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Full Length Research Paper

In vitro microbial efficacy analysis of Supime, a fixed dose combination of cefepime and sulbactam, in comparison with cefepime alone

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Cefepime is a fourth generation cephalosporin having an extended spectrum of activity against gram positive and gram negative bacteria. Sulbactam is a β-lactamase inhibitor similar in structure to clavulanic acid. In presence of sufficient β-lactamase inhibitor, the β-lactamase enzymes are neutralized and thus the drug used in combination with inhibitor has an opportunity to be more bactericidal and is of therapeutic value in treatment of certain microbial infections. This study was aimed at evaluating microbial efficacy of Supime, a fixed dose combination (FDC) of Cefepime and Sulbactam, in comparison with cefepime alone. Efficacy was evaluated on the basis Antibiotic Susceptibility Test (AST), Minimum Inhibitory Concentration (MIC) and Time Kill Curve (TKC) analysis in *Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae* and *Enterobacter cloacae.* In all organisms under study, Supime was found to have more bacterial inhibiting properties than cefepime *in vitro*.

Key words: Cefepime, sulbactam, supime, minimum inhibitory concentration.

INTRODUCTION

Bacteria have acquired a variety of mechanisms to resist the action of antibiotics. The production of β-lactamases, enzymes that destroy penicillins and cephalosporins by hydrolyzing their β-lactam nucleus, is the most common mechanism of resistance (Williams, 1997). β-lactamase was first identified in *Escherichia coli* in 1940 (Rolinson, 1991).

Cephalosporins are used into clinical practice and they have served as efficacious and fairly safe agents for the management of many serious infections (Donowitz and Masndell, 1993). 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 (Clarke et al., 1985; Tsuji et al., 1985). In addition to a very broad antimicrobial spectrum, cefepime appears to be less affected by the non hydrolytic barrier mechanism of resistance in some bacteria (Phelps et al., 1986). Cefepime has high affinity for essential penicillin binding proteins and has zwitter ionic structure (Wynd and Paladino, 1996). Extended spectrum β-lactamases (ESBL) production is one of the main mechanisms of resistance to β-lactam antibiotics among the strains of family Enterobacteriaciaceae (Jacoby and Medeiros, 1991).

Sulbactam is a β-lactamase inhibitor similar in structure to clavulanic acid having very limited antibacterial properties (Levy et al., 1988). Sulbactam combines with some clinically relevant β-lactamases in an irreversible manner. If sufficient inhibitor is present at the site of infection, the β-lactamase enzymes should be neutralized and thus the drug used in combination with inhibitor should have an opportunity to inhibit bacterial growth (Barry and Jones, 1988).

The use of β-lactamase inhibitors in combination with β-lactam antibiotics is currently the most successful strategy to combat a specific resistance mechanism in case of microbial infections (Koch, 2000). Their broad spectrum of activity originates from the ability of respective inhibitors to inactivate a wide range of β-lactamases produced by gram positive, gram negative,

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anaerobic and even acid fast pathogens.

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 sulbactam acts synergistically and has a broad spectrum *in vitro* activity that in encompasses a wide range of gram positive and gram negative bacteria.

Present study is aimed at microbial efficacy analysis of Supime, a fixed dose combination (FDC) of Cefepime and Sulbactam, in comparison with Cefepime alone in *Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae and Enterobacter cloacae.*

MATERIALS AND METHODS

Bacterial strains

Following strains, not tested for β - lactamase production, obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India were used for the study: *S. aureus* (MTCC No. - 737), *P. mirabilis* (MTCC No - 425), *K. pneumoniae* (MTCC No. - 109) and *E. cloacae* (MTCC No. - 509).

Antibiotic

Supime, Cefepime and Sulbactam 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) was used for susceptibility tests and killing curve experiments. Colony counts were determined with MH agar plates.

Antibiotic susceptibility test

The Antibiotic Susceptibility Test (AST) of Cefepime Sulbactam combination and Cefepime alone and against *S. aureus*, *P. mirabilis*, *K. pneumoniae* and *E. cloacae* were determined by measurement test for the lysis zone development in MH agar plates in concentration of 30 µg for Cefepime and 40 µg (in ratio of 3:1 of Cefepime and Sulbactam) per disc.

Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) of Supime and cefepime alone, against *S. aureus*, *P. mirabilis*, *K. pneumoniae* and *E. cloacae* were determined by broth micro dilution method as per the standard National Committee for Clinical Laboratory Standards (NCCLS, 1997). Overnight MH broth cultures were used to prepare inocula of 10^5 CFU/ml. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37°C.

Time kill curve studies

For each strain, time kill curve studies were performed in MH broth with an inoculum of 5×10^6 - 1×10^7 CFU/ ml in the presence of Supime and cefepime individually. A flask of inoculated MH broth with no antibiotic served as a control. The surviving bacteria were counted after 0, 4 and 8 h of incubation at 37°C by subculturing 50 µl serial dilutions (in 0.9% NaCl) in to MH plates with a spiral plater.

Statistical analysis

All values are expressed in mean ± SD. One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between different groups under study. P values <0.05 were considered statistically significant.

RESULTS

Antibiotic susceptibility test (AST)

The AST of all microbial strains under study resulted in statistically significant ($p < 0.001$) increased zone measurement in Supime than cefepime alone (Table 1).

Minimum inhibitory concentration studies

In case of S. *aureus*, *P. mirabilis*, *K. pneumoniae* and *E. cloacae* MIC were found to be 0.5 µg/mL, 1 µg/mL, 8 µg/mL and 4 µg/mL for Supime respectively. In a cefepime alone the MIC was found to be 1, 4, 32 and 8 µg/mL.

Time kill curve analysis

Bactericidal effect with 2 x the MIC of Supime achieved the earliest killing at 4 h. Bacterial killing rate in Supime was distinctly higher at 8 hours than cefepime alone.

In a *S. aureus,* time kill curve analysis demonstrated statistically significant ($p<0.001$) bacterial killing rate at 4 h from 6.20 - 4.11 Log_{10} CFU /ml for Supime when compared to 6.23 - 5.68 Log_{10} CFU /ml by 4 h for cefepime. After 8 h, bacterial count was found to be 4.91 Log_{10} CFU /ml for Supime and for cefepime 5.68 - 6.15 Log_{10} CFU /ml and the difference at this point was marked statistically significant ($p < 0.001$) (Figure 1).

Cefepime has killing of 5.95 - 5.89 Log_{10} CFU /ml, 5.66 - 5.95 Log_{10} CFU /ml and 4.62 - 4.99 Log_{10} CFU /ml after 4 - 8 h in *P. mirabilis*, *K. pneumoniae* and *E. cloacae* respectively. When Supime was tested with organism bacterial killing was found to be 5.56 - 5.74 Log_{10} CFU /ml, 5.58 - 5.85 Log_{10} CFU /ml and 4.18 - 4.29 Log_{10} CFU /ml after 4 - 8 h was in *P. mirabilis*, *K. pneumoniae* and *E. cloacae* respectively (Figure 2, 3 and 4). *P. mirabilis* and *E. cloacae* are recorded with statistically significant ($p < 0.001$) change of bacterial count at 4 h and non significant change at 8 h of time kill study. The change in colony count in *K. pneumoniae* was statistically non significant at both time points.

Table 1. Results of comparative antimicrobial susceptibility test studies of cefepime alone and supime.

S. No.	Microorganism	Zone diameter (mm)	
		Cefepime (30 µg) Mean ± S.D.	Supime (30 µg Cefepime + 10 µg Sulbactam) Mean± S.D.
1	*S. aureus*	24.83 ± 0.56	27.76 ± 0.34
2	*P. mirabilis*	37.23 ± 0.56	40.51 ± 0.50
3	*K. pneumonia*	22.96 ± 0.65	27.61 ± 0.42
4	*E. clocae*	20.71 ± 0.50	23.58 ± 0.07

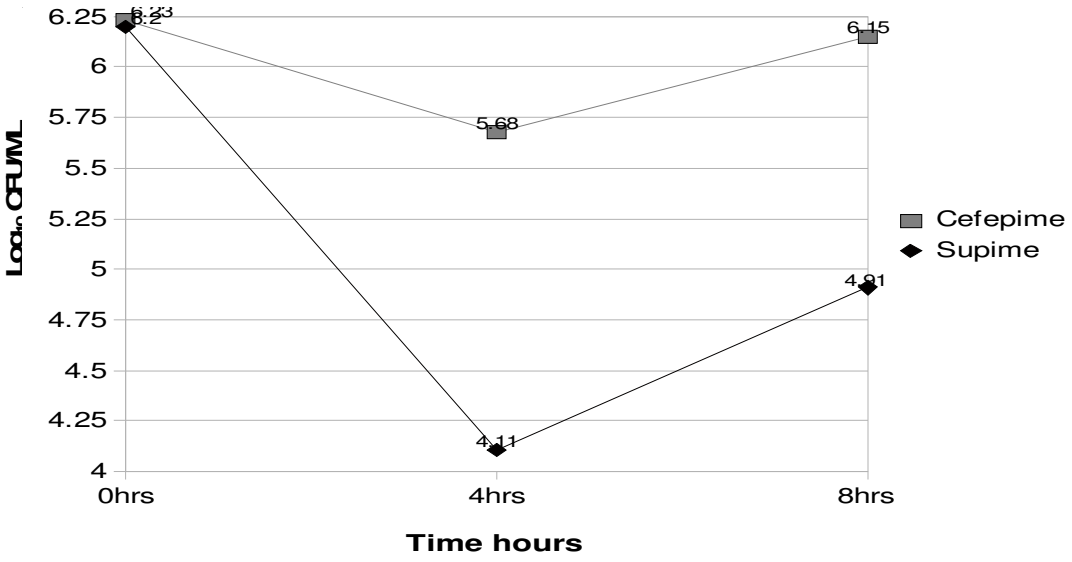

Figure 1.

DISCUSSION

There has been increase of resistance in bacteria against β-lactam antibiotics which causes decreased efficacy of these drugs. The production of β-lactamases is still the main mechanism for resistance of bacteria to β-lactam group of antibiotics. Combination of β-lactam antibiotics with β-lactamase inhibitor such as sulbactam is used to overcome β-lactamase mediated resistance. *In vitro* efficacy of β-lactam antibiotics in combination with sulbactam has been well evaluated (Wang et al., 2004).

Cephalosporins have significant and potential advantages over other broad spectrum nontraditional β-lactam antibiotics (Kessler et al., 1985; Shrivastava et al., 2008). In addition, some cephalosporins appears to have low affinity for major chromosomally mediated, β – lactamases and thus is less affected by the non hydrolytic barrier mechanism of resistance in these bacteria. A combination of β-lactam and β-lactamase inhibitor has shown better bactericidal activity (Phelps et al., 1986).

Cefepime crosses the bacterial outer membrane faster than other beta lactam antibiotics and it is used to achieve better therapeutic efficacy. Cefepime also has advantages of rapid penetration in periplasmic space and extended spectrum of activity that include gram positive and gram negative organisms (Angelescu and Apostol, 2001).

In clinical isolates of Acienetobacter spp combined effect of cefepime and sulbactam has been evaluated and found that the combinations of cefepime with sulbactam have moderate synergistic activity against some carbapenem-resistant strains of Acinetobacter spp., which could be beneficial for the treatment of infections due to multidrug-resistant strains of Acinetobacter spp (Tong et al., 2006). In present study, AST data from demonstrated that Supime, a combination of cefepime and sulbactam has more bactericidal activity than cefepime alone in most of the cases. It is appears that addition of sulbactam, the β-lactamase inhibitor to

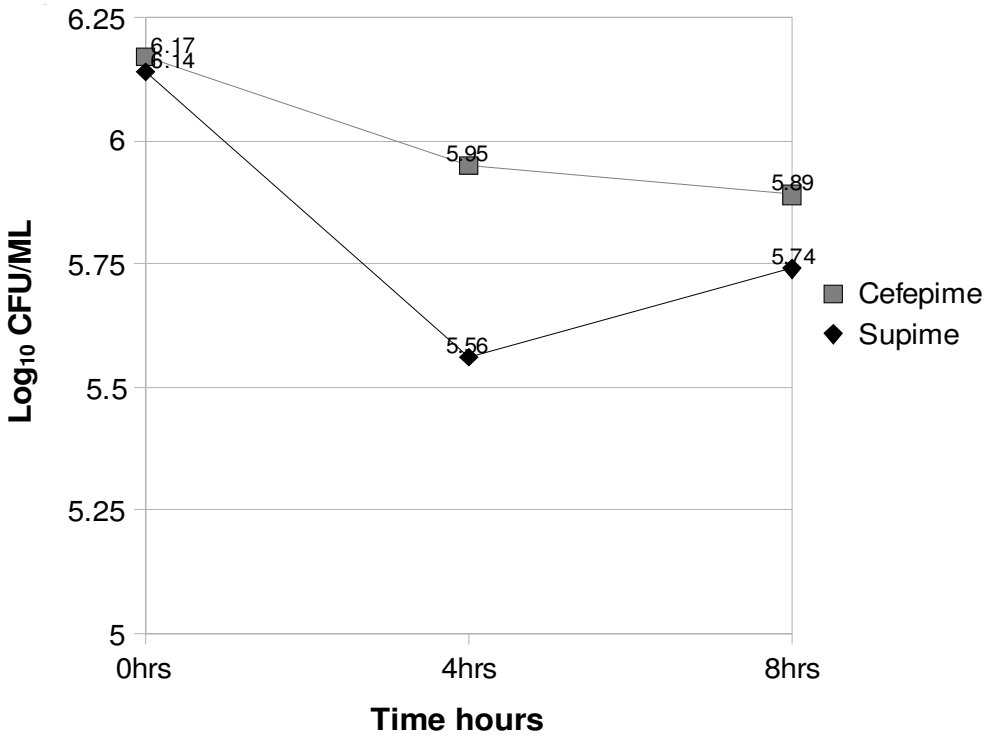

Figure 2. Time kill curve of *P. mirabilis.*

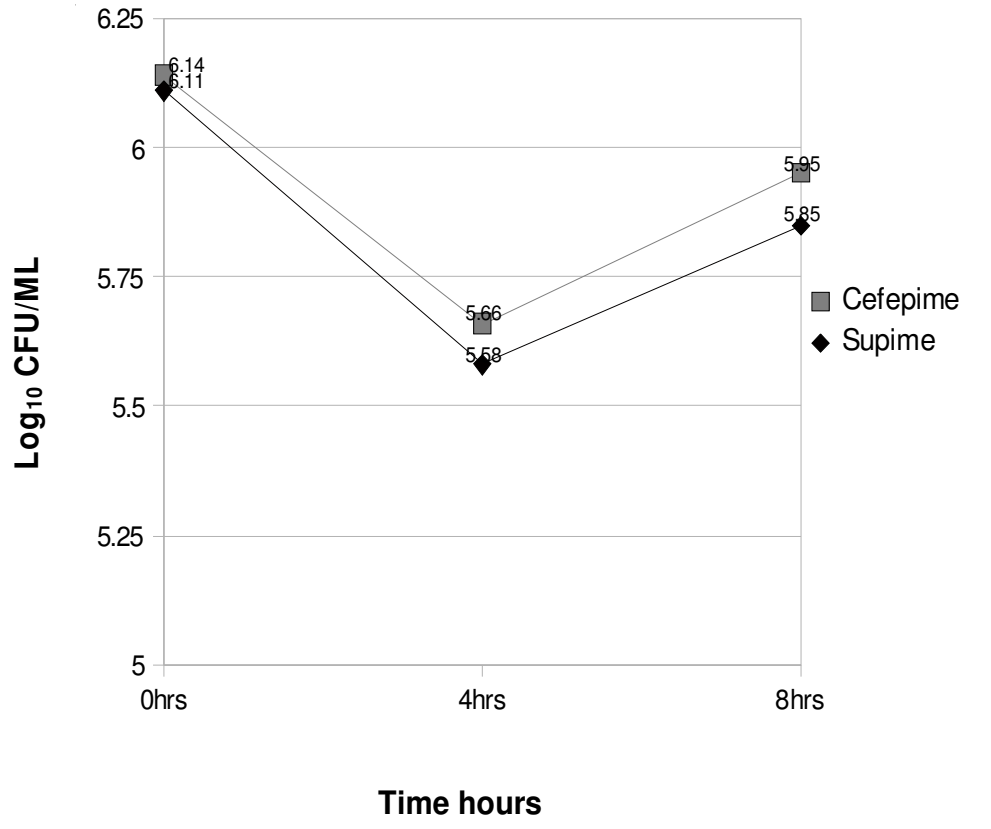

Figure 3. Time kill curve of *K. pneumoniae*.

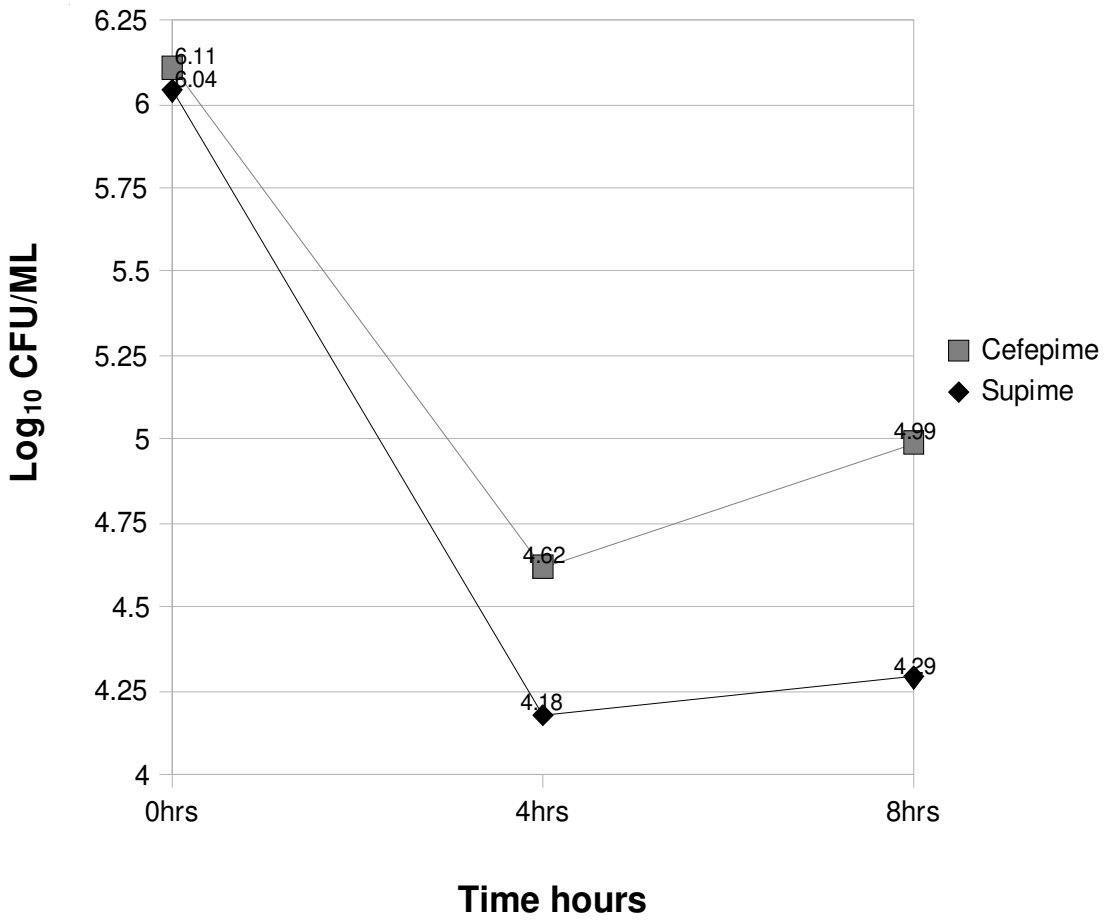

Figure 4. Time kill curve of *E. clocae*.

cefepime adds upon antimicrobial activity of cefepime.

Lower MIC value of Supime than cefepime alone, also suggests higher bactericidal activity in Supime because of addition of sulbactam. This was reconfirmed by the results of time kill analysis even at a concentration of 2x of the MIC after 4 h in all organisms under study. There has been a uniform pattern of regrowth of microorganisms in broth after incubation for 8 h. It appears that in *K. pneumoniae* even if there is significant increase of lytic zone (Table 1), there is regrowth reported in both drugs after 8 h of study in MIC. 2x concentration of MIC is not sufficient enough to achieve complete bactericidal properties in case of both the drugs in all organisms under study.

Conclusion

In conclusion, the results of MIC, AST and TKC studies are in similar pattern for *S. aureus, P. mirabilis, K. pneumoniae* and *E. cloacae.* Supime may be of therapeutic importance in treatment of infections caused by organisms under study as demonstrated by providing better bactericidal effect than cefepime alone.

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Transparency declarations

All authors are staff members of Venus Medicine Research Centre, an independent unit of Venus Remedies Limited. Authors have consent on Management to carryout research and publish results independently. No material support or reimbursements of any kind have accepted in preparing this research paper.

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