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Comparative Studies on Susceptibility and Minimum Inhibitory Concentration of Potentox, a Fixed Dose Combination of Cefepime Amikacin in *Proteus vulgaris*, *Escherichia coli* and *Bacillus subtilis*

S.M. Shrivastava and M. Chaudhary

Present study was undertaken to evaluate antimicrobial response of potentox against *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis*. Minimum Inhibitory Concentration (MIC) and Antibiotic Susceptibility Test (AST) of potentox was performed on test organisms in comparison with cefepime and amikacin individually. In case of *P. vulgaris*, *E. coli*, *B. subtilis*, MIC were found to be 0.25, 0.5 and 1 mg L⁻¹, for potentox, respectively. In cefepime alone the MIC was found to be 1, 2 and 8 mg L⁻¹, respectively and in amikacin alone the MIC was found to be 2, 2 and 4 mg L⁻¹. The AST result shows that potentox is having more lytic zone than cefepime and amikacin alone. In organisms under study, antimicrobial response of Potentox was found to be better than any of it's components.

Key words: MIC, AST, cefepime, amikacin, potentox

INTRODUCTION

Cefepime is a broad spectrum parenteral fourth generation cephalosporin group of antibiotic with significant potential advantages over other antibiotics (Clarke *et al.*, 1985; Kessler *et al.*, 1985; Tsuji *et al.*, 1985). Other than having a very broad antimicrobial spectrum, cefepime appears to have low affinity for major chromosomally mediated β -lactamases and therefore it is less affected by the nonhydrolytic barrier mechanism of resistance in these bacteria (Phelps *et al.*, 1986). Cefepime has high affinity for essential penicillin-binding proteins and it has zwitter ionic structure (Wynd and Paladino, 1996; Barradell and Bryson, 1994). However, resistance of antibiotics has developed during their clinical use (French, 2005). There have been cases of failure of cefepime therapy, which are linked with development of resistance against cefepime (Bhat *et al.*, 2007; Song *et al.*, 2005).

Amikacin is used to treat different types of bacterial infections. Amikacin is not active against anaerobes as it is semi synthetic amino glycoside antibiotic for the treatment some Gram-negative and other infections. Amino glycosides are used in combination with other antibiotics such as penicillin in the treatment of serious infections with aerobic Gram-negative bacilli, including infections caused by *Pseudomonas*, complicated urinary tract infections and enterococcal endocarditis.

Mode of action of amikacin is by binding to the bacteria 30 S ribosome subunit and causing misreading of m-RNA and leaving the bacterium unable to synthesize proteins vital to its growth. It is most often used for treating severe, hospital-acquired infections with multi drug resistant gram negative bacteria such as *P. vulgaris*, *E. coli*, *B. subtilis*.

Combination therapy of cephalosporins with an amino glycoside has commonly been recommended because this approach provides broad-spectrum coverage against infections, bactericidal activity, reduced chances of resistance development and potential synergistic effect. It is evident that the Fixed Dose Combination (FDC) minimizes the development of resistance during treatment (Hughes *et al.*, 1997). Extended spectrum β -lactamases (ESBL) production is one of the main mechanisms of resistance to β -lactam antibiotics among the strains of family Enterobacteriaceae (Jacoby and Medeiros, 1991). The therapeutic choices in infections caused by such strains remain limited because of cross resistance (Brun-Buisson *et al.*, 1987).

Conflicting reports have been published concerning the activities of the broad-spectrum and fourth generation cephalosporins with an explanation of the inoculum effect (Caron *et al.*, 1990; Jett *et al.*, 1995;

Thauvin-Eliopoulos *et al.*, 1997). Cefepime and amikacin acts synergistically and has a broad spectrum *in-vitro* activity that active against a wide range of Gram positive and gram negative bacteria providing a better therapeutic choice. There are some studies available demonstrating antimicrobial activity of potentox was better than cefepime and amikacin alone in *E. cloacae*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* (Shrivastava *et al.*, 2008).

The present study is aimed at evaluating *in vitro* antimicrobial response of potentox, a FDC of Cefepime and amikacin in comparison with cefepime and amikacin alone with the assumption that the combination of cefepime amikacin in potentox will have better antimicrobial activity.

MATERIALS AND METHODS

This study is carried out in the laboratories of Venus Medicine Research Centre, India from January 2009 to February 2009.

Bacterial strains: Strains obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India, used for the study are *Proteus vulgaris* (MTCC No-426), *Escherichia coli* (MTCC No-1687), *Bacillus subtilis* (MTCC No-736).

Antibiotics: Potentox, cefepime and amikacin used in study were provided by manufacturer (Venus Remedies Limited, India) for the study.

Medium: Mueller- Hinton (MH) broth supplemented with calcium (25 mg L⁻¹) and Magnesium (1.25 mg L⁻¹) was used for MIC studies. MH agar was used for AST.

Susceptibility testing: The MIC of cefepime and amikacin, alone and FDC was done against *Proteus vulgaris*, *Escherichia coli* and *Bacillus subtilis*. The MIC was determined by broth micro dilution method as per the standard. Overnight MH broth cultures were used in this study and prepared inocula of 10⁵ cfu mL⁻¹. The MIC was determined after incubation for 24 h at 37°C.

In AST, MH agar plates were prepared which was pre inoculated with test organisms. Eight plates were used for each antibiotic under evaluation. 30/7.5, 30 and 10 μ g of potentox, cefepime and amikacin were added in wells prepared on each MH agar plates, respectively. The plates were incubated at 37°C for 24 h. Lytic zone was measured using zone reader. One-way Analysis of Variance (ANOVA) was used to determine statistical difference between lytic zones of potentox, cefepime and amikacin in all organisms under study. p-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

MIC studies: In case of *P. vulgaris*, *E. coli* and *B. subtilis* MIC were found to be 0.25, 0.5 and 1 mg L⁻¹ for potentox, respectively. In cefepime alone the MIC was found to be 1, 2 and 8 mg L⁻¹, respectively. For amikacin alone, these values were 2, 2 and 4 mg L⁻¹, respectively (Table 1).

Susceptibility studies: Antimicrobial Susceptibility Test (AST) of all microbial strains under study resulted in significant increase in lytic zone size in Potentox when compared with cefepime and amikacin alone.

In the present study, results of lytic zone measurement of *P. vulgaris*, *E. coli*, *B. subtilis* for cefepime were 29.11, 22.87 and 24.53 mm, respectively. Zone size for amikacin was found to be 23.01, 20.49 and 22.21 mm, respectively and for potentox 33.01, 26.49 and 27.21 mm, respectively. In all organisms under study lytic zone of Potentox was found to significantly (p<0.05) more when compared to cefepime or amikacin alone (Table 2).

Cefepime is known to cross the bacterial outer membrane faster resulting in fast bactericidal activity. This unique advantage of rapid penetration against Gram positive and Gram negative organisms results in bactericidal activity of this drug (Angelescu and Apostol, 2001). Amikacin has the property to be active against resistant bacteria to other amino glycoside. There has been practice of using FDC of various classes of drugs to resistance and to provide better bactericidal activity. There has been reduced number of choices for treatment of such resistant bacteria. There has been need to develop a FDC which provides better bactericidal effect, widened spectrum and reduced possibility of development of resistance. Potentox has the potential to be placed under such type of FDC. Earlier reports, have claimed that antimicrobial activity of potentox was better than cefepime and amikacin alone in *E. cloacae*,

S. aureus, *K. pneumoniae* and *P. aeruginosa* (Shrivastava *et al.*, 2008). There are also available evidences of Human trials with FDC of cefepime and amikacin with better results of FDC in clinical conditions (Chaudhary *et al.*, 2008). Present study is aimed at comparing potentox with cefepime and amikacin alone in *P. vulgaris*, *E. coli* and *B. subtilis*. There is no earlier report of evaluating efficacy of Potentox in *Proteus vulgaris*, *Escherichia coli* and *Bacillus subtilis*. Present results demonstrated that potentox has lower MIC value than cefepime and amikacin individually in all organisms under study, suggesting higher bactericidal activity of potentox in comparison to its ingredients. In case of *P. vulgaris*, The lytic Zone size of potentox was found to be more than 10% and more than 40% higher in comparison to cefepime and amikacin suggesting better bactericidal potential of potentox. Similarly, for *E. coli* and *B. subtilis* lytic zone for Potentox was higher than cefepime and amikacin alone. The study demonstrates that cefepime and amikacin acts synergistically and achieves higher *in vitro* antibacterial activities in *Proteus vulgaris*, *Escherichia coli* and *Bacillus subtilis* as evident by better bactericidal activity of potentox.

In conclusion, the results of MIC and AST studies are in similar pattern and potentox has better antimicrobial response in *Proteus vulgaris*, *Escherichia coli* and *Bacillus subtilis* than cefepime and amikacin alone. Use of potentox in clinical condition can be a better choice in combating infections caused by the study organisms.

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Table 1: Results of comparative minimal inhibitory concentration studies of cefepime, amikacin and potentox

Microorganisms	Values of MIC (mg L ⁻¹)		
	Cefepime	Amikacin	Potentox
<i>P. vulgaris</i>	1	2	0.25
<i>E. coli</i>	2	2	0.50
<i>B. subtilis</i>	8	4	1.00

Table 2: Results of comparative antimicrobial susceptibility test studies of cefepime, amikacin and potentox

Microorganisms	Zone diameter (mm)		
	Cefepime	Amikacin	Potentox
<i>P. vulgaris</i>	29.11±0.7015	23.01±0.5934	33.01±0.6834
<i>E. coli</i>	22.87±0.7568	20.49±0.6078	26.49±0.5078
<i>B. subtilis</i>	24.53±0.3760	22.21±0.6330	27.21±0.7530

Data are expressed as Mean±SD

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