Abstract

Background: The emergence and subsequent widespread dissemination of bacterial resistance to a variety of β-lactam antibiotics by the elaboration of ESBL (Extended Spectrum Beta Lactamase) poses a serious threat to the effective use of beta lactam antibiotics and cephalosporins. Cefoperazone combination with the beta lactamase inhibitor tazobactam would be a strong basis for rational therapeutics when dealing with ESBL producing pathogen mediated infections.

Objective: The objective of the study was to investigate the in vitro efficacy of cefoperazone-tazobactam and cefoperazone-sulbactam against ESBL producing respiratory and urinary pathogens.

Methodology: 54 samples (34 samples of urine and 20 samples of sputum) were collected from 9 hospitals in Mumbai. The pathogens isolated from urine included *E.coli* (*n=27*), *K. pneumoniae* (*n=6*), and *Serratia marcescens* (*n=1*). The pathogens isolated from sputum included *Pseudomonas aeruginosa* (*n=5*), *Streptococcus pneumoniae* (*n=1*) and *Klebsiella pneumoniae* (*n=14*). The Kirby Bauer disc diffusion method was used to evaluate the susceptibility of the isolated pathogens to cefoperazone-tazobactam and cefoperazone-sulbactam.

Results: In sputum samples, all the isolates of *Pseudomonas aeruginosa* were susceptible to cefoperazone-tazobactam while one isolate was resistant to cefoperazone-sulbactam. *Streptococcus pneumoniae* and *Klebsiella pneumoniae* were sensitive to both the antibiotic formulations.

In uropathogens, higher susceptibility rates to cefoperazone-tazobactam (96% vs. 89%) were observed for *Escherichia coli*. Similarly higher rates of susceptibility to cefoperazone-tazobactam were observed for *Klebsiella* as compared to cefoperazone-sulbactam (83% vs. 67%).

*Serratia marcescens* was sensitive to cefoperazone-tazobactam but was intermediate resistant to cefoperazone-sulbactam.

The isolates of *Klebsiella pneumoniae* that were resistant to cefoperazone-sulbactam were susceptible to cefoperazone-tazobactam.

Conclusion: The results of the current in vitro study corroborate the efficacy of the beta lactamase inhibitor tazobactam in improving the spectrum of activity and efficacy of cefoperazone.

Introduction

The treatment of infections in India is based on the tenet of empirical therapy. The efficacy of the older broad spectrum antibiotics has been questioned with the observed advent of longer resolution times of infections, treatment relapses and treatment failures resulting in an increased morbidity and mortality.

The emergence and subsequent widespread dissemination of bacterial resistance to a variety of β-lactam antibiotics poses a serious threat to the effective use of these antimicrobial agents.

One of the key resistance mechanisms observed in bacteria is the secretion of Extended Spectrum Beta Lactamase enzymes (ESBL). The destruction of beta lactam antibiotics and cephalosporins by these ESBL producing pathogens has become a cause for grave concern today when dealing with infections in the community, nosocomial infections or infections in critically ill patients.

The ESBL enzymes are plasmid-mediated enzymes and result due to mutations of TEM-1 and TEM-2 and SHV-1. The challenge of ESBL is compounded further by the diagnostic challenge posed by ESBL. Most of the local laboratories are not equipped to detect ESBL.

New antibiotics or new combinations of antibiotics would be required to overcome the current threat posed by ESBL secreting pathogens. Majority of ESBL producing strains are *K. pneumoniae, K. oxytoca* and *E.coli*. Other organisms include *Enterobacter* spp., *Salmonella* spp., *Morganella morganii*, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas aeruginosa*.

One proved strategy is the combination of beta lactamase inhibitors with the beta lactam antibiotics or cephalosporins.
There is an eight fold reduction in the MIC of a cephapirin in presence of beta lactamase inhibitors and this phenomenon can be utilized for detection of ESBL production. With a high prevalence of infections due to ESBL positive bacteria, a parallel increase in the use of these combinations is being observed. Hence there is a need to determine the susceptibility pattern of different microorganisms against the commercially available combination agents, knowledge of which is essential to guide empiric as well as appropriate therapy of severe infections in hospitalized patients.

Amongst the β-lactamase inhibitors, tazobactam shows greater in vitro activity than sulbactam against extended-spectrum and conventional spectrum plasmid-mediated β-lactamases. Cefoperazone is a β-lactam antibiotic particularly effective against the difficult to eradicate pathogens such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii. Cefoperazone is an invaluable drug in the current scenario of infections. But the efficacy of cefoperazone is threatened by the ESBL producing pathogens.

Choosing a 3rd generation cephalosporin such as cefoperazone for combination with tazobactam, a potent beta lactamase inhibitor would be a strong basis for rational therapeutics. The present study was carried out to study the in-vitro activities of cefoperazone tazobactam and cefoperazone sulbactam against ESBL pathogens in respiratory and urinary infections.

Materials and Methods

Bacterial Isolates

A total of 54 samples (34 samples of urine and 20 samples of sputum) were collected from 9 hospitals in Mumbai during January 2010 to March 2010. The pathogens isolated from sputum included Pseudomonas aeruginosa (n=5), Streptococcus pneumoniae (n=1) and Klebsiella pneumoniae (n=14). The pathogens isolated from urine included E.coli (n=27), K. pneumoniae (n=6), and Serratia marcescens (n=1). The strains were identified at a reputed NABL accredited lab in Mumbai.

The methodology of testing

The Kirby-Bauer method was used for antimicrobial susceptibility testing as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The antibiotic susceptibility discs for cefoperazone sulbactam (75/30 µg) were prepared as recommended by the Clinical and Laboratory Standards Institute (CLSI) which was formerly the NCCLS. The discs of cefoperazone-tazobactam were also prepared with similar concentrations (75/30 µg) from Hi-media, Mumbai (India).

The predetermined batteries of antimicrobial discs were dispensed onto the surface of the inoculated agar plate. The plates were placed in an incubator set to 35°C within 15 minutes after the discs were applied.

Reading the Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate was examined. The strains were identified and characterized by standard tests. The control strains used were E.coli NCTC 10418 and Pseudomonas aeruginosa NCTC 10662.

Susceptibility testing

The zones of inhibition were compared with the reference ranges provided by NCCLS for cefoperazone. The E. coli 25922 strain was used as a quality control strain for susceptibility testing. This assumption was based on the premise that the NCCLS break points for defining susceptible and resistant strains of amoxicillin and amoxicillin clavulanic acid do not differ. Therefore the break points for cefoperazone-sulbactam and cefoperazone-tazobactam would be similar to those of cefoperazone.

The susceptibilities were classified as sensitive (S), intermediate resistant (I) and resistant (R) depending upon the reported break points for cefoperazone. The term non-susceptible was used for both resistant and intermediate susceptible strains.

Results

The chief pathogens isolated from sputum specimens were Streptococcus pneumoniae (5%), Pseudomonas aeruginosa (25%) and Klebsiella pneumoniae (70%).

All the isolates of Pseudomonas aeruginosa were susceptible to cefoperazone-tazobactam while one isolate was resistant to cefoperazone-sulbactam.

Streptococcus pneumoniae and Klebsiella pneumoniae were sensitive to both the antibiotic formulations.

The chief pathogens isolated from urine specimens were Escherichia coli (79%) and Klebsiella pneumoniae (18%). Serratia marcescens (3%) was isolated from one sample only.

Higher susceptibility rates to cefoperazone tazobactam (96% vs. 89%) were observed for Escherichia coli in the urine isolates. Similarly higher rates of susceptibility to cefoperazone-tazobactam were observed for Klebsiella as compared to cefoperazone-sulbactam (83% vs. 67%) (Table 1).

Serratia marcescens was sensitive to cefoperazone-tazobactam but showed intermediate resistance to cefoperazone-sulbactam.

The isolates of Klebsiella pneumoniae that were resistant to cefoperazone-sulbactam were susceptible to cefoperazone-tazobactam.

Discussion

The current trends of infections in India portend the presence of ESBL producing pathogens. Resistance to the common beta lactam antibiotics and cephalosporins is the consequent outcome of this trend.

Emergence of multi and pan-drug resistant gram-negative bacteria causing nosocomial infections in intensive care settings has become a challenge for clinicians. The mortality rate of ventilator-associated pneumonia (VAP) is known to increase when the initial microbiological diagnosis and antimicrobial therapy are inappropriate.

Major outbreaks involving ESBL strains have been reported from all over the world, thus making them emerging pathogens. Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of resistance to third generation cephalosporin use and instrumentation or catheterization.

The limitations in the Indian scenario include the high cost
of the tests for detection of ESBL production, non-availability of higher antibiotics in rural areas and lack of awareness about antibiotic resistance poses serious problems to clinicians during their therapeutic practice. With increasing resistance pattern amongst penicillins and cephalosporins, there is a need for development of more effective as well as affordable therapeutic options.

Beta lactam - beta lactamase inhibitor combinations can be viewed as antimicrobial agents of potential use in the treatment of multidrug resistance.

Cefoperazone achieves high concentrations in the lung tissues as well as in the urinary tract. These values are higher than MIC90 values of cefoperazone for pathogens such as Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Haemophilus influenzae and Enterobacteriaceae.5,6

The combination of cefoperazone tazobactam is a true synergistic combination because of the absence of functional antagonism between cefoperazone and tazobactam. Tazobactam does not induce AMPC enzymes that attack the cephalosporin but Clavulanate induces AMPC enzymes which attack the cephalosporins.7 The rising rates of resistance to sulbactam make tazobactam an important beta lactamase inhibitor for combining with cefoperazone.

In this study, we found a trend towards improved susceptibility of common respiratory and urinary tract infection related pathogens to cefoperazone-tazobactam as compared to cefoperazone-sulbactam.

Our results corroborate the earlier reports of superior efficacy of tazobactam as a beta lactamase inhibitor as compared to sulbactam.2 These differences were found to be greater for E.coli, P. aeruginosa and K. pneumoniae. However, the clinical implications of these findings remain to be investigated in large, randomized controlled clinical trials.

The encouraging finding in our study was the low resistance to cefoperazone-tazobactam in gram negative pathogens. Our study highlights the need for antimicrobial susceptibility pattern determination from time to time so that proper guidelines for hospital antibiotics policies can be developed.

The limitations of the current in-vitro study were the small sample size. Further studies are required to define the break points for cefoperazone-tazobactam as it has not been listed in the NCCLS guidelines. Validation of these in vitro results in controlled clinical trials will be required to be undertaken in the future.

**Conclusion**

The results of the current in-vitro study corroborate the efficacy of the beta lactamase inhibitor tazobactam in improving the spectrum of activity and efficacy of cefoperazone. Thus the combination of cefoperazone-tazobactam is likely to be a useful addition to the therapeutic armamentarium of the Indian clinicians. The difference in activities in these combination agents needs to be evaluated further by ascertaining their efficacy in clinical study.

**References**