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COMPARISON OF SENSITIVITY OF CEFUROXIME-CLAVULANIC ACID COMBINATION V/S CEFUROXIME ALONE IN BIOFILM AND/OR BETA-LACTAMASE-PRODUCING BACTERIA

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ABSTRACT

Introduction: Bacterial resistance against cephalosporins is most often mediated by β-lactamases in both Gram-positive and Gram-negative bacteria. A novel approach to countering this is the delivery of a β-lactam antibiotic in combination with a β-lactamase inhibitor. **Objectives:** We intended to study the synergistic effect of Cefuroxime-Clavulanic Acid combination in biofilm and/or β-lactamase producing *Staphylococcus aureus* and common Gram-negative bacilli isolated from clinical specimens and compare its efficacy with Cefuroxime alone. **Methods:** It was a retrospective study for a period of 3 months. Bacteria isolated from cases of urinary tract infections (UTIs), skin and soft tissue infections (SSTIs) and post-operative infections were identified by standard techniques. Out of these, 100 biofilm-producing and/or β-lactamase producing bacteria were selected for this study. Detection of in-vitro biofilm formation was done by Congo Red Agar (CRA) method. Beta-lactamase production was tested by Clover leaf method. The antibiotics tested were cefuroxime (30µg) and cefuroxime-clavulanic acid combination in the ratio of 2:1. **Results:** With Cefuroxime-Clavulanic acid combination, overall susceptibility was 24% while for Cefuroxime alone, susceptibility was only 2%. Maximum combination susceptibility was seen in only biofilm producers (42.85%) which was statistically significant. Isolates from SSTIs showed 43.75% sensitivity to Cefuroxime-Clavulanic acid combination which was again statistically significant. Methicillin sensitive *Staphylococcus aureus* showed most significant response to treatment with this combination. All Gram negative bacteria showed less than 25% sensitivity to the Cefuroxime-Clavulanic acid combination. **Conclusion:** This combination can be recommended in SSTIs caused by *Staphylococcus aureus* and in infections where biofilm formation acts as a triggering factor for increased drug resistance. This combination cannot be recommended in Gram-negative bacterial isolates – β-lactamase and/or biofilm producers.

KEY WORDS: Cefuroxime-Clavulanic acid, Biofilm, beta-lactamase, drug resistance.

INTRODUCTION

Biofilms are defined as microbially derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. They are embedded in a matrix of extracellular polymeric substances (EPS) they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.[1] Bacteria growing in a biofilm are highly resistant to microbial agents, known to be associated with severe human diseases which are far more difficult to eradicate. According to National Institutes of Health, more than 80% of all microbial infections are caused by biofilms.[1] Formation of biofilms by Gram-negative bacilli in the urinary tract – the catheter related infections, is one of the first recognized and most studied biofilm associated diseases.[2] Clinical isolates of staphylococci also has a propensity to form biofilms.[3] Various screening methods for detection of biofilm production include Congo red agar (CRA) method, Tube method (TM) by staining with 0.1% crystal violet and Tissue Culture Plate (TCP) method.[1-3]

Bacterial resistance against cephalosporins is most often mediated by β-lactamases in both Gram-positive and Gram-negative bacteria. A novel approach to countering this is the delivery of a β-lactam antibiotic in

combination with a β-lactamase inhibitor.[4] There are reports that addition of clavulanic acid to cefuroxime has caused visible synergism in the activity of cefuroxime against *Staphylococcus aureus* and *Escherichia coli*.[4,5]

Therefore this study was undertaken to study the synergistic effect of Cefuroxime-Clavulanic acid combination in biofilm and/or β-lactamase producing bacteria isolated from clinical specimens and compare the efficacy of this combination with Cefuroxime alone.

MATERIALS AND METHODS

Study design- Retrospective

Duration of Study- 03 months (May to July 2012)

Inclusion criteria- Bacteria isolated from cases of urinary tract infections (UTIs), skin and soft tissue infections (SSTIs) and post-operative infections were included in this study. They were either biofilm-producing and/or β-lactamase-producing bacteria.

Exclusion criteria- Bacteria from infections other than that mentioned above were excluded from the study.

Methodology- Laboratory isolated and preserved strains of Gram-positive cocci and Gram-negative bacilli from cases of UTIs, SSTIs and post-op. infections were identified by standard techniques. Of the 100 biofilm and/or β-lactamase producing isolates included in the study, 44% (44) were from UTI, 40% (40) from post operative infections and 16% (16) from SSTIs.

Biofilm detection- Detection of in-vitro biofilm formation was done by Congo Red Agar (CRA) method.[1,2] It uses brain heart infusion (BHI) broth supplemented with 5% sucrose and Congo red stain (0.8 grams/litre). All isolates were inoculated on CRA plates and incubated aerobically at 37°C for 24 hours. They were performed in duplicate and repeated twice for all isolates. A positive result was indicated by black colonies with a dry crystalline consistency. CRA is an established phenotypic method for detection of biofilms in vitro. It has good sensitivity and specificity and can be routinely used in resource limited settings for detection of biofilm production.

Detection of beta-lactamase production- Beta-lactamase production was tested by Clover leaf method.[6] An agar plate was spread with standard strain of *Staphylococcus aureus* ATCC 25923 strain and an ampicillin disc (30 µg) was placed in the centre. Heavy inoculum of test strain was streaked radially from the disc and the plate was incubated at 37 °C overnight. Deep indentation of growth of *S. aureus* in an otherwise circular zone of inhibition is indicative of beta-lactamase production.

Antibiotic susceptibility- Antibiotic susceptibility testing was done on Mueller Hinton agar by Kirby-Bauer disc diffusion method (KBDDM), according to Clinical Laboratory Standards Institute (C.L.S.I.) guidelines.[7] The antibiotics tested were Cefuroxime (30µg) and Cefuroxime-Clavulanic acid combination in the ratio of 2:1.

Statistical Analysis- Data were entered into Microsoft Excel Worksheet. Statistical analysis was performed using IBM Statistical Package for the Social Sciences version 20 (SPSS v20, IBM). Categorical variables between two groups were compared with Chi-square test and Fisher exact test. A p value of <0.05 was considered significant.

RESULTS

Out of the 44 Urinary Tract Infection (UTI) isolates, 22 (50%) were both beta-lactamase and biofilm producers and 13 (29.55%) were only beta-lactamase producers. Both these findings are statistically significant. In Soft Tissue Infections (STIs), only 25% produced both beta-lactamase and biofilms, but in post-operative infections, majority were only beta-lactamase producers (70%) which was statistically significant (Table 1). Photo 1 and 2 show beta-lactamase production and biofilm production respectively.

Table 1. Overall beta-lactamase and biofilm producers in different types of infections

Infections (Total)	Beta-lactamase producer No. (%)	Biofilm producer No. (%)	Both beta-lactamase and biofilm producer No. (%)
Urinary tract infections (44)	13 (29.55) **p≤0.001**	09 (20.45) **p=0.453**	22 (50) **p≤0.001**
Skin & Soft tissue infections (16)	06 (37.5) **p=0.203**	06 (37.5) **p=0.081***	04 (25) **p=0.256**
Post-operative infections (40)	28 (70) **p≤0.001**	06 (15) **p=0.115**	06 (15) **P=0.001**
Total (100)	**47**	**21**	**32**

(p=Chi-square test, p*=Fischer's exact test)

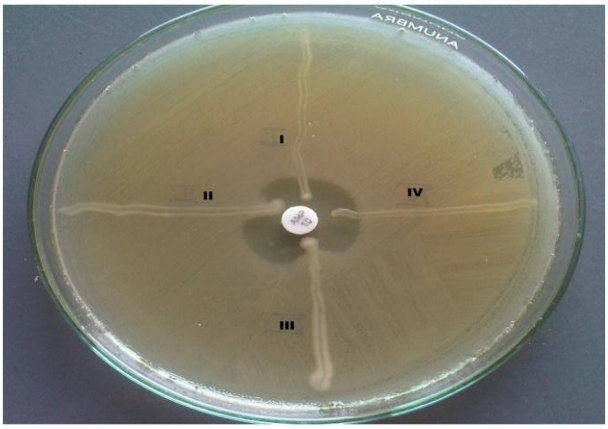

Fig. 1 Clover leaf method for beta-lactamase detection, showing isolates II and III as beta-lactamase producers and isolates I and IV as non producers

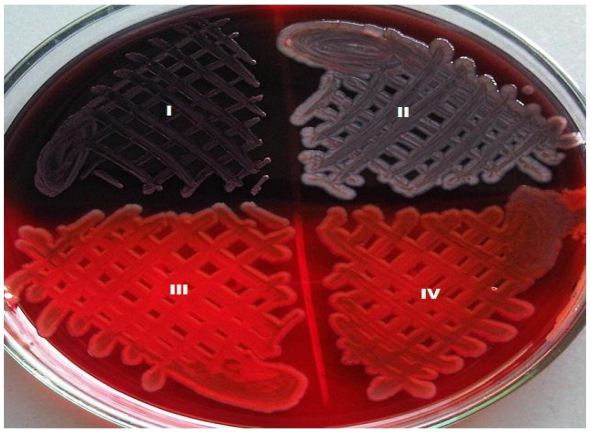

Fig. 2 Congo red agar method for biofilm detection, showing isolate I as biofilm producer, isolate II as indeterminate and III & IV as biofilm non producers

Maximum Cefuroxime-Clavulanic Acid combination sensitivity was seen in only biofilm producers (42.85%) (p value= 0.011), followed by only beta-lactamase producers (19.15%) (Table 2).

Table 2. Sensitivity of Cefuroxime-Clavulanic acid combination against beta-lactamase and/or biofilm producers

	Total	No. positive	Percentage	*p-value*
Beta-lactamase producer only	47	09	19.15%	0.143
Biofilm producer only	21	09	42.85%	**0.011 (Significant)**
Both beta-lactamase and biofilm producer	32	06	18.75%	0.200
Total	**100**	**24**	**24%**	

(p value- Chi Square test)

Sensitivity to Cefuroxime-Clavulanic Acid combination was encountered in 43.75% (7/16) isolates from SSTIs which was statistically significant (p value = 0.04) (Table 3).

Table 3: Source of bacterial isolates and susceptibility to cefuroxime-clavulanic acid combination

Type of infection	Total isolates	Susceptible isolates	p value
SSTIs	16	07	**0.04 (significant)**
UTIs	44	09	0.65
Post-op infections	40	08	0.44
Total	100	24	

(p value- Chi Square test)

Eleven of the 27 isolates of *Staphylococcus aureus* tested showed susceptibility to cefuroxime-clavulanic acid combination which was a statistically significant finding (p value= 0.009). None of the other bacterial isolates gave statistically significant results when tested with the drug combination. (Table 4).

Table 4. Sensitivity of bacterial isolates to Cefuroxime-Clavulanic acid combination

Bacteria	No. tested	No. positive	Percentage	*p-value*
Staphylococcus aureus	27	11	40.74%	**0.009 (significant)**
Escherichia coli	33	08	24.25%	0.484
Pseudomonas aeruginosa	10	01	10.00%	0.253*
Klebsiella pneumoniae	07	01	14.28%	0.464*
Enterobacter species	10	01	10.00%	0.253*
Acinetobacter species	09	00	00.00%	-
Proteus species	04	02	50.00%	0.243*
Total (%)	**100**	**24**	**24.00%**	

(p=Chi-square test, p*=Fischer's exact test)

On testing with Cefuroxime-Clavulanic Acid combination, overall sensitivity was 24% (24/100) (Photo 3), while for Cefuroxime alone, sensitivity was only 2%. The latter were two isolates from Surgical Site Infections (SSIs) and both the isolates were MSSA and both produced beta-lactamases as well as biofilms.

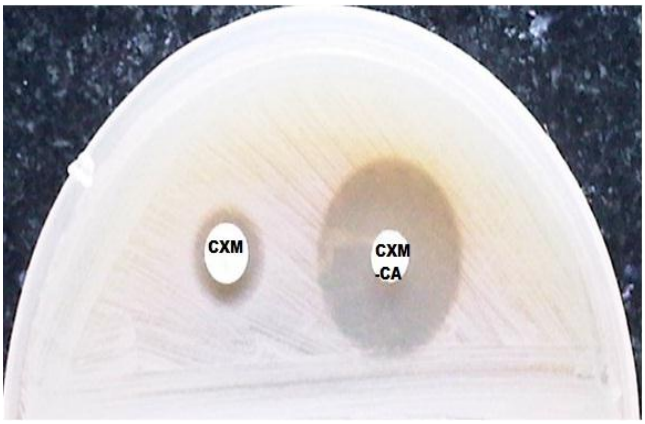

Fig. 3 An isolate of *Escherichia coli* showing susceptibility to Cefuroxime-Clavulanic acid combination and resistance to Cefuroxime alone

Escherichia coli was the most common isolate (33%) followed by Methicillin Sensitive *Staphylococcus aureus* (MSSA) (27%). In UTIs, maximum *Escherichia coli* isolates were found 25/44 (56.82%). In SSTIs, Methicillin Sensitive *Staphylococcus aureus* (MSSA) was predominant 13/16 (81.25%) and in post-operative infections also, MSSA was the predominant isolate 14/40 (35%). Only MSSA isolated from SSTIs and Post-operative infections showed a statistically significant susceptibility to cefuroxime-clavulanic acid combination. (Table 5)

Table 5: Bacteria isolated from different types of infections and their sensitivity to Cefuroxime-clavulanic acid combination

Bacteria	UTI	+	P value	SSTI	+	P value	Post-op	+	P value	Total	+
MSSA	00	00	-	13	08	**0.03**	14	03	**0.03**	27	11
Escherichia coli	25	04	0.05	00	00	-	08	04	0.05	33	08
Pseudomonas aeruginosa	02	00	-	00	00	-	08	01	-	10	01
Klebsiella pnuemoniae	07	01	-	00	00	-	00	00	-	07	01
Enterobacter species	08	01	-	00	00	-	02	00	-	10	01
Acinetobacter species	01	00	-	01	00	-	07	00	-	09	00
Proteus species	01	00	-	02	02	-	01	00	-	04	02
Total	**44**	**06**		**16**	**10**		**40**	**08**		**100**	**24**

(p value= Chi Square test)

DISCUSSION

A novel approach of counteracting bacterial β-lactamases is the delivery of a β-lactam antibiotic in combination with a β-lactamase inhibitor. Commercially clavulanic acid has been combined with amoxycillin and ticarcillin and sulbactam has been combined with ampicillin and cefoperazone.[8] With this combination, not only did the clinical usefulness of amoxicillin become evident but also it showed activity against penicillin resistant staphylococci, *Klebsiella species* and *Bacteroides fragilis*, which had never shown activity in the past.[8]

It is therefore likely that the resistance to 2nd generation cephalosporin like cefuroxime can be overcome by using it in combination with clavulanic acid in some appropriate ratio. In a study in 2008, the combined effect of cefuroxime with clavulanic acid in 2:1 ratio caused visible synergism in the activity of cefuroxime against *Staphylococcus aureus*.[4] So we intended to use cefuroxime and clavulanic acid in 2:1 ratio in this study. In another study conducted by Jalil et al, synergistic effect of cefuroxime and clavulanic acid against *Escherichia coli* were shown.[5] Therefore we intended to study the same synergistic effect against *Staphylococcus aureus* and against common Gram-negative bacilli isolated from clinical specimens.

Majority of the isolates tested were only β-lactamase producers (47%). Out of the 100 isolates tested with the combination antibiotic, 79 were beta-lactamase producing bacteria and 53 produced biofilms (Table 1). Among all isolates, 42.85% of only biofilm producers showed susceptibility to this drug combination which was statistically significant. Only beta-lactamase producers or biofilm and beta-lactamase producing bacterial isolates did not respond favourably to this combination (Table2). Hence, use of cefuroxime-clavulanic acid combination may be recommended in biofilm producing organisms. This is accordance with the findings published in a study by Zubair et al in 2011 where promising results were seen for treatment of biofilms in cases of diabetic foot with the use of cephalosporin-clavulanic acid combination (Cefotaxime-clavulanic acid resistance- 12.2%, ceftazidime-clavulanic acid resistance 9.2%) as compared to cephalosporins alone.[9]

Overall sensitivity to Cefuroxime-Clavulanic acid combination was only 24%. Isolates from SSTIs showed 43.75% sensitivity to Cefuroxime-Clavulanic Acid combination which was statistically significant (Table 3). This again suggests that like the findings of the study conducted by Zubair et al [9], cefuroxime-clavulanic acid combination can be used in the treatment of skin and soft tissue infections (SSTIs).

Overall, MSSA susceptibility to the combination antibiotic was 40.74% (p value = 0.009). All Gram negative bacteria showed less than 25% sensitivity to the Cefuroxime-Clavulanic acid combination and statistical analysis also did not reveal any significant correlation. (Table 4).

On analysis of the susceptible strains according to source of the clinical isolate, it was observed that only MSSA from SSTIs and post operative infection sites showed statistically significant susceptibility to the drug combination tested (Table 5). Therefore, this combination may be recommended in SSTIs caused by *Staphylococcus aureus*. Even though the association is

statistically significant, the overall sensitivity is less than 50% even with *Staphylococcus aureus* in SSTIs, so whether it can become the drug of choice in SSTIs due to *Staphylococcus aureus* is questionable.

According to the findings of the present study, this combination cannot be recommended in Gram-negative bacterial isolates – β-lactamase and/or biofilm producers as no statistically significant correlation could be found for any Gram negative clinical isolate from any infection source. Thus the combination of Cefuroxime and Clavulanic Acid can only be given if tested isolates show in vitro sensitivity to it, after proper antibiotic susceptibility testing.

CONCLUSION

As biofilm formation has an important role in pathogenicity of infections, its detection should be mandatory in a laboratory set up. Though Congo red agar method has disadvantage of subjective evaluation, it is one of the simple, cost-effective phenotypic methods used for screening biofilm formation and does not require technical expertise. Cefuroxime-Clavulanic acid combination can be recommended in SSTIs caused by *Staphylococcus aureus* and in infections caused by biofilm producing organisms. Treatment of the patients with cefuroxime-clavulanic acid combination may help to reduce the morbidity and mortality in these patients, reduce dependence on higher level antibiotics and help in improving patient care. However, according to the present study, the above combination cannot be recommended in Gram-negative isolates – β-lactamase and/or biofilm producers. More studies in this regard should be carried out to meet the challenge of β-lactamase mediated resistance in cefuroxime and other second generation cephalosporins by combining with clavulanic acid or sulbactam, by following a similar strategy.

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REFERENCES

1. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods for biofilm formation in the clinical isolates. Brazilian J Infect Dis., 2011; 15: 305-11.
2. Sevanan M, Pongiya U, Peedikayil NJ. Antibiotic susceptibility pattern of biofilm producing Escherichia coli of urinary tract infections. Current Res Bacteriol., 2011; 4: 73-80.
3. Mathur T. Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: An evaluation of three different screening methods. Indian J Med Microbiol., 2006; 24: 25-9.
4. Jalil A, Niazi ID, Khan SU. Evaluation of restoration of sensitivities of resistant Staphylococcus aureus isolates by using cefuroxime and clavulanic acid in combination. J Ayub Med Coll Abbottabad., 2008; 20: 28-30.
5. Jalil A, Wadood A, Naseem R. Evaluation of synergistic effect of cefuroxime and clavulanic acid against *Escherichia coli*. J Med Sci., 2005; 13: 132-5.
6. Bergan T, Bruun JN, Digranes A, Lingaas E, Melby KK, Sander J. Susceptibility testing of bacteria and fungi. Report from "the Norwegian Working Group on Antibiotics". Scand J Infect Dis Suppl., 1997; 103: 1-36.
7. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute (C.L.S.I.). 2011; M 100- S 21. 31: pp 42-44, 68: 72.
8. Aldridge KE, Savders CV, Marier RL. Variation in the potentiation of β-lactam antibiotic activity by clavulanic acid and sulbactam against multiple antibiotic resistant bacteria. J Antimicrob Chemother., 1986; 17: 463-9.
9. Zubair M, Malik A, Ahmad J, Rizvi M, Farooqui KJ, Rizvi MW. A study of biofilm production by gram-negative organisms isolated from diabetic foot ulcer patients. Biol Med., 2011; 3: 147-57.