ORIGINAL ARTICLE

In Vitro Activity of Piperacillin/Tazobactam against Pseudomonas Species in Paediatric Population

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ABSTRACT

Aim: To determine the susceptibility of piperacillin/ tazobactam against *Pseudomonas* species
Methods: This cross sectional observational study was conducted during April 2011 to March 2012 at Microbiology department of The Children's Hospital and Institute of Child Health, Lahore. A total number of 10761 samples were collected and processed for microbiological investigations. The organisms were identified by routine laboratory tests including biochemical tests.
Results: Out of 10,761 samples 2,375(22%) were positive for bacterial growth, among them 200(9.2%) were *Pseudomonasspp*. and 2,175(90.8%) were other than *Pseudomonasspp*. The highest proportion of *Pseudomonas spp*. was isolated from urine samples 69(34.5%) followed by blood 60(30%), swab 50(25%) and 21(10.5%) from the samples of sputum, secretion and tips. From a total 200 positive cultures of*Pseudomonas spp.* urology ward accounted for the highest proportion 44(18%), followed bymedical ward 36(18%). The proportions of other wards were as: OPD 30(15%), neonatology 29(14.5%), ICU 16(8%), hematologyoncology ward 14(7%), surgical ward 13(6.5%), developmental ward 7(3.5%), M/E 6(3%) and orthopedics ward 5(2.5%).
Pseudomonas spp. was found highly sensitive to piperacillin/tazobactam (87.5%). Sensitivity to other antimicrobial discs was meropenem (86.5%), sulbactam/cefoperazone (82.5%), amikacin (74.5%), ciprofloxacin (64.5%), cefotaxime (17.5%), ceftriaxone (20.0%), ceftazidime (60.5%), pipemedic acid (21.75%), nitrofurantoin (18.8%) and norfloxacin (46.4%). Sensitivity of norfloxacin, nitrofurantoin.
Conclusion: Piperacillin/tazobactam is the most active combination against *Pseudomonas spp.*and remains a very active beta-lactam when tested in vitro against clinical isolates of *Pseudomonas spp.*
Keywords: Piperacillin/tazobactam, *Pseudomonas spp.*

INTRODUCTION

Over the past century, *Pseudomonas* species have become an increasingly important pathogen, leading to the characteristically high level of lethality and persistent infections[1]. *Pseudomonas aeruginosa*, called the "epitome" of opportunistic pathogen, almost never infects uncompromised tissue. However it can infect practically any type of tissue if that tissue has some type of compromised defenses[2]. Patients who are hospitalized for extended periods are frequently colonized by this organism and are at high risk of developing infections[3].

It is a notoriously difficult to control this organism with antibiotics or disinfectants[4]. There are three basic mechanisms by which organisms resist the action of antimicrobial agents: restricted uptake and efflux, drug inactivation and changes in target. The innate resistance of *Pseudomonas aeruginosa* to all classes of antibiotics has generally been attributed to the low permeability of its cell wall[5].

Piperacillin /tazobactam is a β-lactamase inhibitor combination with a broad spectrum of antibacterial activity encompassing most Gram positive and Gram negative aerobic and anaerobic bacteria, including many pathogens producing β-lactamases[6]. For β-lactamase inhibitor combinations, susceptibility rates are higher for piperacillin/tazobactam when compared in all regions with piperacillin alone. Piperacillin/tazobactam remained a very active β-lactam drug when tested in vitro against clinical isolates of *Pseudomonas aeruginos*a[7].

Combining tazobactam, a beta-lactamase inhibitor, with the ureidopenicillin- piperacillin, successfully restores the activity of piperacillin against beta-lactamase producing bacteria. Anextremely high level of antimicrobial resistance was seen in hospital acquired infections, especially among non-enteric Gram-negative bacilli. Due to this high level of resistance, piperacillin/tazobactam represents an important contribution to the treatment of nosocomial infections[8].

METHEDOLOGY

This cross sectional study was performed at the Microbiology Department of the Children's Hospital and The Institute of Child Health Lahore, Pakistan, from July 2012 to November 2012. A total number of

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10,761 clinical samples of blood, urine, swab, sputum, tracheal secretions and tips collected from various wards were analyzed. The samples were cultured on Blood, Chocolate, MacConkey and CLED agar (Cystine Lysine Electrolyte Deficient Medium). CLED was specifically used for urine sample. *Pseudomonas* species were identified by Gram's staining,Oxidase test, Citrate test, Triple Sugar Iron test, Urease test and API 20NE system. Isolated organism was tested against various antibiotics in vitro by Kirby-Bauer disc diffusion method.

By using a sterile wire loop 3-5 well isolated colonies of similar appearance were picked up and emulsified in 3-4 ml of sterile physiological saline. In a good light the turbidity of suspension were matched against McFarland 0.5 turbidity standard.The appropriate antimicrobial discs amikacin (AK), ceftazidime (CAZ), ceftriaxone (CRO), cefotaxime (CTX), ciprofloxacin (CIP), meropenem (MEM), sulbactam/cefoperazone (SCF), piperacillin/tazobactam/ (TZP), norfloxacin (NOR), nitrofuranton (F) and pipemidic acid (PIP)were placed and evenly distributed. The plates were incubated at 37^{o}C overnight.After overnight incubation the diameter of each zone of inhibition was measured in mm. The endpoint of inhibition was where the growth started. Interpretation of zone sizes of each antimicrobial disc was made, as Sensitive, Intermediate Sensitive or Resistant using interpretation chart of zone sizes[9].

RESULTS

Out of 10,761 samples 2,375(22%) were found positive for bacterial growth, out of which 200(9.2%) were *Pseudomonasspp*. and 2,175(90.8%) were other than *Pseudomonasspp*. In this study 133 samples from male patients and 67 from female patients were positive for*Pseudomonas spp.* thus giving the ration of 1:1.9.

The highest frequency of *Pseudomonas spp*. was isolated from urine samples 69(34.5%) followed by blood 60(30%), swab 50(25%) and 21(10.5%) from the samples of sputum, secretion and tips.From a total of 200 positive cultures of *Pseudomonas spp.* urology ward accounted for the highest proportion 44(22%), followed bymedical ward 36(18%). The proportions of other wards were as OPD 30(15%), neonatology Unit 29(14.5%), ICU 16(8%), hematology Oncology ward 14(7%), Surgical Ward 13(6.5%), developmental Ward 7(3.5%), M/E 6(3%) and orthopedics ward 5(2.5%).

Antimicrobial sensitivity testing was performed by Kirby-Bauer disc diffusion method.*Pseudomonas spp*. was found highly sensitive to piperacillin/ tazobactam (87.5%) followed by meropenem (86.5). Sensitivity to other antimicrobial discs was as sulbactam/cefoperazone (82.5%), amikacin (74.5%), ciprofloxacin (64.5%), cefotaxime (17.5%), ceftriaxone (20.0%), ceftazidime (60.5%), pipemidic acid (21.75%), nitrofurantoin (18.8%) and norfloxacin (46.4%). Sensitivity of norfloxacin, nitrofurantoin and pipemidic acid was being tested only on urine samples.

Table 1: Frequency of organism

Organism	Frequency	Percentage
Pseudomonas	200	9.2
Other than *Pseudomonas*	2175	90.8
Total	2375	100

Table 2: Frequency of Sample

Sample	Frequency	Percentage
Blood	60	30.0
Swab	50	25.0
Tips/Sputum/secretion	21	10.5
Urine	69	34.5
Total	200	100

Table 3: Antibiotic Susceptibility Pattern

Antibiotics	Sensitive n (%)	Intermediate Sensitive n (%)	Resistant n (%)
piperacillin/tazobactam (TZP)	175 (87.5%)	9(4.5%)	16(8.0%)
meropenem(MEM)	173(86.5%)	6(3.0%)	21(10.5%)
sulbactam/ cefoperazone (SCF)	165 (82.5%)	10(5.0%)	25(12.5%)
ciprofloxacin(CIP)	129(64.5%)	4(2.0%)	67(33.5%)
amikacin(AK)	149(74.5%)	10(5.0%)	41(20.5%)
cefotaxime(CTX)	35 (17.5%)	5(2.5%)	160(80.0%)
ceftazidime(CAZ)	121(60.5%)	7(3.5%)	72(36.0%)
ceftriaxone(CRO)	40(20.0%)	4(2.0%)	156(78.0%)
pipemidic acid(PIP)	15(21.75%)	4(5.8%)	50(72.5%)
nitrofurantoin(F)	13(18.8%)	1(1.4%)	55(79.8%)
norfloxacin(NOR)	32(46.4%)	3(4.3%)	34(49.3%)

DISCUSSION

Over the past century, *Pseudomonas spp.* has become an increasingly important pathogen particularly in individuals with cystic fibrosis and in intensive care units worldwide.Patients who are hospitalized for extended periods are frequently colonized by this organism and are at high risk of developing infections.[10]According to the present study, 10,761 samples were collected from which 2,375 (22%) were positive and 8,386(78%) were negative for bacterial growth.*Pseudomonas spp*. was isolated in 200(9.2%) samples whereas 2,175 (90.8%) samples contained organisms other than *Pseudomonasspp*. The highest proportion of *Pseudomonas spp*. was from urology ward 44(22%) followed by medical ward 36(18%), OPD 30(15%) and neonatology Unit 29(14.5%). The highest percentage of *Pseudomonas spp.* was isolated from urine sample 34.5% followed by blood 30%, swabs 25% and 10.5% from samples of tips, sputum and secretion.

A similar study was performed in the microbiology section of Burgor Ankles aria Hospital Karachi. A total 2800 clinical specimens were received out of which 1,008 were *Pseudomonas aeruginosa.* The highest proportion 403(40%) was isolated from urine sample followed by 258(26%) from ear swab. These results are very close to the present study.[7]According to present study piperacillin/tazobactam showed highest sensitivity (87.5%) against *Pseudomonas spp.* While the sensitivity pattern of other antimicrobial discs was as; meropenem (86.5%), sulbactam/cefoparazone (82.5%), amikacin (74.5%), ciprofloxacin (64.5%), cefotaxime (17.5%), ceftriaxone (20.0%), ceftazidime (60.5%), pipemidic acid (21.75%), nitrofurantoin (18.8%) and norfloxacin (46.4%).

A similar study was conducted to investigate the susceptibility pattern of antibiotics against different strains of *Pseudomonas* isolated from medical facilities in Gifu and Aichi. According to this study piperacillin/tazobactam gave the highest susceptible rate (93.1%).[11]A similar study was performed in Department of Microbiology, Chhatrapati Shahuji Maharaj Medical University, Lucknow India. Cefepime and ceftazidime were more resistant to *Pseudomonas aeruginosa* with resistance rate 36.27 and 35.30% respectively. Least resistancewas noted for piperacillin/tazobactam andImipenem that is, 4.90% and 5.88%, respectively.[12]

Another study was performed in the Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad.In this study ticarcillin/Clavulanic acid showed just 4% sensitivity against *Pseudomonas aeruginosa* while sensitivity of piperacillin/tazobactam was 39%. The isolates exhibited high resistance to all the generations of Cephalosporins and the other groups of antibiotics except Carbapenems.[13]Another study was performed in Federal University of Sao Paulo, SP, Brazil.*Pseudomonas aeruginosa* was highly resistant to all beta-lactams evaluated and piperacillin/ tazobactam was the most active compound against this species[14].

CONCLUSION

This study shows that piperacillin/tazobactam is the most active combination against *Pseudomonas spp.*and remains a very active beta/lactam when tested in vitro against clinical isolates of *Pseudomonas spp.*However, caution is required; the use of this drug must be restrictive and discriminative to prevent a rapid development of drug resistance.

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