

## EVALUATION OF RESTORATION OF SENSITIVITIES OF RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES BY USING CEFUROXIME AND CLAVULANIC ACID IN COMBINATION

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**Background:** The present study was planned to observe the activity of cefuroxime, a second generation cephalosporin after combining it with a  $\beta$ -lactamase inhibitor clavulanic acid. The study was conducted to evaluate the restoration or increase in sensitivity of  $\beta$ -lactamase producing isolates of Staphylococcus aureus. **Methods:** Staphylococcus aureus were identified by standard procedures. For  $\beta$ -lactamase detection chromogenic Nitrocefin impregnated sticks were used. The sensitivity of the bacteria to the antibiotic disks was measured by disk diffusion method using standard zone diameter criteria given by National Committee of Clinical Laboratory Standards. **Results:** The disks of cefuroxime with clavulanic acid had developed larger zones of inhibition. The activity of cefuroxime against Staphylococcus aureus was significantly increased by clavulanic acid. **Conclusion:** Clavulanic acid if used in combination with cefuroxime, can improve the antimicrobial activity of cefuroxime against  $\beta$ -lactamase producing Staphylococcus aureus.

**Keywords:** Staphylococcus aureus, cefuroxime, clavulanic acid.

### INTRODUCTION

In fact as early as in 1940, a bacterial enzyme capable of neutralizing antibiotic drug effect was described.<sup>1</sup> With the continued medical use of antibiotics, bacterial resistance quickly became apparent<sup>2</sup> and it was proved that the major factor involved in the development of antibiotic resistance is antibiotic usage.<sup>3</sup> Now this global emergence and spread of microbial resistance is posing a major risk for human health due to the impact on morbidity, mortality and health care costs.<sup>4</sup>

The  $\beta$ -lactam antibiotics are the largest and currently most widely used antibacterial agents. Their antibacterial spectrum is the widest of all antibiotics groups and can range from very narrow to extremely broad for individual agents.<sup>5</sup> More over  $\beta$ -lactam antibiotics have actually revolutionized the treatment of infections.<sup>6</sup> Bacterial resistance against cephalosporins is most often mediated by  $\beta$ -lactamases which have emerged and evolved rapidly in both Gram positive and Gram negative bacteria.

A novel approach to countering of bacterial  $\beta$ -lactamases is the delivery of a  $\beta$ -lactam antibiotic in combination with a  $\beta$ -lactamase inhibitor.<sup>7</sup> The first inhibitor of  $\beta$ -lactamase, clavulanic acid, became available for clinical use in 1994.<sup>8</sup>  $\beta$ -lactamases often exhibit a high affinity for these compounds, become irreversibly bound and are there by inactivated.<sup>9</sup> It thus allows the  $\beta$ -lactam antibiotic to act as if the organism was fully sensitive. Therefore in treatment of infections caused by  $\beta$ -lactamase producing strains, the combination of  $\beta$ -lactamase inhibitor with a  $\beta$ -lactam has been successful.<sup>10</sup>

Commercially Clavulanic acid has been combined with amoxicillin and ticarcillin.<sup>11</sup> In combination with clavulanic acid, the clinical usefulness

of amoxicillin is restored to that which was present when the product was first introduced, prior to acquired drug resistance becoming a major clinical problem. In addition certain infections caused by bacteria against which amoxicillin had never shown useful activity, such as penicillin resistant Staphylococci, Klebsiella and Bacteriodes fragilis, can now be treated with the combination.<sup>11</sup> It is therefore likely that the resistance to 2<sup>nd</sup> generation cephalosporin like cefuroxime can be overcome by using it in combination with clavulanic acid in some appropriate ratio. The objective of the present study was to evaluate the antibiotic activity of cefuroxime against Staphylococcus aureus using the antibiotic alone and then the restoration of its activity by using its combination with clavulanic acid.

### MATERIALS AND METHODS

This study was carried out at Clinical Microbiology Laboratory, Khyber Medical College, Peshawar. Out of the clinical specimens isolated and identified by their morphology, staining reactions, culture characteristics and bio-chemical tests, forty isolates of  $\beta$ -lactamase producing Staphylococcus aureus were taken. The  $\beta$ -lactamase production by these isolates was confirmed by the rapid chromogenic method<sup>12</sup> in which Nitrocefin containing sticks change their colours to pink or red by hydrolysis of the Nitrocefin and production of acidic derivative.

Bacterial inoculation of Staphylococcus aureus was done in the Petri dishes containing a layer of Mueller Hinton agar.<sup>13</sup> It has become the standard medium for Baur-Kirby method and its performance is specified by National Committee for Clinical laboratory standards (NCCLS). Small disks with different concentrations were prepared and placed in these Petri dishes 24-30 cms apart.<sup>14</sup> The disks were

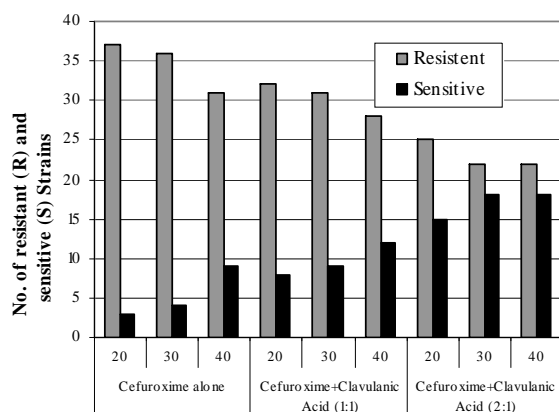
incubated at 37 °C for about 16-18 hours. After this period the diameter of zone of inhibition of each disk was measured. The zone of inhibition corresponded to the antibiotic activity of each disk<sup>15</sup>. For statistical evaluation, a modified form of X<sup>2</sup> test was applied, because this special form of X<sup>2</sup> test is used in medical practice. This is 2x2 contingency table in which positive and negative values are compared<sup>16</sup>. On this basis the respective P-values were worked out which were all less than 0.001, and were thus not significant.

## RESULTS

The antibiotic disks containing 20, 30 and 40 µg of the cefuroxime and those containing cefuroxime and clavulanic acid in 1:1 and 2:1 ratios were used for recording their activity against all 40 β-lactamase producing isolates of *Staphylococcus aureus*. The results obtained by measuring the zones of inhibition of different disks are shown in Table 1 and Fig 1. Zones of inhibition of inhibitor of diameter more than 14 mm were taken as sensitive.

**Table-1: Sensitivities of *Staphylococcus aureus* to Cefuroxime alone and in combination with Clavulanic acid**

Cefuroxime (Control)		Cefuroxime+Clavulanic Acid (1:1)			Cefuroxime+Clavulanic Acid (2:1)		
Cone (µg)	Sensitive	Sensitive	Increase in Sensitivity	% Increase in sensitivity	Sensitive	Increase in Sensitivity	% Increase in Sensitivity
20	3	8	5	12.5	15	12	30
30	4	9	5	15	18	14	35
40	9	12	3	7.5	18	9	22.5



**Figure-1: Sensitivities of *Staphylococcus aureus* to Cefuroxime alone and in combination with Clavulanic acid**

## DISCUSSION

Certain bacteria now defy nearly all antibiotics. World wide strains of *Staphylococcus aureus* resistant to all antibiotics except vancomycin are encountered<sup>3</sup>. Ironically *Staphylococcus aureus* is one of the most frequently isolated human pathogens.<sup>3</sup> On the other hand the β-lactam antibiotics are the most frequently used antimicrobial agents. Unfortunately the efficiency of these drugs is increasingly being challenged by the emergence of resistant bacteria<sup>3</sup>. The production of β-lactamases is the main cause of bacterial resistance.<sup>17</sup>

The novel therapy of combining an established β-lactam antibiotic with β-lactamase inhibitor, neutralizes the effect of β-lactamases.<sup>18</sup> This approach has largely overcome the resistance which has been developed in bacteria including *Staphylococcus aureus* to commonly used antibiotics, such as ampicillin and amoxicillin.<sup>10</sup> Clavulanic acid

has been combined with amoxicillin and ticarcillin, while sulbactam has been combined with ampicillin and cefoperazone.<sup>11</sup> But the result of the combination of clavulanic acid with cefuroxime is still to be ascertained. New cephalosporins are being developed for their anti *Staphylococcus aureus* activity, notably Ceftobiprole and Ceftaroline. These are also vulnerable to extended spectrum β-lactamases<sup>19</sup> and manufacturers may seek to formulate them with Clavulanic acid and other β-lactamase inhibitors to widen their spectra.<sup>20</sup>

In this study the result of combining cefuroxime with clavulanic acid has been evaluated.

According to our results, it is evident that the second generation cephalosporin, cefuroxime alone has very little effect in all three concentrations (20, 30, 40µg). however, as expected, higher concentrations have progressively shown an increase in sensitivity of *Staphylococcus aureus*.

When cefuroxime was combined with clavulanic acid in 1:1 ratio, the sensitivities of *Staphylococcus aureus* increased to some extent. The increase in sensitivity in this ratio was 12.5% with 20µg, 15% with 30µg and 7.5% with 40µg of each component. The combined effect of cefuroxime with clavulanic acid in 2:1 ratio produced respectively 30, 35 and 22.5% increase in sensitivity. In other words, addition of clavulanic acid has caused visible synergism in the activity of cefuroxime against *Staphylococcus aureus*. Beside, formulations which are approved for use in the USA, other combinations, such as mezlocillin-sulbactam and cefoperazone sulbactam are currently used in medical practice in other countries as separate agents or are being tested in preliminary clinical trails.<sup>21, 22</sup>

Therefore it can be hoped that the wide spread resistance to a  $\beta$ -lactam, cefuroxime against *Staphylococcus aureus* can be expected to overcome by combining cefuroxime with clavulanic acid.

Taking into account this model and also studying the synergism against other bacteria, it is suggested that more clinical studies in this regard should be carried out to meet the challenge of  $\beta$ -lactamase mediated resistance in cefuroxime and other second generation cephalosporins by following a similar strategy.

## CONCLUSION

The  $\beta$ -lactamase inhibitor clavulanic acid if used along with cefuroxime in appropriate proportion, can restore the sensitivities of  $\beta$ -lactamase producing *Staphylococcus aureus* isolates.

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