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Research Article

COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF CEFTRIAXONE IN COMBINATION WITH SULBACTAM AND TAZOBACTAM USING DISC DIFFUSION METHOD

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ABSTRACT
The present study includes comparative analysis of the antimicrobial effect of combination of ceftriaxone/sulbactam and ceftriaxone/tazobactam against *Staphylococcus aureus* (*S. aureus).*
Isolate of *β*-lactamase producing *S. aureus was* cultured. Antibiotic discs of ceftriaxone/sulbactam and ceftriaxone/tazobactam combinations were prepared and their antimicrobial activity was compared against zone of inhibition produced in cultured isolates. National Committee for Clinical Laboratory Standards zone diameter criteria was used to measure and evaluate the diameter of zones of inhibition.
Zones of inhibition produced by ceftriaxone/tazobactam combination were larger than those produced by ceftriaxone/sulbactam combination indicating comparative advantages. The study reveals that as compared to sulbactam, tazobactam is adding more synergistic action to the ceftriaxone activity against *S. aureus*.
KEYWORDS: Antibiotic susceptibility disc; ceftriaxone; sulbactam; *S. aureus*; tazobactam.

INTRODUCTION

The *β*-lactam antibiotics are the largest and currently most widely used antibacterial agents. The third generation cephalosporins have been proved as the safe & effective antibiotics for treatment of many infections. But, the non-rational use of *β*-lactam antibiotics is causing resistance in bacteria against these antibiotics. Therefore, their resistance means a level of antimicrobial activity associated with a high likelihood of therapeutic failure. Bacteria have acquired a variety of mechanisms to resist the action of antibiotics. One of the most common mechanisms of resistance is production of *β*-lactamase enzyme by bacteria. This *β*-lactamase enzyme destroys cephalosporins by hydrolyzing their *β*-lactam nucleus.[1-8]

Usually *β*-lactam antibiotics are administered in combination with a *β*-lactamase inhibitor that results into neutralization of *β*-lactamase effect.[9,10] *β*-lactamase inhibitors are *β*-lactam antibiotics only having very low or almost no antibacterial activity. But, upon combining with certain *β*-lactam antibiotics, they add to the potency of *β*-lactam antibiotics against *β*-lactamase producing bacteria.

Ceftriaxone, a third generation cephalosporin, is being most commonly used antibiotic in the treatment of bacterial infections caused by susceptible organisms. Ceftriaxone has *in vitro* activity against gram-positive and gram-negative aerobic and anaerobic bacteria. The bactericidal activity of ceftriaxone results from the inhibition of bacterial cell wall synthesis Because of this widespread use of cephalosporin, many of the bacteria including *S. aureus* have become resistant to this *β*-lactam antibiotic.[11]

Sulbactam, a member of *β*-lactamase inhibitor family, possess good inhibitor activities against the plasmid mediated *β*-lactamase.[12] Like other *β*-lactamase inhibitors, upon combining with *β*-lactam antibiotics, sulbactam prevent destruction of *β*-lactam antibiotics by *β*-lactamase and thus synergies the activity of *β*-lactam antibiotic. Sulbactam has been approved in many countries, including India, to be combined with *β*-lactam antibiotics.[9,13] Tazobactam, another effective *β*-lactamase inhibitor, acts synergistically with many *β*-lactamase labile drugs such as cephalosporins. Chemically, tazobactam is triazolylmethyl penicillanic acid sulfone. Many animal model studies have been conducted to evaluate the efficacy of a combination of Ceftriaxone-Tazobactam. Some studies also evaluated benefits of this combination in bacterial species.[8,14]

Different methods to test the antimicrobial susceptibility have been suggested by researcher and governing bodies. Disc diffusion method for antimicrobial susceptibility testing, which is most commonly used technique was selected for the present study as it is simple, sensitive, dependable, and effective.[15-17]

The objective of the present study was to compare the *in-vitro* antibacterial activity of the two combination of *β*-lactamase inhibitor antibiotic with ceftriaxone against *S. aureus.* Generally, ceftriaxone is used in combination with sulbactam and tazobactam in the ratio of (2:1) and (8:1) respectively.[18-20] 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, Bangalore, Karnataka, India. Blank sterile discs were procured from HiMedia. Ceftriaxone sodium, sulbactam sodium and tazobactam sodium were procured from Kilitch Drugs (I) Ltd, Mumbai, India. Antibiotic assay medium no. 1 (USP) was used as culture media. *S. aureus* NCIM 2901 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.[21]

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 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

Pipette delivery method, as suggested by *Velmonte et al*, was used to prepare antibiotic discs.[22] The blank discs were impregnated with the antibiotic solutions. The sterile discs were placed in petri dishes approximately 5mm apart. A fixed volume delivery of 0.02 ml antibiotic solution was loaded to the discs using a mechanical pipettor. During loading of solution, precaution was taken to avoid excessive pressure on disc by pipette tip.

The discs were allowed to dry in a clean incubator at 35°C for 90 min. After drying, 50 discs were placed in small sterile airtight-labeled containers with a desiccant at the bottom. Sterile cotton layer was placed over the desiccant to avoid contact with the discs. The discs were stored in refrigerator at 2-8°C until further use.[23]

Before using, the antibiotic discs were left at room temperature for about 1-2 hour to ensure temperature equilibrium between disc and atmosphere. It minimizes the amount of condensation that may occur when warm room air reaches the cold containers.

Antibiotic Susceptibility Testing

The discs were placed in the culture plates with adequate distance between two consecutive discs and incubated at 37^{o}C for about 24 hours. After adequate incubation, zones of inhibition were measured with the use of a digital caliper and recorded. These zones of inhibition diameters were correlated to the activity of each disc.[10,24]

Student *'t'* test with 95% confidence interval was applied to compare and evaluate the results.[25]

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 discs are shown in Table-1. The mean zone of inhibition of ceftriaxone/sulbactam and ceftriaxone/tazobactam were 34.28 and 37.87 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 8.77, which is greater than the critical *'t' value* i.e. 2.10. The result reveals the significant difference between the antibacterial activity of antibiotic combinations. Figure 1 shows comparative effectiveness of the two combinations of antibiotics against *S. aureus* at different time interval.

For stability analysis of discs, the mean zone diameter produced by the antibiotic discs was measured at different time interval up to 6 months. Samples were analyzed at different time interval during stability study and at each point of analysis, no significant difference was observed in the mean zone diameter from the initial mean zone diameters [Table 2]. Stability of both the discs at 2-8^{0}C is shown graphically in figure 2.

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 was found to be 11.82 and 10.47 after 3 and 6 month study respectively, which is greater than the critical '*t*' value i.e. 2.78. This also indicates the significant difference between activity of ceftriaxone/sulbactam and ceftriaxone/tazobactam

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.

Stability study data revealed that the potency of antibiotic discs was maintained even after storage for 6 months. At every point of analysis during stability study, tazobactam has been shown to be a more effective than sulbactam against *S. aureus* when combined with ceftriaxone. Further testing in the year ahead may substantially lengthen the stability period.

CONCLUSION

The present study leads to the conclusion that the combination of ceftriaxone with tazobactam is showing improved efficacy with lower concentration as compared to the combination of ceftriaxone with sulbactam. However, further studies with other bacterial species are also proposed.

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Table 1: ANTIMICROBIAL ACTIVITY OF CSD AND CTD

S. no.	**Zone of Inhibition (mm)**	
	CSD[a]	**CTD**[b]
1.	34.21	38.99
2.	33.15	37.56
3.	33.72	37.92
4.	34.59	37.21
5.	35.00	37.19
6.	34.84	38.26
7.	34.91	37.73
8.	33.48	38.45
9.	34.11	37.41
10.	34.72	37.94
Mean ± SD	**34.27 ± 0.65**	**37.87 ± 0.57**
***t*- value**	**8.46**	

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

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.	34.21	38.99	33.11	37.28	32.98	37.01
2.	33.15	37.56	33.84	37.64	33.42	37.36
3.	33.72	37.92	33.42	37.31	32.84	37.21
Mean	33.69	38.16	33.46	37.41	33.08	37.19
SD**	0.53	0.74	0.37	0.20	0.30	0.18
t_{cal} value	8.77		11.82		10.47	

*Average of triplicates,
**Standard Deviation

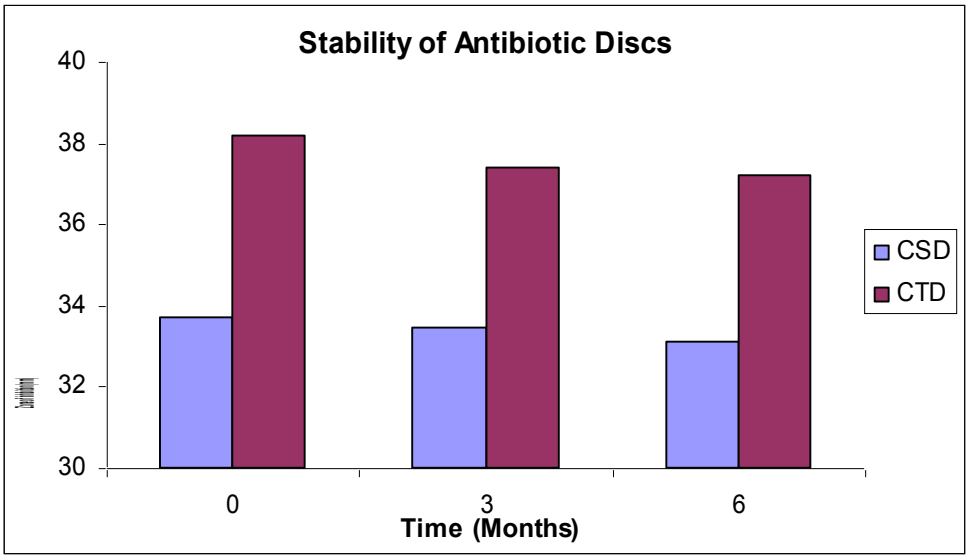

Figure 1: Zone diameter for antibiotic combinations at different time

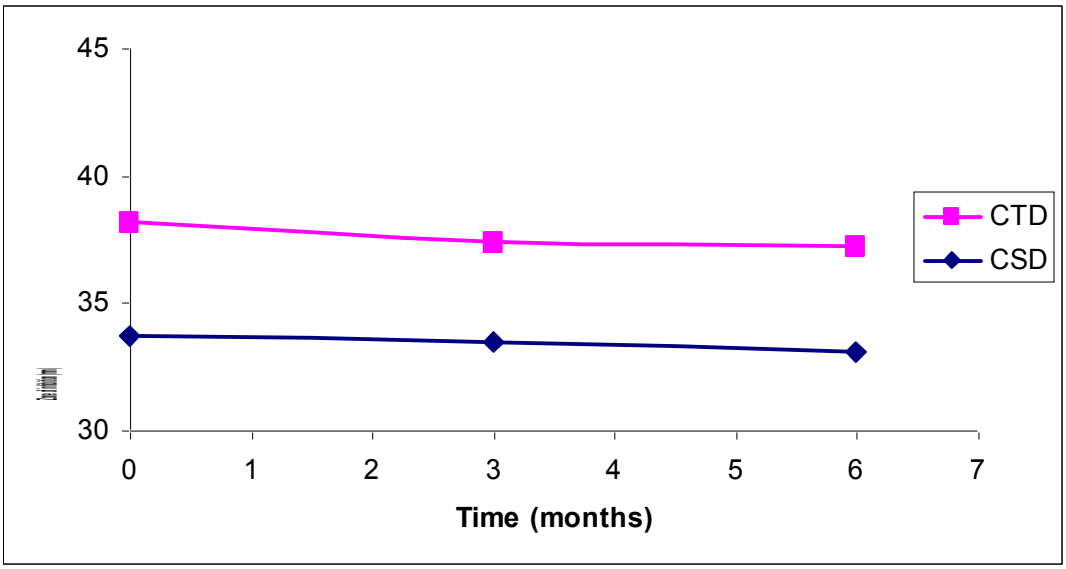

Figure 2: Stability data of antibiotic combinations in disc

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