

Microbial efficacy analysis of potentox, a fixed dose combination of cefepime amikacin with cefepime and amikacin alone in a *Citrobacter braaki*, *Mycobacterium smegmatis*, *Acinetobacter baumannii* and *Neisseria mucosa*

SANJAY MOHAN SHRIVASTAVA,* SANJEEV KUMAR SHUKLA,
SHAILESH KUMAR and MANU CHAUDHARY

Venus Medicine Research Centre, Hill Top Industrial Estate, Jharmajri EPIP,
Phase I (Extn) Bhatoli Kalan, Baddi - 173 205 (India).

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ABSTRACT

A newly developed extended spectrum fourth generation cephalosporins cefepime, has been shown to have good activity against both gram negative organisms. Amikacin is an aminoglycoside antibiotic used to treat different types of bacterial infections. Potentox, a Fixed Dose Combination (FDC) of cefepime and amikacin has a wider range of susceptibility to any of these drugs individually. The *in vitro* and *in vivo* effectiveness of Potentox was studied using a high inoculum of an extended spectrum β -lactamase producing *Citrobacter braaki*, *Mycobacterium smegmatis*, *Acinetobacter baumannii* and *Neisseria mucosa* strain. This study was aimed at evaluating microbial efficacy of Potentox, a FDC of cefepime and amikacin, in comparison with cefepime and amikacin alone. Efficacy was evaluated on the basis of Minimum Inhibitory Concentration (MIC), Antibiotic Susceptibility Test (AST) analysis in *C. braaki*, *M. smegmatis*, *A. baumannii* and *Neisseria mucosa*. In case of *C. braaki*, *M. smegmatis*, *A. baumannii* and *Neisseria mucosa* MIC were found to be in potentox 0.421mg/l, 0.625mg/l, 0.342mg/l and 0.423 mg/l. In cefepime alone the MIC were found to be 1.67mg/l, 0.52mg/l, 0.84mg/l and 2.67 mg/l respectively and in amikacin alone the MIC were found to be 3.34mg/l, 1.67mg/l, 2.67mg/l and 1.0mg/l respectively. Antibiotic susceptibility result are given below. In all organisms under study, potentox was found to have more bacterial inhibiting properties than cefepime and amikacin alone *in vitro*.

Key words: MIC, Cefepime, Amikacin, Potentox.

INTRODUCTION

Cefepime is a new broad spectrum parenteral fourth generation cephalosporin antibiotic with significant potential advantages over other broad spectrum cephalosporins and some nontraditional β -lactam antibiotics^{1, 2, 3}. In addition to a very broad antimicrobial spectrum, cefepime appears to have low affinity for major chromosomally mediated, β -lactamases and those it less affected by the nonhydrolytic barrier mechanism of resistance in these bacteria⁴. Its high affinity for essential penicillin binding proteins, and its zwitterionic structure^{5, 6}. Cefepime also appears to have a low propensity toward the development of resistance. Cefepime is generally active against gram negative bacteria resistance to other broad

spectrum cephalosporins. Broad spectrum cephalosporins and aminoglycosides are highly active against aerobic gram negative bacteria. However, resistance to these agents has developed during their clinical use. These *in vitro* advantages have been borne out in a number of *in vivo* infection modal⁷. It is a active against a wider range of gram positive and gram negative aerobic organism.

Combination therapy with an aminoglycoside plus an anti pseudomonal β -lactam has commonly been recommended because this approach provides broad spectrum coverage, bactericidal activity and potential synergistic effects, and minimizes the development of resistance during treatment⁸. Extended spectrum β -lactamases (ESBL) production is one of the main mechanisms

of resistance to β -lactam antibiotics among the strains of family Enterobacteriaceae⁹. The therapeutic choices in infections caused by such strains remain limited because of cross resistance¹⁰.

Conflicting reports have been published concerning the activities of the broad-spectrum and fourth-generation cephalosporins with an explanation of the inoculum effect^{11, 12, 13}. Cefepime and amikacin act synergistically and have a broad spectrum *in vitro* activity that encompasses a wide range of gram-positive and gram-negative bacteria. Cefepime has a low affinity for chromosomally encoded β -lactamases.

Amikacin is an aminoglycoside antibiotic used to treat different types of bacterial infections. Amikacin works by binding to the bacteria 30S ribosome subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth. Amikacin is most often used for treating severe, hospital-acquired infections with multi-drug resistant gram-negative bacteria such as *C. braaki*, *M. smegmatis*, *A. baumannii* and *Neisseria mucosa*.

Amikacin is a semi-synthetic aminoglycoside antibiotic for the treatment of some gram-negative

and other infections. Amikacin belongs to the aminoglycosides and is active against aerobic gram-negative bacilli, including *Pseudomonas*. It does not have activity against anaerobes, and alone they are inactive against streptococci. Aminoglycosides are usually used in the treatment of serious infections with aerobic gram-negative bacilli, including *Pseudomonas*, complicated urinary tract infections.

MATERIAL AND METHODS

Bacterial Strains

Following strains obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India were used for the study, *Citrobacter braaki* (MTCC No. - 2690), *Mycobacterium smegmatis* (MTCC No. - 995), *Acinetobacter baumannii* (MTCC No. - 1425) and *Neisseria mucosa* (MTCC No. - 1722).

Antibiotic

Cefepime, amikacin and potentox 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) and Magnesium (1.25 mg/l)

Table 1 : Results of Minimal Inhibitory Concentration Studies of potentox, a fixed dose combination of cefepime amikacin with cefepime and amikacin alone

S. No.	Micro-organisms	Mean Values of MIC (mg/l)		
		Cefepime	Amikacin	Potentox
1	<i>C. braaki</i>	1.67	3.34	0.421
2	<i>M. smegmatis</i>	0.52	1.67	0.625
3	<i>A. baumannii</i>	0.84	2.67	0.342
4	<i>N. mucosa</i>	2.67	1.00	0.423

Table 2: Results of Antimicrobial Susceptibility Test studies of potentox, a fixed dose combination of cefepime amikacin with cefepime and amikacin alone

S. No.	Microorganism	Zone diameter (mm)		
		Cefepime Avg. \pm S.D	Amikacin Avg. \pm S.D	Potentox Avg. \pm S.D
1	<i>C. braaki</i>	30.71 \pm 0.7560	21.70 \pm 0.5960	33.20 \pm 0.5853
2	<i>M. smegmatis</i>	23.50 \pm 0.3760	22.63 \pm 0.7350	29.11 \pm 0.6230
3	<i>A. baumannii</i>	30.70 \pm 0.6330	17.65 \pm 0.3870	32.80 \pm 0.6280
4	<i>N. mucosa</i>	32.70 \pm 0.5078	21.80 \pm 0.3652	34.15 \pm 0.5162

Mean value \pm Standard deviation value

was used for susceptibility tests. Colony counts were determined with MH agar plates.

Susceptibility Testing

The Minimum Inhibitory Concentration (MIC) of potentox, cefepime and amikacin alone, against *C. braaki*, *M. smegmatis*, *A. baumannii* and *N. mucosa* were determined by broth microdilution method as per the standard National Committee for Clinical Laboratory Standards¹⁴. Overnight MH broth cultures were used to prepare inocula of 10⁵ CFU/ml. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 hours of incubation at 37°C.

RESULTS

MIC studies

In case of *C. braaki*, *M. smegmatis*, *A. baumannii* and *N. mucosa* MIC were found to be in potentox 0.421mg/l, 0.625mg/l, 0.342mg/l and 0.423 mg/l. In cefepime alone the MIC were found to be 1.67mg/l, 0.52mg/l, 0.84mg/l and 2.67 mg/l respectively and in amikacin alone the MIC were found to be 3.34mg/l, 1.67mg/l, 2.67mg/l and 1.0mg/l. (Table 1).

Susceptibility Studies

Antimicrobial Susceptibility Test of all microbial strains under study resulted in significant reduction in potentox when compared with cefepime and amikacin alone. (Table 2)

In case of *C. braaki*, *M. smegmatis*, *A. baumannii* and *N. mucosa* AST were found of zone diameter to be 30.71mm, 23.50mm, 30.70mm and 32.70mm respectively of cefepime, for amikacin 21.70mm, 22.63mm, 17.65mm and 21.80mm respectively and for potentox 33.20mm, 29.11mm, 32.80mm and 34.15mm respectively.

DISCUSSION

The inappropriate use of antibiotics has contributed to the emergence of resistance globally with gram negative bacilli and gram positive bacteria¹⁵. The emerging mechanism of antibacterial resistance have compromised the effectiveness of the β -lactam. Cefepime is a newly developed fourth generation cephalosporins with an extended spectrum of activity against many gram positive

bacteria and gram negative organism, including multi resistance gram negative bacteria. Amikacin is particularly effective when used against bacteria that are resistant to other aminoglycosides, since its chemical structure makes it less susceptible to several inactivating enzymes. Antibiotic combinations including a β -lactam and an aminoglycoside have frequently produced an increased bactericidal effect in *in vivo* experimental models of aerobic gram negative bacillary infections which has generally paralleled an increased rate of killing *in vitro*¹⁶.

Combination therapy with an aminoglycoside plus an anti pseudomonal β -lactam has commonly been recommended because this approach provides broad spectrum coverage, bactericidal activity and potential synergistic effects, and minimizes the development of resistance during treatment. To start with mono therapy/combination broad spectrum empiric antibiotics are used, then switching to narrow spectrum specific therapy as guided by microbiological result. Appropriate β -lactam antibiotics are recommended in international and German guidelines for the treatment of mono therapy and combination therapy.¹⁷ In comparison with older cephalosporins, cefepime crosses the bacterial outer membrane faster. Cefepime has advantages of rapid penetration in periplasmic space and extended spectrum of activity that include gram positive and gram negative organisms¹⁸.

The therapeutic choices in infections caused by such strains remain limited because of cross resistance. Potentox acts synergistically and has a broad spectrum *in vitro* activity that encompasses a wide range of gram positive and gram negative bacteria. Susceptibility data from our study demonstrated that potentox has lower MIC value than cefepime and amikacin alone, suggesting higher bactericidal activity in potentox. The *in vitro* susceptibilities of ESBL producing strains to cefepime have been found to be 52 or 90%.¹⁹ Cefepime was recommended for the treatment based on this *in vitro* susceptibility²⁰. Cefepime and amikacin acts synergistically and has a broad spectrum *in vitro* activity that encompasses a wide range of gram positive and gram negative bacteria. Cefepime has a low affinity for chromosomally encoded β -lactamases.

In conclusion, the results by the statistical analysis of MIC and AST studies are in similar pattern for *C. braaki*, *M. smegmatis*, *A. baumannii*

and *N. mucosa* Potentox has shown better bactericidal effect than cefepime and amikacin alone in organisms under study.

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