%).

This study shows that the clinical isolates of *Pseudomonas aeruginosa* are becoming resistant to commonly used antibiotics and gaining resistance to newer antibiotics. The antimicrobial agents are losing their efficacy because of the spread of resistant organisms due to indiscriminate use of antibiotics, lack of awareness, patient non compliance and unhygienic conditions. It is the need of the hour that antibiotic policies should be formulated and rationale use of drugs should be implemented to resist and overcome this emerging problem. Every effort should be made to prevent spread of resistant organisms.[34]

CONCLUSION

Prevalence of MBL producing clinical isolates of *Pseudomonas aeruginosa* have been continuously reported globally with some disparity in the rates of resistance. In present stdy Colistin and Polymyxin-B are the main drugs to treat multidrug resistant *Pseudomonas aeruginosa* as they are showing highest sensitivity and we recommand early detection of Metallo beta lactamase production to identify the resistance of *P. aeruginosa*. . This is our initial step towards controlling the spread of MDR (Multi Drug Resistant) strains by detecting their incidence in our hospital and appropriate treatment of *Pseudomonas aeruginosa* infection.

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