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In-Vitro Activity of Ceftriaxone in Combination with Sulbactam and Tazobactam against *Escherichia Coli*

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ABSTRACT

The aim of the study was to compare the antimicrobial effect of combination of ceftriaxone/sulbactam and ceftriaxone/tazobactam against Escherichia coli (E. Coli). Isolate of β-lactamase producing E. coli was cultured and their sensitivity tests were done with both combinations using specially prepared antibiotic disks. National Committee for Clinical Laboratory Standards zone diameter criteria was used to measure and evaluate the zones of inhibition. The disks containing the combination of ceftriaxone with tazobactam produced larger zones of inhibition than those containing combination of ceftriaxone with sulbactam. Addition of tazobactam with ceftriaxone adds more to the efficacy of ceftriaxone against E. Coli as compared to the efficacy of ceftriaxone with sulbactam..

KEYWORDS: *E. coli, ceftriaxone, sulbactam, tazobactam, susceptibility disc.*

INTRODUCTION

Since the introduction of third generation cephalosporins in 1980, these have served as the efficacious & safe antibiotics for treatment of many infections. But, bacteria have acquired a variety of mechanisms to resist the action of antibiotics. The production of β-lactamase, an enzyme that destroys cephalosporins by hydrolyzing their β-lactam nucleus, is the most common mechanism of resistance.[1-4]

The extensive use of β-lactam antibiotics is creating major evolutionary changes in bacteria to evolve towards resistance. This increased resistance results into increased morbidity, mortality and healthcare costs.[5,6] The β-lactam antibiotics are the largest and currently most widely used antibacterial agents. Therefore their resistance means a level of antimicrobial activity associated with a high likelihood of therapeutic failure.[7] The combination of an established β-lactam antibiotic with a β-lactamase inhibitor neutralizes the effect of β-lactamase. It thus allows the β-lactam antibiotic to act as if the organism was fully sensitive. [8,9] β-lactamase inhibitors are themselves β-lactam antibiotics usually with minimal or no antibacterial activity. When combined with certain β-lactam antibiotics, they augment the potency of these against β-lactamase producing bacteria.

Ceftriaxone is a third generation cephalosporin, which has become the most common antibiotic for empirical use. Because of this reason, bacteria including *E. coli* have become resistant to this broad-spectrum β-lactam antibiotic.

Sulbactam is a competitive, irreversible β - lactamase inhibitor and has good inhibitor activities against the clinically important plasmid mediated β-lactamase and most frequently responsible for transferred drug resistance.[10] Like other β-lactamase inhibitors, Sulbactam can also be combined with β-lactam antibiotics and thus prevent their destruction by β-lactam for the treatment of infections. Sulbactam has been approved in many countries, including India, to be combined with β-lactam antibiotics. [9,11] Chemically, Tazobactam is triazolylmethyl penicillanic acid sulfone. Tazobactam is β-lactamase inhibitor that acts synergistically with many β-lactamase labile drugs such as cephalosporins. The efficacy of a combination of

Ceftriaxone-Tazobactam has been evaluated in animal models and bacterial species.[7,12]

There are various methods available to test the antimicrobial susceptibility. Among those methods, Disc diffusion method is most commonly used technique for antimicrobial susceptibility testing because of its convenience, simplicity, sensitivity, efficiency and dependability.[13-15]

The objective of the present study was to compare the *in-vitro* efficacy of the two combination of *β*-lactamase inhibitor antibiotic with ceftriaxone against *E. coli.* Generally sulbactam and tazobactam are used in combination with *β*-lactam antibiotic in the ratio of (2:1) and (8:1) respectively.[16-18] In the present study, two combinations of antibiotics, ceftriaxone/sulbactam (2:1) and ceftriaxone/tazobactam (8:1) were studied. The rationale behind selection of these antibiotics in the given ratios is based on the available literature in order to get comparative data on the antimicrobial efficacy.

MATERIALS AND METHODS:

All the studies were performed in Provimi India Innovation Centre, Bengalore-Karnataka. Blank sterile discs were procured from HiMedia. Ceftriaxone sodium, Sulbactam sodium and Tazobactam sodium were procured from Kilitch Drugs (I) Ltd, Mumbai. Antibiotic assay medium no. 1 (USP) was used as culture media. *E. coli* NCIM 2563 was used as organisms to test the susceptibility of antibiotics.

SUSCEPTIBILITY DISC PREPARATION

Preparation of antibiotic stock solutions

Dissolve ceftriaxone sodium equivalent to 500mg of ceftriaxone in Phosphate buffer (pH 6.0) and make up the volume up to 50 ml with phosphate buffer.[19]

Similarly, Stock solutions of sulbactam and tazobactam were prepared by dissolving weight equivalent to 500mg of base in distilled water separately and make up the volume up to 50 ml with distilled water.

Preparation of ceftriaxone/Sulbactam solution

Transfer 5 ml of ceftriaxone sodium stock solution and 7.5 ml of sulbactam sodium stock solution to 100 ml volumetric flask. Mix well and make up the volume with distilled water.

Preparation of ceftriaxone/Tazobactam solution

Transfer 15 ml of ceftriaxone sodium stock solution and about 1.875 ml of tazobactam sodium stock solution to 100 ml volumetric flask. Mix well and make up the volume with distilled water.

Preparation of antibiotic susceptibility discs

The discs were impregnated with the antibiotic solutions by pipette delivery method.[20] The sterile discs were placed in petri-dishes approximately 5mm apart. Using a mechanical pipettor with a fixed volume delivery of 0.02 ml, the disks were loaded with antibiotic solutions. During loading of solution, precaution was taken to avoid excessive pressure on disc by pipette tip.

The disks were allowed to dry in a clean incubator at 35°C for 1-2 hours. After drying, 50 disks were placed in small sterile airtight-labeled containers with a desiccant at the bottom. A layer of sterile cotton was placed over the desiccant to avoid contact with the disks. The disks were stored in refrigerator at 2-8°C.[21]

The discs were left at room temperature for about 1-2 hour before use to allow the temperature to equilibrate. It minimizes the amount of condensation that may occur when warm room air reaches the cold containers.

Antibiotic Susceptibility Testing

The disks were placed in the culture plates with adequate distance between two consecutive disks. The plates were incubated at 37^{o}C for about 24 hours. The diameter of zone of inhibition around each disk was measured which corresponded to the activity of each disk. With the use of a digital caliper the zones of inhibition were measured after 18 hours of incubation and recorded.[8,22]

For evaluation and comparison of the results, student *'t'* test was applied with 95% confidence interval.

STABILITY STUDY

Stability study for both the discs were performed up to six month and the zone of inhibition was observed at different time interval i.e. initial, 3 month and 6 month. The discs were stored in refrigerator at 2-8^{0}C up to 6 months.

RESULTS

The performance of both the discs was measured as diameter of zone of inhibition. The results obtained by measuring the zones of inhibition of both the disks are shown in **Table-1.** The mean zone of inhibition of ceftriaxone/sulbactam and ceftriaxone/tazobactam were 29.3 and 34.04 mm respectively. The '*t*'-value for combination of antibiotics also supports the significant difference in effectiveness of two combinations. The *'t' value* at 95% confidence interval for the zone diameters was obtained as 9.05, which is greater than 2.10 i.e. tabulated value. The results obtained by this exercise reveal that there is significant difference between the effects of antibiotic combinations. **Figure 1** shows comparative effectiveness of the two combinations of antibiotics against *E. coli* at different time interval.

Table 1: Antimicrobial activity of CSD and CTD

S. no.	Zone of Inhibition (mm)	
	CSD[a]	CTD[b]
1.	29.6	34.2
2.	28.9	34.9
3.	29.4	34.6
4.	30.8	33.7
5.	29.8	34.1
6.	29.1	35.9
7.	29.3	34.9
8.	28.8	32.8
9.	27.6	32
10.	29.7	33.3
Mean ± SD	**29.3 ± 0.82**	**34.04 ± 1.14**
***t*- value**	**9.05**	

[a] Ceftriaxone/ sulbactam disc
[b] Ceftriaxone/ tazobactam disc

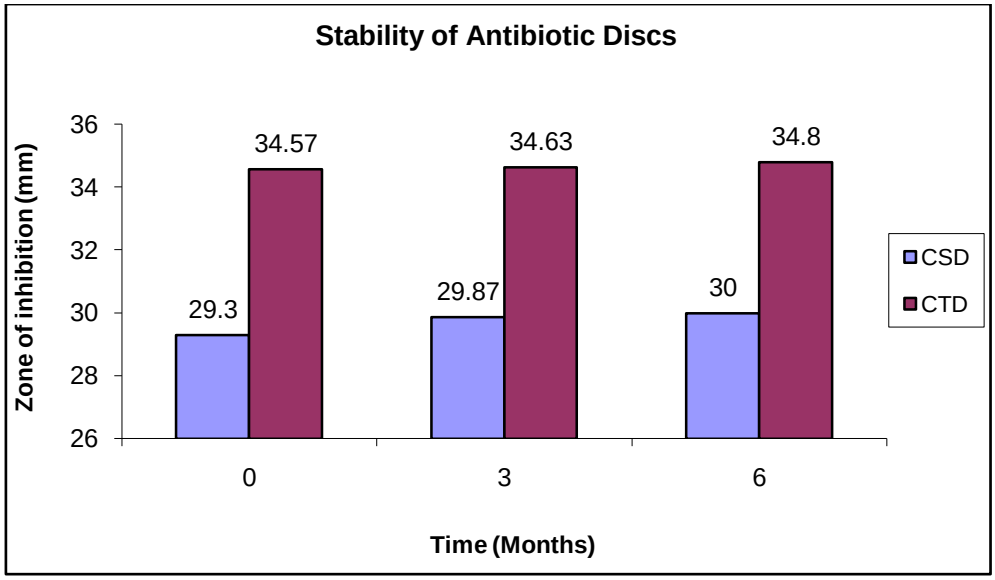

Figure 1: Zone Diameter for Antibiotic Combinations at Different Time

For stability analysis of discs, the mean zone diameter produced by the antibiotic disks was measured at different time interval up to 6 months. As indicated in **Table 2**, no significant difference was observed in the mean zone diameter after 6 months from the initial mean zone diameters. **Figure 2** gives a graphical comparison of the stability for both the discs at 2-8^{0}C.

Table 2: Zone Diameter of antibiotic discs (Stability Study)

S. no	Zone of Inhibition (mm)					
	0 month		3 month		6 month	
	CSD	CTD	CSD	CTD	CSD	CTD
1.	29.6	34.2	29.5	34.3	30.2	33.9
2.	28.9	34.9	29.7	35	30.7	34.7
3.	29.4	34.6	30.4	34.6	29.1	35.8
Mean	29.30	34.57	29.87	34.63	30.00	34.80
SD**	0.36	0.35	0.47	0.35	0.82	0.95
t_{cal} value	4.33		4.99		11.48	

*Average of triplicates, **Standard Deviation

Simultaneously, the difference between the activities of two different antibiotic combinations was found to be significant at each time interval of stability study. The't'-value after 3 and 6 month study was found to be 4.99 and 11.38, which is greater than the tabulated '*t*' value i.e. 2.78. This also reveals the significant difference between activity of ceftriaxone/sulbactam and ceftriaxone/tazobactam

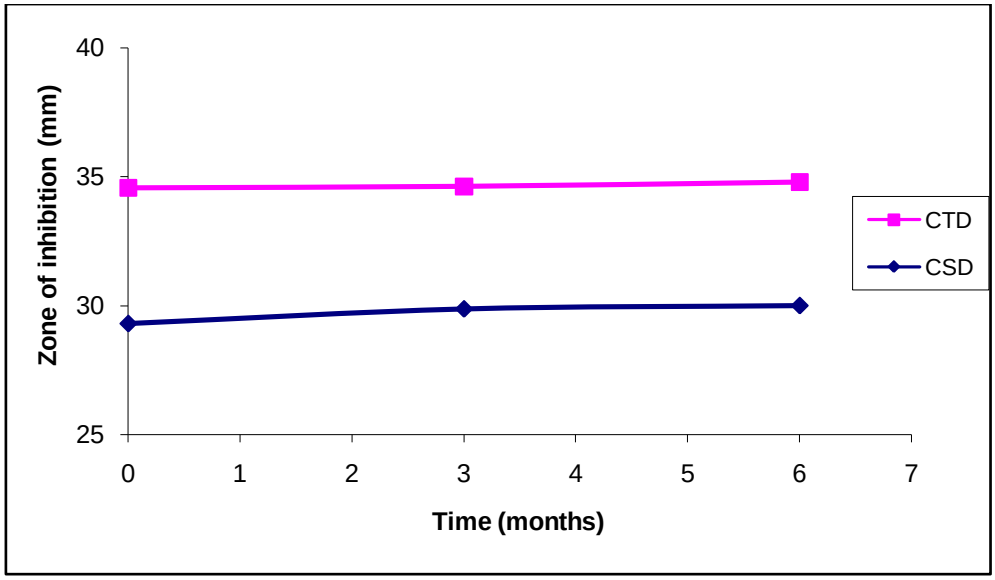

Figure 2: Stability data of Antibiotic combinations

DISCUSSION

Resistance to third and fourth generation cephalosporins has become a major concern worldwide. In many of the developing countries where laboratory-testing facilities are not sufficient, broad-spectrum antibiotics are often used for suspected bacterial infections and selection of these antibiotics depends upon the site of infection, sign & symptoms of infection and patient's illness status. This practice results in increased resistance of bacteria towards antibiotics. Now a days, ceftriaxone is the most commonly used antibiotic. Consequently bacteria are becoming more resistant to these broad-spectrum cephalosporins. Also the emergence of extended spectrum *β*-lactamases is a new aspect of resistance to ceftriaxone. Therefore, the use of some *β*-lactamase inhibitors in combination with ceftriaxone may solve in part, the problem of resistance. Data obtained from stability study revealed that the antibiotic disks maintained their potency even after storage for 6 months. At each time interval of stability, tazobactam has been shown to be a more effective than sulbactam against *E. coli* when combined with ceftriaxone. Further testing in the year ahead may substantially lengthen the stability period.

CONCLUSION

It is inferred that the combination of ceftriaxone with tazobactam improved the efficacy as compared to the combination of ceftriaxone with sulbactam. However, additional studies with other bacterial species are also required.

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