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The effect of inoculum size on the activity of different ratios of Ceftriaxone – Sulbactam [(1:1) vs. (2:1)] against ESBL producing organisms

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Abstract

Background & Objective: Antibiotic resistance has become the most importunate health issue and remains a major public health concern worldwide. Production of extended spectrum *β-lactamases* (ESBL) is one of the major causes of resistance to *β*-lactam antibiotics. Several *in-vitro* studies have demonstrated the significant impact of inoculum size on the effectiveness of *β*-lactam-*β-lactamase* inhibitor combination. Higher concentration of *β-lactamase* inhibitor (Sulbactam) will be needed to combat the increased production of ESBLs in infections with high inoculum. The primary objective of the present study is to evaluate Minimum Inhibitory Concentration (MICs) of ESBL producing *Enterobacteriaceae,* against Ceftriaxone alone and Ceftriaxone with Sulbactam combination (1:1 and 2:1) in presence of normal and higher inoculum of these bacteria. The secondary objective of the study is to demonstrate the *in-vitro* efficacy of Ceftriaxone-Sulbactam (CS) in presence of higher inoculum of bacteria.

Method: ESBL producing strains of *Enterobacteriaceae* (*E. coli, K pneumoniae* 30 each) were isolated from patient samples. MIC of Ceftriaxone and Sulbactam alone and in combination (1:1 and 2:1) was determined in presence of both normal (10^5CFU/ml) and higher inoculum (10^7CFU/ml) of these bacteria using micro broth dilution method of antimicrobial susceptibility testing.

Results & Conclusion: The study finds an inoculum effect with Ceftriaxone. After addition of Sulbactam, MIC value for Ceftriaxone was reduced by 4-128 folds and 2-32 folds in ESBL producing strains of *E. coli* and *K. pneumoniae* respectively. The findings of the present study

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demonstrated that CS at 1:1 ratio has better *in-vitro* activity against ESBL producing strains of *E. coli* and *K. pneumoniae* than at a 2:1 ratio in both normal bacterial inoculum and higher bacterial inoculum. This is suggestive of the need for the 1:1 ratio combination of Ceftriaxone-Sulbactam in management of severe infections caused by ESBL producing organisms.

Keywords: Ceftriaxone-Sulbactam combination, *Extended-spectrum β-lactamases* (ESBL), Inoculum effect, Antibiotic resistance, *Escherichia coli, Klebsiella pneumonia*

Introduction

The cephalosporins, particularly third and fourth generation cephalosporins are widely used in clinical practice as these are relatively safe and effective agents for the treatment of various serious infections [1].Antibiotic resistance has become the most importunate health issue and remains a major public health concern worldwide. The increasing rate of antibiotic resistance in community settings and health facilities globally can be attributed to a number of factors including overuse, misuse and abuse of antibiotics [2, 3].One of the major mechanisms of development of antibiotic resistance is emergence of *extended spectrum β-lactamases* (ESBL), which are capable of conferring resistance to cephalosporins including third generation cephalosporins, which has reduced the efficacy of these agents [1]. To overcome this resistance, the current trend is to use *β-lactamase* inhibiting agents such as Sulbactam in combination with *β*-lactam antibiotics. This strategy, have been successfully used in clinical practice to manage and treat infections, including that of resistant bacteria.

Currently, carbapenems are the first-line drug of choice for management of infections caused by ESBL producing pathogens [4]. However, increased emergence of resistance to carbapenems as a result of its overuse warrants consideration for usage of *β*-lactam- *β-lactamase* inhibitors as a carbapenem-sparing strategy for the management of severe infections caused by ESBL producing organisms [5].

Several combinations of Ceftriaxone (a third generation cephalosporin) and Sulbactam are being used in clinical practice. The Central Drugs Standard Control Organization (CDSCO) approved combinations of these agents are available in the ratio of 2:1 for Ceftriaxone: Sulbactam (CS) [6].

Recently, numerous studies showed the inoculum effects (IE), that the minimal inhibitory concentration (MIC) of an antibiotic would increase as the microbial burden increased in the inoculum [2,7]. Several studies have demonstrated an observable inoculum effect with Ceftriaxone and Sulbactam [8]. One of the factors for IE may be the increased release of ESBL, which demands the new strategy for effective inhibition of ESBL in managing severe infections. One of the strategies for effective management of severe infections with high inoculum would be to increase the amount of *β-lactamase* inhibitor.

To the best to our knowledge, there has been no comparative study made to understand impact of different ratios of CS (2:1 vs. 1:1) on its antimicrobial efficacy. So the Present study has been undertaken to understand the effect of different ratios of CS in relation to IE to justify the need of higher concentration of Sulbactam in the CS preparations for effective inhibition of ESBL.

The primary objective of this study is to evaluate MICs of ESBL producing *Enterobacteriaceae* (*Escherichia coli [E. coli], Klebsiella pneumoniae [K. pneumoniae]*), against Ceftriaxone alone, sulbactam alone and Ceftriaxone with Sulbactam combination (1:1 and 2:1) using Broth micro dilution method of antimicrobial susceptibility testing in presence of both normal (10^5 CFU/ml) and higher inoculum (10^7 CFU/ml) of these bacteria. The secondary objective of the study is to demonstrate the *in-vitro* efficacy of CS in presence of higher inoculum of bacteria.

Materials and Methods

The study was performed at Department of Microbiology, Indraprastha Apollo Hospitals, New Delhi. ESBL producing strains of *Enterobacteriaceae* (*E. coli, K. peumoniae.* 30 each) were isolated from patient samples (already fully processed and reported). Stock solution of Ceftriaxone and Sulbactam were prepared, filter sterilized and dilutions prepared from 0.25 to 256 mg/L. Appropriate controls were included with every batch of MIC determinations. All the tests were performed in triplicates and the results were pooled for that isolate to take the average MIC. Testing with Quality control strains were done simultaneously with each batch. MIC of Ceftriaxone and Sulbactam alone and in combination (1:1 and 2:1) was determined in presence of both normal (10^5CFU/ml) and higher inoculum (10^7 CFU/ml) of these bacteria using microbroth dilution method of antimicrobial susceptibility testing.

Results

In consistent with findings of previous studies, present study also finds inoculum effect with Ceftriaxone. *In-vitro* efficacy of addition of Sulbactam to Ceftriaxone against ESBL producing clinical isolates of *Enterobacteriaceae* was studied in the present study.

In ESBL producing strains of *E. coli*, there was a 4 to 125 fold decrease in MIC value of Ceftriaxone on the addition of Sulbactam. The *in-vitro* effect of CS combination on ESBL producing strains of *E. coli* was more pronounced in 1:1 combination at both normal as well as higher bacterial inoculum. The results are shown in the Table 1.

Table 1: MIC range Ceftriaxone, Sulbactam, Ceftriaxone-Sulbactam (1:1) and Ceftriaxone-Sulbactam (2:1) against *E. coli*

Sr. No.	Ceftriaxone		Sulbactam		Ceftriaxone + Sulbactam			
					1:1	1:1	2:1	2:1
	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
1.	>128	>128	=32	=64	=4/4	=8/8	=16/8	=32/16
2.	>128	>128	=64	=128	=2/2	=2/2	=8/4	=16/8
3.	>128	>128	=32	=128	=4/4	=32/32	=16/8	=32/16
4.	=64	>128	=32	=64	=1/1	=2/2	=4/2	=32/16

Sr. No.	Ceftriaxone		Sulbactam		Ceftriaxone + Sulbactam			
5.	>128	>128	=32	=128	=8/8	=32/32	=16/8	=32/16
6.	>128	>128	=32	=64	=4/4	=8/8	=16/8	=32/16
7.	>128	>128	=32	=64	=4/4	=8/8	=16/8	=32/16
8.	=128	>128	=32	=32	=4/4	=8/8	=16/8	=64/32
9.	=32	>128	=32	=32	=1/1	=2/2	=1/0.5	=2/1
10.	=16	>128	=128	>128	=2/2	=16/16	=8/4	=16/8
11.	>128	>128	=32	=32	=16/16	=16/16	=16/8	=16/8
12.	=16	>128	=128	>128	=8/8	=16/16	=8/4	=32/16
13.	>128	>128	=32	=32	=16/16	=16/16	=16/8	=16/8
14.	=128	>128	=32	=32	=8/8	=16/16	=16/8	=16/8
15.	>128	>128	=64	=64	=32/32	=32/32	=64/32	=64/32
16.	>128	>128	=32	>128	=8/8	=32/32	=8/4	=64/32
17.	>128	>128	=128	>128	=32/32	=32/32	=32/16	=32/16
18.	>128	>128	=128	>128	=8/8	=16/16	=16/8	=32/16
19.	>128	>128	=32	=32	=4/4	=32/32	=8/4	=32/16
20.	>128	>128	=32	=32	=4/4	=4/4	=16/8	=32/16
21.	=128	>128	=32	=32	=16/16	=16/16	=64/32	=16/8
22.	>128	>128	>128	>128	=16/16	=16/16	=64/32	>128/64
23.	>128	>128	=128	>128	=8/8	=8/8	=32/16	>128/64
24.	>128	>128	=32	>128	=4/4	=16/16	=8/4	=32/16
25.	=8	>128	=16	>128	=2/2	=4/4	=2/1	=64/32
26.	>128	>128	=64	>128	=4/4	=32/32	=16/8	=32/16
27.	>128	>128	=32	>128	=4/4	=32/32	=16/8	=32/16
28.	>128	>128	=32	>128	=4/4	=16/16	=8/4	=32/16
29.	>128	>128	=32	>128	=8/8	=32/32	=8/4	=64/32
30.	>128	>128	=32	>128	=8/8	=32/32	=8/4	=64/32

In ESBL producing strains of *K. pneumoniae* MIC value for Ceftriaxone was reduced by 2-32 folds with the addition of Sulbactam. The *in-vitro* effect of CS combination on ESBL producing strains of *K. pneumoniae* was more pronounced in 1:1 combination at both normal bacterial inoculum and higher bacterial inoculum. The results are shown in Table 2

Table 2: MIC range Ceftriaxone, Sulbactam, Ceftriaxone-Sulbactam (1:1) and Ceftriaxone-Sulbactam (2:1) against *K. pneumoniae*

Sr. No	Ceftriaxone		Sulbactam		Ceftriaxone + Sulbactam			
					1:1	1:1	2:1	2:1
	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
1.	>128	>128	=128	>128	=4/4	=16/16	=16/8	=32/16
2.	>128	>128	=128	=128	=4/4	=8/8	=16/8	=32/16
3.	=128	>128	=64	=128	=4/4	=32/32	=16/8	=64/32
4.	>128	>128	=32	=16	=4/4	=4/4	=8/4	=16/8
5.	>128	>128	=32	=16	=8/8	=8/8	=16/8	=16/8
6.	=128	>128	=32	>128	=4/4	=32/32	=16/8	=64/32
7.	>128	>128	=32	>128	=8/8	=8/8	=16/8	=32/16
8.	=128	>128	=64	>128	=2/2	=2/2	=4/2	=4/2
9.	=128	>128	=32	>128	=8/8	=8/8	=16/8	=32/16
10.	>128	>128	=32	>128	=16/16	=16/16	=32/16	=32/16
11.	=128	>128	=32	=32	=16/16	=32/32	=8/4	=8/4
12.	>128	>128	=64	=64	=2/2	=2/2	=4/2	=4/2
13.	=128	>128	=32	=32	=8/8	=8/8	=16/8	=16/8
14.	>128	>128	=128	>128	=32/32	=32/32	=32/16	>128/64
15.	>128	>128	=32	=32	=4/4	=64/64	=8/4	=128/64
16.	>128	>128	=32	>128	=16/16	=32/32	=32/16	=32/16
17.	>128	>128	>128	>128	=8/8	=8/8	=64/32	=64/32
18.	=128	>128	=32	=32	=8/8	=8/8	=16/8	=16/8
19.	>128	>128	=32	>128	=4/4	=64/64	=4/2	=64/32
20.	>128	>128	=64	>128	=8/8	=16/16	=16/8	=32/16
21.	>128	>128	=32	=32	=4/4	=8/8	=8/4	=64/32
22.	>128	>128	>128	>128	=8/8	=8/8	=32/16	=64/32
23.	>128	>128	=32	=32	=4/4	=8/8	=8/4	=16/8
24.	>128	>128	>128	>128	=8/8	=8/8	=64/32	=64/32
25.	=128	>128	=32	=32	=8/8	=8/8	=16/8	=32/16
26.	>128	>128	=32	=32	=4/4	=8/8	=8/4	=16/8
27.	>128	>128	=64	>128	=8/8	=16/16	=16/8	=32/16
28.	>128	>128	=128	>128	=32/32	=32/32	=32/16	>128/64
29.	>128	>128	>128	>128	=64/64	=64/64	=8/4	=8/4
30.	>128	>128	=32	=32	=4/4	=8/8	=8/4	=16/8

Discussion

Inoculum effect is defined as an increase in the MIC (eightfold or greater) with increasing bacterial inoculum size.9 If there is an inoculum effect, bacteria may be susceptible in normal inoculum (10^5 CFU/ml) but resistant if the inoculum size is increased. Several studies have proved that inoculum size has a significant impact on the effectiveness of an antibiotic [9, 10].

β-lactam antibiotics demonstrate significant *in-vitro* inoculum effect. The production of *β-lactamases* (including ESBL) is one of the primary mechanisms responsible for inoculum effect.

Caron et al, (1990) evaluated the CS combination in rabbit endocarditis caused by a strain of *K. pneumoniae* producing extended-broad-spectrum TEM-3 *β-lactamase*. They found a significant inoculum effect against ESBL producing *E. coli and K. pneumoniae* with Ceftriaxone. The effective concentrations of Sulbactam needed to reduce the MIC and Minimum Bactericidal Concentration (MBC) of Ceftriaxone to similar levels increased from 1 µg/ml in the presence of an inoculum of $5x10^5$ CFU/ml to 20 µg/ml in the presence of an inoculum of $1x10^7$ CFU/ml [11].

We had conducted an in vitro study (data not published) to understand the inoculum effect on CS combination 1:1 ratio. We found a clear effect of inoculum size on MIC. scientific@aristopharma.co.in. At higher inoculum (i.e. 10^6 CFU/ml) level there was up to 2 fold increase in MIC against *E. coli* and up to 16 fold increase in MIC against *K. pneumoniae* as compared at normal inoculum (10^5 CFU/ml) (study not published). These finding are consistent with previous studies reporting an inoculum effect with Ceftriaxone [12].

This study investigated the *in-vitro* activities of different compositions of Ceftriaxone-Sulbactam and Ceftriaxone alone against ESBL producing strains of *E. coli* and *K.pneumoniae* has significant findings. Ceftriaxone alone was ineffective against ESBL producing strains of *E. coli* and *K. pneumoniae* at both the inoculum strengths. After the addition of Sulbactam, the *in-vitro* activity of Ceftriaxone against ESBL producing strains of *E. coli* and *K. pneumoniae* improved which was demonstrated by a significant decrease in MIC values. In this study, we found a 4 to 128 fold decrease in MIC against ESBL producing *E. coli* and a 2 to 32 fold decrease in MIC against ESBL producing *K. pneumoniae*. This effect was more pronounced with the 1:1 combination than with 2:1 ratio in both normal bacterial inoculum and higher bacterial inoculum.

These findings are consistent with previous study done by Bruno Fauntin et al (1990) [13, 14], wherein it was demonstrated that addition of Sulbactam lowered the MICs of Ceftriaxone against ESBL producing pathogens. The study also emphasised that the bactericidal effects of Ceftriaxone in the presence of Sulbactam was not modified by the high inoculum tested. Time – kill – curve studies demonstrated a concentration dependent effect of Sulbactam with the highest inoculum [13]. The greatest reduction in MICs was documented with the highest concentration of both Ceftriaxone and Sulbactam. The efficacy of the higher concentrations of Ceftriaxone and Sulbactam would underline the importance of *β*-lactam-*β-lactamase* inhibitor concentration ratio in constantly providing sufficient levels of *β*-lactam antibiotic free from *β-lactamase* hydrolysis *in-vivo*.

In a study from Chang et al (2018) [2] Cefoperazone-Sulbactam combinations were evaluated with the aim to assess *in-vitro* activity of Cefoperazone alone and in combination with Sulbactam in different ratios against different inoculum size of multidrug resistant organisms They found that, Cefoperazone-Sulbactam at a 1:1 ratio had greater *in-vitro* activity against most multidrug resistant organisms than at a 2:1 ratio of Cefoperazone-Sulbactam. Moreover, the efficacy of 1:1 ratio was not influenced by the inoculum size of ESBL producing *E. coli* and *K. pneumoniae*. The relative greater *in-vitro* efficacy of 1:1 ratio of Cefoperazone-Sulbactam compared to 2:1 ratio suggested that there is a need of higher concentration of Sulbactam to counter the inoculum effect in severe infections.

Similar findings were observed in our study, which support the justification for requirement of higher strength of Sulbactamin combination with *β*-lactam antibiotics such as Ceftriaxone for the management and treatment of severe infections caused by ESBL producing multidrug resistant organisms.

Conclusion

β-lactam and *β-lactamase* inhibitors have long been used as an effective alternative in the management of infections caused by ESBL producing organisms. The present study has emphasised the need for greater concentrations of Sulbactam in combination with Ceftriaxone (i.e. Ceftriaxone-Sulbactam in the ratio 1:1) for the management of severe infections with higher inoculum caused by ESBL producing organisms. In view of increasing resistance to carbapenems, this combination of Ceftriaxone-Sulbactam (1:1) would be a more effective alternative than Ceftriaxone-Sulbactam (2:1) for management of infections caused by ESBL producing organisms and reserve carbapenems for more severe infections.

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Abbreviations

CFU: Colony forming units; CDSCO: Central Drugs Standard Control Organisation; CS: Ceftriaxone-Sulbactam; ESBL: Extended-Spectrum *β-lactamase*; IE: Inoculum effect; MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration

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