Original Article

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IN VITRO EFFICACY OF PIPERACILLIN/SULBACTAM, PIPERACILLIN / TAZOBACTAM AND CEFOPERAZONE/SULBACTAM AGAINST CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA – A COMPARATIVE STUDY

Umme Farwa, Irfan Ali Mirza, Shahid Ahmad Abbasi, Alina Amjad, Zeeshan Ahmed Qureshi, Bushra Sultan

Armed Forces Institute of Pathology Rawalpindi

ABSTRACT

Objective: To determine the in vitro efficacy of Piperacillin / Sulbactam, Piperacillin/ Tazobactam and Cefoperazone / Sulbactam against clinical isolates of *Pseudomonas aeruginosa*.

Study Design: Cross-sectional study

Place and duration of study: Department of Microbiology, Armed Forces Institute of Pathology from January 2010 to September 2010.

Material and Methods: A total of 287 isolates of *Pseudomonas aeruginosa* recovered from various clinical specimens were taken under consideration. Routine microbiological methods were used to identify the organism. Susceptibility of the isolates was carried out by modified Kirby-Bauer disc diffusion method against piperacillin 100/sulbactam 30 (SPR130µg), cefoperazone 75/sulbactam 30 (SCF105µg) and piperacillin 100/tazobactam 10 (TZP110µg), according to the guidelines provided by Clinical and Laboratory Standards Institute (CLSI).

Results: The highest numbers of *Pseudomonas aeruginosa* isolates were found in pus swabs, followed by urine and endobronchial washings. Seventy five percent of clinical isolates of *P. aeruginosa* were susceptible to tazobactam/piperacillin, 71% to piperacillin/sulbactam and 70% to cefoperazone/sulbactam. The difference between the susceptibility of isolates to these three antimicrobials was statistically not significant (p>0.05).

Conclusion: We conclude that there was very little difference in the antimicrobial susceptibility of P. *aeruginosa* to the three beta-lactam/beta-lactamase inhibitor combination drugs studied. Periodic susceptibility testing should be carried out over a period of two to three years, to detect the current resistance trends. Moreover, a rational strategy on the limited and prudent use of anti-Pseudomonal agents is urgently required.

Keywords: Antimicrobial susceptibility, combination antibiotics, Kirby-Bauer disc diffusion method, *Pseudomonas aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa is primarily an opportunistic nosocomial pathogen which is able to grow in moist environment[1]. It has a remarkable ability to withstand disinfectants and antiseptic solutions commonly used in hospitals. According to Centre for Disease control (CDC) released details, the overall incidence of *P. aeruginosa* infections in U.S. hospitals averages about 0.4% (4 per 1000 discharges), and that it is the fourth most commonly isolated nosocomial pathogen accounting for 10.1% of all hospital-acquired infections[2].

The high mortality associated with *P. aeruginosa* is due to a combination of weakened host defenses, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes and toxins[3]. Resistance of this notorious bacterium to commonly used antimicrobial agents is becoming an increasing clinical problem and a recognised public health threat because of limited number of antimicrobial agents including antipseudomonal penicillins, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones[4]. The emergence of multidrug resistance (MDR) *Pseudomonas aeruginosa* has become a serious problem[5]. There are several mechanisms which may contribute to the antimicrobial resistance among *Pseudomonas aeruginosa* including the production of chromosomally encoded Amp C beta-lactamases[6,7]. Hypermutable strains of *Pseudomonas aeruginosa* with defects in the

Correspondence: Dr Irfan Ali Mirza, Consultant Microbiologist, AFIP Rawalpindi

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methyl directed mismatch repair (MMR) system are also being frequently isolated from the lungs of cystic fibrosis (CF) patients[6,8].

The older antipseudomonal penicillins (carbenicillin, ticarcillin) have largely been replaced by newer compounds (piperacillin, mezlocillin, azlocillin) which are thought to be more potent in vitro against *P. aeruginosa*[9]. Therefore, the present study was conducted to compare the in vitro efficacy of three antipseudomonal beta-lactam/beta-lactamase inhibitor combination drugs which include piperacillin/sulbactam, piperacillin/ tazobactam and cefoperazone/sulbactam against clinical isolates of *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

This cross sectional comparative study was carried out in the department of microbiology, Armed Forces Institute of Pathology, Rawalpindi. A total of 287 isolates of *Pseudomonas aeruginosa* recovered from January 2010 to September 2010 from various clinical samples, were included in the study. For primary isolation of *Pseudomonas aeruginosa* all the clinical specimens (pus, urine, sputum, pus swabs, endobronchial washings, blood, catheter tips, fluids and tissue) were inoculated on 5% Sheep Blood Agar (Oxoid, UK), MacConkey agar (Oxoid, UK), Chocolate agar (Oxoid, UK) and incubated at 37°C for 24 hours. All the blood samples were incubated in BacT/ALERT bottles (Biomerieux, Brasil) and after positive growth, were subcultured on 5% Sheep Blood Agar and MacConkey agar. Urine samples were inoculated on to Cysteine lactose electrolyte deficient (Oxoid, UK) medium and incubated at 37°C for 24 hours.

P. aeruginosa was identified on the basis of colonial morphology, pigment production, gram stain, motility, oxidase and catalase tests[1]. Final confirmation was done with biochemical tests by using API 20NE (Biomerieux, France)[1,10].

Antibacterial susceptibility testing of selected *Pseudomonas aeruginosa* isolates was done on Mueller Hinton agar (MHA) (Oxoid, UK). Bacterial suspensions were prepared taking one to two colonies of pure growth from overnight cultures of test strains and then transferring into a tube containing five millilitres of sterile normal saline, to match the turbidity with McFarland's index of 0.5. Lawns of each bacterial suspension were made on MHA using sterile cotton swabs. Commercially available standard antibiotic discs of standardised concentrations of Piperacillin 100 /sulbactam 30 (SPR 130µg) (Oxoid, UK) Cefoperazone 75 /sulbactam30 (SCF 105µg) (Oxoid UK), Piperacillin 100 /tazobactam10 (TZP 110µg) (Oxoid UK), were positioned at appropriate distances on the bacterial lawns and incubated at 37 °C for 24 hours. The growth inhibition zones were carefully measured and interpreted according to the standard Kirby-Bauer disc diffusion method[11,12] and CLSI guidelines 2010[6,12].

ATCC Control Strain of *P. aeruginosa* (27853) was used as control strain to check the efficacy of antibiotic discs.

Biostatistical evalution was done using SPSS version 16.0. Descriptive statistics were used to describe the data. Frequencies and percentages were obtained for qualitative variables. Mean and standard deviation were calculated for qualitative variables. To test the significance, Cochran, s Q test was applied and p-value of <0.05 was considered to be significant.

All ethical considerations and obligations have been duly addressed and the study was conducted after approval of ethical committee.

RESULTS

Out of total 287 isolates, 205 (71%) were from male patients and 82 (29%) from female patients. Male to female ratio was 3.5:1 showing male predominance. *P. aeruginosa* was isolated from specimen of patients belonging to all age groups with maximum number of isolates recovered from patients in the fourth decade and mean age was 44 years (SD= 19). (Fig 1).

Antibacterial susceptibility of 287 isolates of *P. aeruginosa* against three antimicrobials was determined, using modified Kirby Bauer disc diffusion method[12, 13]. These isolates were recovered from different specimens including

pus swabs, urine, endobronchial washings, tissue, blood, tips, pure pus, fluids and sputum. The maximum number of test isolates were recovered from pus swabs 102 (36%), while only 8 (3%) were isolated from different catheter tips. The antimicrobial susceptibility pattern of *P. aeruginosa* isolated from different clinical specimen against three studied combination compounds is shown in (Table 1)

Seventy five percent of clinical isolates of *P. aeruginosa* were susceptible to tazobactam/piperacillin, followed by piperacillin/sulbactam 71% and cefoperazone/sulbactam 70%. The susceptibilities of these three drugs were compared and the difference were found insignificant (p>0.05). Similarly statistically there was no difference in the susceptbilities of the isolates (p>0.05), when the three antimicrobials were compared specimen wise.

DISCUSSION

Pseudomonas aeruginosa is a leading cause of nosocomial infections, accounting for 20% of pneumonia and 16% of urinary tract infections according to recent data from the National Nosocomial Infections Surveillance System[14].

Treatment of *Pseudomonas aeruginosa* is a challenge because rapidly emerging resistance has limited the therapeutic options. A number of combination compounds containing beta-lactam/beta-lactamase inhibitors combinations are available in Pakistan which are being used in different clinical settings. Piperacillin/ Tazobactam and Cefoperazone/Sulbactam are extensively used as beta-lactam/beta-lactamase inhibitor combination compounds for treatment of life threatening *P. aeruginosa* infections. At present there is very little published data available from Pakistan and this is the first time that the three beta-lactam/beta-lactamase inhibitors combinations have been studied together and compared.

We found a high male predominance with a maximum number of isolates recovered from pus swabs and urine. Smilar results were found

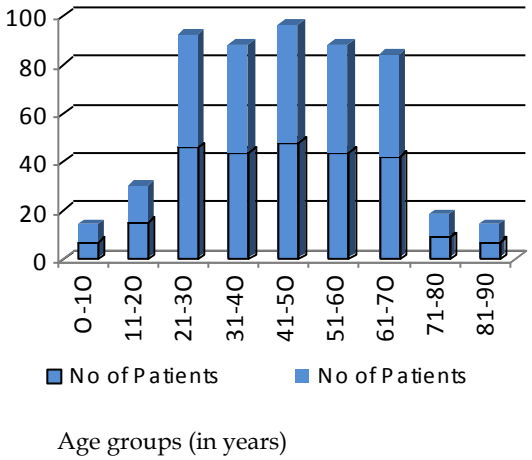

Figure: Age distribution of patients with clinical isolates of P. aeruginosa.

Table-1: Antimicrobial susceptibility of P. aeruginosa against three beta-lactam/beta-lactamase combination drugs.0

Clinical specimen source of *P.aeruginosa* n=287)	Piperacillin/sulbactam 130µg		Piperacillin/tazobactam 110µg		Cefoperazone/ sulbactum 105 µg		p-value
	S	R	S	R	S	R	
Pus swab (n=102)	80 (78%)	22(22%)	83(81%)	19 (19%)	80 (78%)	22(22%)	1
Urine (n=67)	32 (48%)	35(52%)	43(64%)	24 (36%)	41 (61%)	26 (39%)	1
EB Washing (n=33)	29 (88%)	4 (12%)	30(91%)	3 (9%)	26 (79%)	7 (21%)	1
Pure Pus (n=21)	18 (86%)	3 (14%)	16(76%)	5 (24%)	15 (71%)	6 (29%)	1
Tissue (n=17)	15 (88%)	2 (12%)	15 (88%)	2 (12%)	15 (88%)	2 (12%)	0.99
Fluids (n=17)	11 (65%)	6 (36%)	8 (47%)	9 (53%)	9 (53%)	8 (47%)	1
Blood (n=11)	5 (45%)	6 (55%)	3 (27%)	8 (73%)	2 (18%)	9 (82%)	1
Sputum (n=11)	8 (73%)	3 (27%)	8 (73%)	3 (27%)	7 (64%)	4 (36%)	0.99
Tips (n=8)	6 (75%)	2 (25%)	8 (100%)	0 (0%)	5 (62%)	3 (38%)	0.99
Total (n=287)	**204(71%)**	**83 (29%)**	**214(75%)**	**73 (25%)**	**200(70%)**	**87 (30%)**	**1**

in a study conducted in Karachi in Dec 2009 by Nadeem et al[15], which also reported male predominance with maximum number of isolates from urine and ear swab. The authors in that study evaluated in vitro efficacy of Cefoperazone/Sulbactam only and 84% of P. aeruginosa isolates in that study were susceptible to this compound compared to 70% in our study.

In 2008 a study conducted in Gujrat India by Javiya et al[16] reported that highest number of Pseudomonas isolates were recovered from urine, followed by pus and sputum. It was reported that *Pseudomonas* species demonstrated marked resistance against penicillins, cephalosporins and fluoroquinolones. Only beta-lactam/beta-lactamase inhibitor combination compounds like Ticarcillin/Clavulanic acid, Piperacillin/ Tazobactam, Cefoperazone/ Sulbactam, Cefotaxime/ Sulbactam, Ceftriaxone/ Sulbactam and Amikacin showed higher susceptibility results when these isolates of *Pseudomonas aeruginosa* were tested against these antimicrobials.

A study conducted in Turkey in 2004[17] and presented in 14th European congress of clinical microbiology and infectious disease Prague revealed that 71% of their isolates were susceptible to Tazobactam/Piperacillin while our results show that 75% of isolates were susceptible to this combination. A previous study also conducted in Turkey in 2002[18] revealed that 60% of their *P. aeruginosa* isolates were susceptible to Piperacillin/Tazobactam, and 59% to Cefoperazone/sulbactam while in our study much higher percentage of isolates were sensitive to Piperacillin/Tazobactam and Cefoperazone/sulbactam. This difference in susceptibility percentage might be either due to different geographical distribution of the isolates or more importantly to the rapid emergence of resistance seen in *P. aeruginosa globally.*

Our study was the first study to compare the three antipseudomonal combination drugs. Cefoperazone/Sulbactam and Piperacillin/ Tazobactam have been tested in multiple studies in the past but Piperacillin/Sulbactam (Combicin) has not been compared and studied so far. Our results have shown that there was very slight difference in the susceptibility of *P. aeruginosa* against the three beta-lactam/beta-lactamase inhibitor combinations studied. As 70-75% of the clinical isolates of *P. aeruginosa* are susceptible to these compounds, these antimicrobials can be administered therapeutically or empirically for the treatment of resistant strains. Statistically there was no difference in the susceptibilities of the isolates (p>0.05), when the three antimicrobials were compared in total as well as specimen wise

Optimum use of antimicrobials against *Pseudomonas aeruginosa* starts with the initial empirical antibiotic choice. It is imperative that surveillance data and hospital antibiograms are available to the clinicians so that choice of the initial regimen on the basis of this information may benefit the patients having such infections. Combinations of antibiotics are often required empirically, and "combination antibiograms" may need to be developed for this purpose.

CONCLUSION

From the present study, we conclude that Pipercillin/ Tazobactam, Piperacillin/ Sulbactam and Cefoperazone/ Sulbactum are equally effective as there was very little difference in the susceptibility of *P. aeruginosa* against these antimicrobials. Periodic susceptibility testing should be carried out over a period of two to three years, to detect the current resistance trends. Moreover, a rational strategy on the prudent use of anti-Pseudomonal agents is urgently required.

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