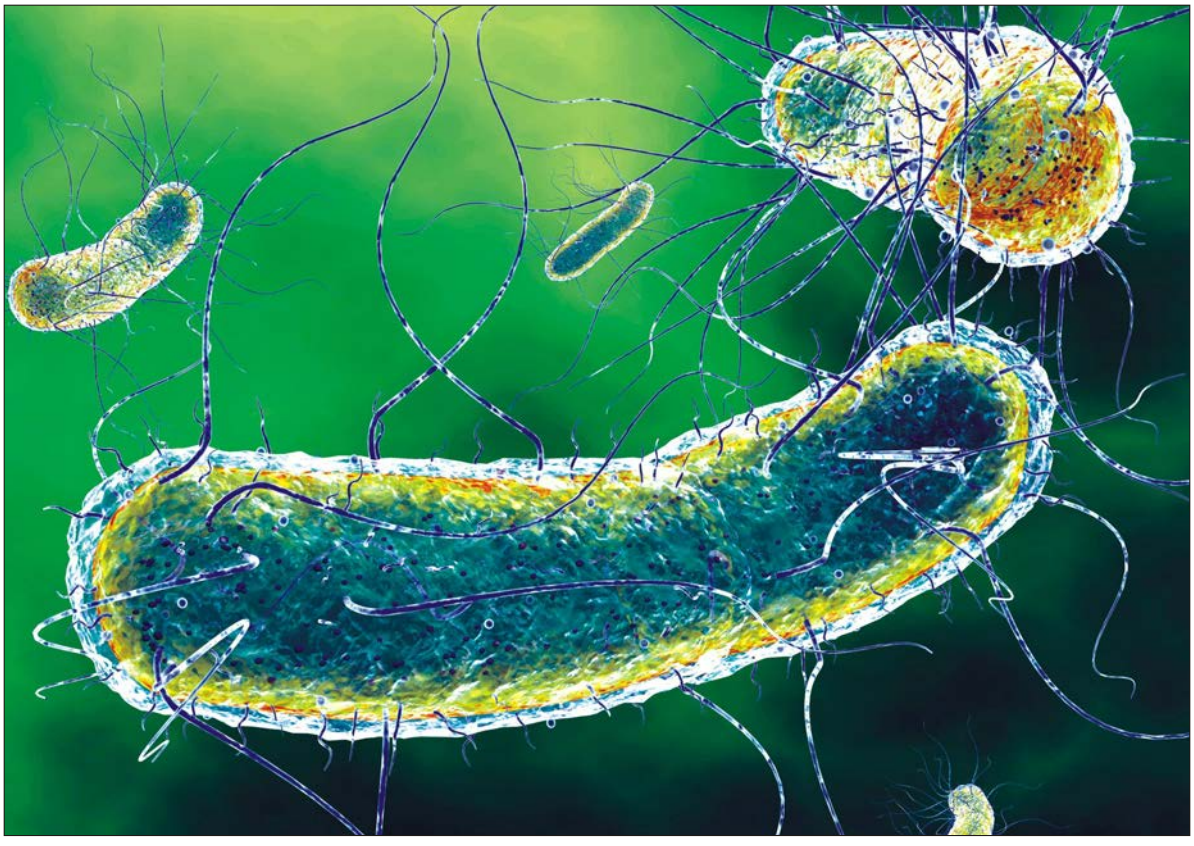

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Ceftriaxone-Sulbactam Combination: Microbial Analysis by Variation of Ratios and Comparative Disc Diffusion

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Abstract: Development of β-lactamase provides resistance to bacteria against cephalosporins. Ceftriaxone, a third generation cephalosporin has also lost it's effectiveness in clinical practices. However, it is the current trend to use combinations of β-lactam antibiotics and β-lactamase inhibitors as they have come up as the ideal solution. The potential combination with ceftriaxone is of sulbactam, a β-lactamase inhibitor. This combination is used in clinical practice for achieving better therapeutic value. In present study, comparative microbial analysis of various ratios of ceftriaxone, sulbactam and sulbactomax, a Fixed Dose Combination (FDC) of ceftriaxone and sulbactam has been performed by Minimum Inhibitory Concentration (MIC) analysis. Comparative evaluation of susceptibility discs of FDC of ceftriaxone and sulbactam with ceftriaxone is done under time stress to find out possibility of development of resistance in *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Escherichia coli* and Methicillin Resistant *Staphylococcus aureus* (MRSA). In the results of MIC, 2:1 ratio of ceftriaxone: sulbactam has shown better bactericidal activity than the ratio of 1:6.66 and 1:3.33. Antibiotic Susceptibility Test (AST) demonstrated that ceftriaxone-sulbactam, apart from being more bactericidal, has less chances of resistance development, when compared with ceftriaxone alone. It may be concluded that ceftriaxone-sulbactam in the ratio of 2 :1 has better bactericidal properties and reduces the probability of resistance development.

Key words: MIC, AST, sulbactomax, ceftriaxone, sulbactam

INTRODUCTION

The third generation cephalosporins are considered good in clinical and therapeutic conditions as they are fairly safe agents for the treatment of many serious infection (Donowitz and Mandell, 1998). There has been emergence of extended spectrum β-lactamases (ESBLs), which are capable of conferring resistance to cephalosporins including third generation cephalosporins, which has reduced the efficacy of these agents (Philippon *et al*., 1989; Bush, 1998; Brinas *et al*., 2005). It is the current trend to use β-lactamase inhibiting agents such as sulbactam to overcome such resistance. The combinations of β-lactam antibiotics and β-lactamase inhibitors have been successfully used in clinical practice to treat and manage infections, including that of resistant bacteria (Philippon *et al*., 1989; Levy *et al*., 1988).

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Potential combinations are ceftriaxone, a third generation cephalosporin and tazobactam and ceftriaxone with sulbactam, β-lactamase inhibitor (Robert, 1993; Philippon *et al*., 1989) along with certain other combinations such as clavulanic acid. Addition of these β-lactamase inhibitors to β-lactam antibiotics are capable of inhibiting β-lactamase produced by many bacteria (Shrivastava *et al*., 2009).

The efficacy of combination of ceftriaxone with tazobactam has already been evaluated in certain animal models and in certain bacterial species (Frank *et al*., 2003). Sulbactomax is a Fixed Dose Combination (FDC) of ceftriaxone, third generation β-lactam antibiotic and sulbactam, a potent β-lactamase inhibitor along with suitable agent, which is used to overcome common bacterial infections. Attempts have also been taken to evaluate Sulbactomax in comparison with ceftriaxone in *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *K. pneumoniae* in only one ratio i.e., 2:1 for ceftriaxone: sulbactam (Shrivastava *et al*., 2009). There has been no comparative study made to understand impact of different ratios of ceftriaxone and sulbactam on it's antimicrobial efficacy. The ratio of ceftriaxone with sulbactam has been a point of concern in using the combinations of ceftriaxone and sulbactam as the bactericidal efficacy depends on the ratio of ceftriaxone and sulbactam in the preparation. Present study, has been undertaken to compare Sulbactomax with various ratios of ceftriaxone and sulbactam and to evaluate commercially available susceptibility disc of ceftriaxone-sulbactam with ceftriaxone alone under normal and time stress condition.

MATERIALS AND METHODS

All the studies were performed in the Laboratories of Venus Medicine Research Center, India from January 2009 to May 2009.

Bacterial Strains

The following strains obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India were used for the study. *Staphylococcus aureus* (MTCC No.-737), *Klebsiella pneumoniae* (MTCC No.-109), *Proteus vulgaris* (MTCC No.-426) and *Escherichia coli* (MTCC No.-1687). Methicillin Resistant *Staphylococcus aureus* (MRSA) used was a clinical isolate obtained from Post Graduate Institute (PGI) of Medical Education and Research, Chandigarh, India.

Antibiotic

Sulbactomax, ceftriaxone and sulbactam used in this study were provided by manufacturer, Venus Remedies Limited, India. 1.5 g pack of sulbactomax contains 1 g of ceftriaxone and 500 mg of sulbactam with suitable agent. Susceptibility discs of combination of ceftriaxone and sulbactam (CIS; 30+10 μg) and ceftriaxone (CI; 30 μg) used in the study was procured from Hi Media Lab. Ltd., India.

Medium

Mueller-Hinton (MH) agar obtained from Hi Media Lab. Ltd., India was used for Antibiotic Susceptibility Test (AST) and Minimum Inhibitory Concentration (MIC).

Antibiotic Susceptibility Testing

The comparative Antibiotic Susceptibility Testing (AST) of Susceptibility discs were placed on agar plates pre inoculated with the test organisms. The plates were incubated at 37°C for 24 h and the lytic zone size was measured. Further, the plates were incubated for

72 h more to provide stress condition for evaluating any possible regeneration of organisms in the lytic zone of inhibition. Lytic zones were read with help of zone reader. The statistical method used to determine significance level of difference in Zone Size of AST was one-way Analysis of Variance (ANOVA). The p-values <0.05 were considered statistically significant.

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of ceftriaxone and sulbactam taken in ratio of 1:6.66, 1:33 of ceftriaxone and sulbactam. Sulbactomax containing ceftriaxone and sulbactam in the ratio of 2:1 along with suitable agent was also taken for the studies. Range of concentration used for each antibiotic was 0.0625 to 256 mg L^{-1}. The MIC were determined by broth method as per the standard of Clinical and Laboratory Standard Institute (NCCLS, 1997). Final inocula of 10^5 cfu mL^{-1} was used for the studies.

RESULTS

In case of use of the susceptibility discs, clear susceptible zone and intermediate zone was found in CIS and CI of *S. aureus*, *P. vulgaris* and *E. coli* (Table 1). The susceptibility zone of these organisms were much higher in CIS when compared with CI. In case of MRSA there was no development of intermediate zone for CIS but the intermediate zone was present in CI. In all organisms under study, the lytic zone of CIS was found to be higher than CI. Under stress condition, there has been growth of MRSA and *S. aureus* in CI lytic zone but no growth has been reported in CIS (Fig. 1, 2). In *P. vulgaris* susceptible zone was clearly seen in CIS but no susceptible zone was evident in CIS (Fig. 3). In case of *E. coli* and *K. pneumoniae* there has been regrowth on susceptible zone in CI but growth was less evident in susceptible zone of CIS (Fig. 4, 5).

Results of MIC demonstrated that in all organisms of study, the ratio of 1:6.66 of ceftriaxone: sulbactam was least bactericidal. Maximum bactericidal activity was seen in case of Sulbactomax. There has been intermediate response of ratio of 1:3.33 (Table 2).

Table 1: Susceptibility zone (mm) of organisms with ceftriaxone (CI) and ceftriaxone-sulbactam (CIS)

	Ceftriaxone CI (30 mcg)		Ceftriaxone-Sulbactam CIS (30+15 mcg)	
Organisms	Susceptibility zone	Intermediate zone	Susceptibility zone	Intermediate zone
S. aureus	14.55±1.3	40.50±2.1	20.85±1.6	42.35±1.5
K. pneumoniae	7.90±2.3	No zone	25.35±2.9	No zone
P. vulgaris	7.34±1.5	29.81±2.8	15.69±2.5	29.92±3.4
E. coli	15.24±2.1	38.14±2.8	21.13±2.5	42.70±2.6
MRSA	7.80±1.6	9.80±2.1	16.45±2.8	No zone

Fig. 1: MRSA zone under time stress. growth of colonies seen with CI but no growth seen in CIS

Fig. 2: *Staphylococus aureus* zone under time stress. Growth of colonies seen with CI but no growth seen in CIS

Fig. 3: *Proteus vulgaris* zone under time stress. Susceptible zone was clearly seen in CIS but no susceptible zone was evident in CIS

Fig. 4: *Escherichia coli* Under time stress. There has been regrowth on susceptible zone in CI but growth was less evident in susceptible zone of CIS

Fig. 5: *Kelbsiella pneumoniae* Under time stress. There has been regrowth on susceptible zone in CI but growth was less evident in susceptible zone of CIS

Table 2: MIC of organisms with different ratios of ceftriaxone and ceftriaxone-sulbactam compared with sulbactomax

	MIC (mg L^{-1})		
Organisms	Ceftriaxone:sulbactam (1:6.66)	Ceftriaxone: sulbactam (1:3.33)	Sulbactomax having Ceftriaxone: sulbactam (2:1)
S. aureus	16	16	8.000
K. pneumoniae	8	4	2.000
P. vulgaris	32	32	16.000
E. coli	4	2	0.125
MRSA	128	32	16.000

DISCUSSION

Resistance development in bacteria to third generation cephalosporins has become a major concern worldwide. Infections caused by such bacteria offering resistance cephalosporins are traeted with a combination of β-lactam antibiotic with β-lactamase inhibitor. This type of practice has become progressively widespread due to increased use of cephalosporins, particularly ceftriaxone and development of bacterial resistance to this antimicrobial agent. Extended Spectrum β-lactamases (ESBLs) have more ability to deactivate antibiotics with β-lactam ring. Major antibiotics included in this group are cefotaxime, ceftazidime, ceftriaxone, cefpodoxime and aztreonam, but with development of resistance in bacteria they are usually inactivated (Bradford, 2001).

There have been evidences that the combination of beta lactam and beta lactamase inhibitor has shown better bactericidal activities with baseline microbiological studies of efficacy of combination of ceftriaxone and sulbactam (Shrivastava *et al*., 2009). Results of the present study show that in case of Sulbactomax, with ceftriaxone-sulbactam ratio of 2:1 has better microbial efficacy than the ratio of 1:6.66 and 1:3.33. Moreover, the best ratio of use of ceftriaxone and sulbactam for getting better therapeutic effect has not been determined by current literature. Microbial studies for Sulbactomax in comparison with Ceftriaxone has been reported for *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *K. pneumoniae* only in the ratio of 2:1 (Shrivastava *et al*., 2009). The MIC results of present study clearly demonstrate that the bactericidal killing effect is least in the case of ratio of 1:6.66 and very less in 1:3.3 of ceftriaxone : sulbactam when compared with Sulbactomax containing ratio of ceftriaxone: sulbactam of 2:1. Results of AST studies have demonstrated that the susceptibility zone is more in case of CIS than CI in all organisms under study. There has been development of intermediate zone which suggests that the possibility of development of resistance is more in case of CI. Time stress results also demonstrates that there has been development of resistant organisms over a period of time. The development of resistance is more in case of CI than CIS.

In summary, 2:1 of ceftriaxone and sulbactam has the best *in vitro* efficacy in organisms under study and the combination of ceftriaxone and sulbactam has less chances of development of resistance than ceftriaxone.

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