

## Minimum inhibitory concentration and time kill curve studies of tobracef, a fixed dose combination of ceftazidime and tobramycin

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### ABSTRACT

Ceftazidime belongs to cephalosporin class of antibiotics with broad spectrum activity. Tobramycin is an aminoglycoside antibiotic used to treat various types of bacterial infections, particularly gram negative infections. This study was aimed at evaluating microbial efficacy of Tobracef, a Fixed Dose Combination (FDC) of ceftazidime and tobramycin in comparison with ceftazidime and tobramycin alone. Efficacy was evaluated on the basis of Minimum Inhibitory Concentration (MIC) and time kill curve analysis in *Acinetobacter baumannii*, *Mycobacterium smegmatis*, *Citrobacter braaki* and *Pseudomonas aeruginosa*. In case of *A. baumannii*, *M. smegmatis*, *C. braaki* and *P. aeruginosa*, MIC were found to be 0.0625 mg/l, 0.125 mg/l, 0.03125mg/l and 0.25 mg/l in Tobracef respectively. In ceftazidime alone the MIC were found to be 0.25mg/l, 0.25mg/l, 1mg/l and 1mg/l respectively. For tobramycin alone the MIC were found to be 0.125mg/l, 0.25mg/l, 0.0625mg/l and 0.5mg/l respectively. In all organisms under study, time-kill curve analysis demonstrated bacterial maximum killing at 4 hours. In conclusion, Tobracef, a FDC of ceftazidime and tobramycin was found to have more bacterial inhibiting properties than ceftazidime and tobramycin alone.

**Key words:** Minimum Inhibitory Concentration (MIC), ceftazidime, tobramycin and tobracef.

### INTRODUCTION

Ceftazidime belongs to cephalosporin class of antibiotics with broad spectrum activity<sup>1, 2</sup>. It is stable to both plasmid and chromosomal  $\beta$ -lactamase resistance than other cephalosporins<sup>3-4</sup>. Ceftazidime is a third generation cephalosporin and is resistant to hydrolysis. It is effective against a broad range of gram positive and gram negative bacteria and also against bacteria resistant to cephalosporins.

Tobramycin is an aminoglycoside antibiotic used to treat various types of bacterial infections, particularly gram negative infections. It is often used concomitantly with other antibacterials to extend its spectrum of efficacy or increases its effectiveness. Treatment with a combination of an

aminoglycoside with a  $\beta$ -lactam has showed increased efficacy.

Ceftazidime and tobramycin combination therapy is considered by some clinicians to be the clinical standard.<sup>5</sup> Antibacterial drugs have been highly successful in controlling the morbidity and mortality that accompany serious bacterial infections. Some of the exiting antibiotics may cause adverse effects in some patients. Some of these side effect may be significant enough to require that therapy should be discontinued<sup>6,7</sup>.

Combination therapy of cephalosporins and aminoglycosides is also used to broaden the antimicrobial spectrum in critically ill patients while awaiting a bacteriological diagnosis or proven polymicrobial infection. Synergism appears to be

maintained even at very high MIC with drug combinations within achievable therapeutic ranges<sup>8, 9, 10, 11</sup>.

The fight against bacterial infection represents one of the highest point of the modern medicine. Since the development of antibiotics, this powerful tool has saved millions of lives. However, because of inappropriate and large use of antibiotics, many antibiotic resistant strains are growing in number. The resistant bacteria pose a significant threat to human health and a challenge to researches.<sup>12, 13</sup> Keeping this in the view, the present study was planned to evaluate efficacy of Tobracef, FDC of ceftazidime and tobramycin against some clinically significant microorganisms.

## MATERIAL AND METHODS

### Bacterial Strains

Following strains obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India were used for the study, *Acinetobacter baumannii* (MTCC No. - 1425), *Mycobacterium smegmatis* (MTCC No. - 995), *Citrobacter braaki* (MTCC No. - 2690) and *Pseudomonas aeruginosa* (MTCC No. - 1688)

### Antibiotic

Tobracef, ceftazidime and tobramycin used in study were provided by manufacturer, Venus Remedies Limited, India.

### Medium

Mueller Hinton (MH) media supplemented with Calcium (25 mg/l) and Magnesium (1.25 mg/l)

was used for MIC and susceptibility tests experiments. Colony counts were determined with MH agar plates.

### Susceptibility Testing

The MIC of ceftazidime and tobramycin alone and in a Tobracef against *A. baumannii*, *M. smegmatis*, *C. braaki* and *P. aeruginosa* were determined by broth micro dilution method as per the standard National Committee for Clinical Laboratory Standards.<sup>14</sup> Overnight MH broth cultures were used to prepare inocula of 10<sup>5</sup> CFU/ml. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 hours of incubation at 37 °C.

## RESULTS

### MIC studies

In case of *A. baumannii*, *M. smegmatis*, *C. braaki* and *P. aeruginosa* MIC were found to be 0.25 mg/l, 0.25 mg/l, 1 mg/l and 1 mg/l for ceftazidime respectively and in tobramycin alone the MIC were found to be 0.125 mg/l, 0.25 mg/l, 0.0625 mg/l and 0.5 mg/l respectively. In a tobracef MIC were found to be 0.0625 mg/l, 0.125 mg/l, 0.03125mg/l and 0.25 mg/l respectively.

The MIC of all microbial strains under study resulted in significant reduction in ceftazidime, tobramycin alone and tobracef (Table 1).

### Time kill curve analysis

Bactericidal effect, with 2X the MIC of tobracef, ceftazidime and tobramycin achieved the earliest killing at 4 hours. Bacterial killing rate in

**Table 1: Minimum Inhibitory Concentrations determination of ceftazidime, tobramycin and Tobracef with *A. baumannii*, *M. smegmatis*, *C. braaki* and *P. aeruginosa***

S. No.	Microorganism	MICs(mg/L)		
		Ceftazidime	Tobramycin	Tobracef
1.	<i>A. baumannii</i>	0.25	0.125	0.0625
2.	<i>M. smegmatis</i>	0.25	0.25	0.125
3.	<i>C. braaki</i>	1	0.0625	0.03125
4.	<i>P. aeruginosa</i>	1	0.5	0.25

toracef were distinctly higher than ceftazidime and tobramycin alone in all the strains under study. (Fig-1, Fig - 2, Fig - 3 and Fig - 4)

In *A. baumannii* time kill curve analysis demonstrated bacterial killing in 0 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs and 12 hrs for ceftazidime alone 6.31 log<sub>10</sub>CFU/ml, 5.94 log<sub>10</sub>CFU/ml, 4.95 log<sub>10</sub>CFU/ml, 5.40 log<sub>10</sub>CFU/ml, 5.62 log<sub>10</sub>CFU/ml, 6.27 log<sub>10</sub>CFU/ml and 6.46 log<sub>10</sub>CFU/ml, for a tobramycin alone 6.15 log<sub>10</sub>CFU/ml, 5.81 log<sub>10</sub>CFU/ml, 4.70 log<sub>10</sub>CFU/ml, 5.32 log<sub>10</sub>CFU/ml, 5.76 log<sub>10</sub>CFU/ml, 6.26 log<sub>10</sub>CFU/ml and 6.40 log<sub>10</sub>CFU/ml, and tobracef were 6.09 log<sub>10</sub>CFU/ml, 5.76 log<sub>10</sub>CFU/ml, 4.30 log<sub>10</sub>CFU/ml, 5.08 log<sub>10</sub>CFU/ml, 5.59 log<sub>10</sub>CFU/ml, 6.09 log<sub>10</sub>CFU/ml and 6.33 log<sub>10</sub>CFU/ml. (Fig - 1)

In *M. smegmatis* time kill curve analysis demonstrated bacterial killing in 0 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs and 12 hrs for tobracef were 5.99 log<sub>10</sub>CFU/ml, 5.43 log<sub>10</sub>CFU/ml, 4.30 log<sub>10</sub>CFU/ml, 5.08 log<sub>10</sub>CFU/ml, 5.48 log<sub>10</sub>CFU/ml, 5.92 log<sub>10</sub>CFU/ml and 6.10 log<sub>10</sub>CFU/ml respectively, for ceftazidime alone 6.25 log<sub>10</sub>CFU/ml, 5.49 log<sub>10</sub>CFU/ml, 4.48 log<sub>10</sub>CFU/ml, 5.18 log<sub>10</sub>CFU/ml, 5.48 log<sub>10</sub>CFU/ml, 5.96 log<sub>10</sub>CFU/ml and 6.16 log<sub>10</sub>CFU/ml receptively and tobramycin alone 6.01 log<sub>10</sub>CFU/ml, 5.49 log<sub>10</sub>CFU/ml, 4.48 log<sub>10</sub>CFU/ml, 5.18 log<sub>10</sub>CFU/ml, 5.48 log<sub>10</sub>CFU/ml, 5.96 log<sub>10</sub>CFU/ml and 6.10 log<sub>10</sub>CFU/ml. (Fig - 2)

In *C. braaki* time kill curve analysis demonstrated bacterial killing in 0 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs and 12 hrs for ceftazidime alone

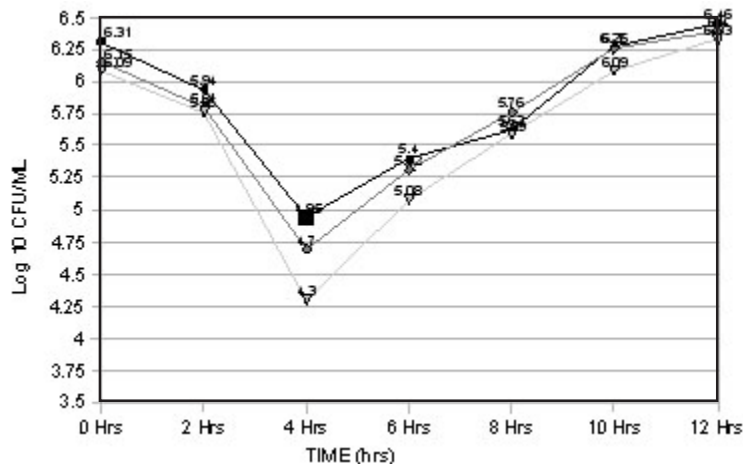


Fig. 1:

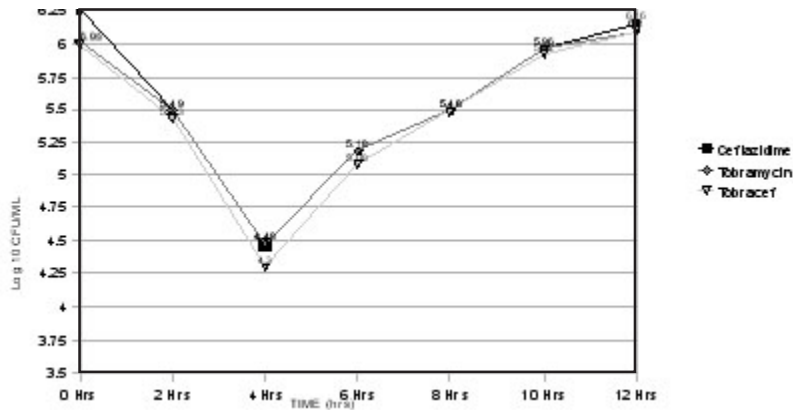


Fig. 2: Time Kill Curve of *M. smagmatis*

6.33 log<sub>10</sub>CFU/ml, 5.99 log<sub>10</sub>CFU/ml, 4.90 log<sub>10</sub>CFU/ml, 5.46 log<sub>10</sub>CFU/ml, 5.83 log<sub>10</sub>CFU/ml, 6.04 log<sub>10</sub>CFU/ml and 6.29 log<sub>10</sub>CFU/ml, for a tobramycin alone 6.22 log<sub>10</sub>CFU/ml, 6.00 log<sub>10</sub>CFU/ml, 4.85 log<sub>10</sub>CFU/ml, 5.54 log<sub>10</sub>CFU/ml, 5.77 log<sub>10</sub>CFU/ml, 6.07 log<sub>10</sub>CFU/ml and 6.32 log<sub>10</sub>CFU/ml, and tobracef were 6.11 log<sub>10</sub>CFU/ml, 5.98 log<sub>10</sub>CFU/ml, 4.70 log<sub>10</sub>CFU/ml, 5.45 log<sub>10</sub>CFU/ml, 5.65 log<sub>10</sub>CFU/ml, 6.02 log<sub>10</sub>CFU/ml and 6.27 log<sub>10</sub>CFU/ml. (Fig - 3)

In *P. aeruginosa* time kill curve analysis demonstrated bacterial killing in 0 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs and 12 hrs for tobracef were 6.46 log<sub>10</sub>CFU/ml, 6.26 log<sub>10</sub>CFU/ml, 4.78 log<sub>10</sub>CFU/ml, 5.96 log<sub>10</sub>CFU/ml, 6.26 log<sub>10</sub>CFU/ml, 6.46 log<sub>10</sub>CFU/ml and 6.55 log<sub>10</sub>CFU/ml respectively, for ceftazidime alone 6.47 log<sub>10</sub>CFU/ml, 6.28 log<sub>10</sub>CFU/ml, 5.00 log<sub>10</sub>CFU/ml, 5.80 log<sub>10</sub>CFU/ml, 6.12 log<sub>10</sub>CFU/ml, 6.48 log<sub>10</sub>CFU/ml and 6.60 log<sub>10</sub>CFU/ml

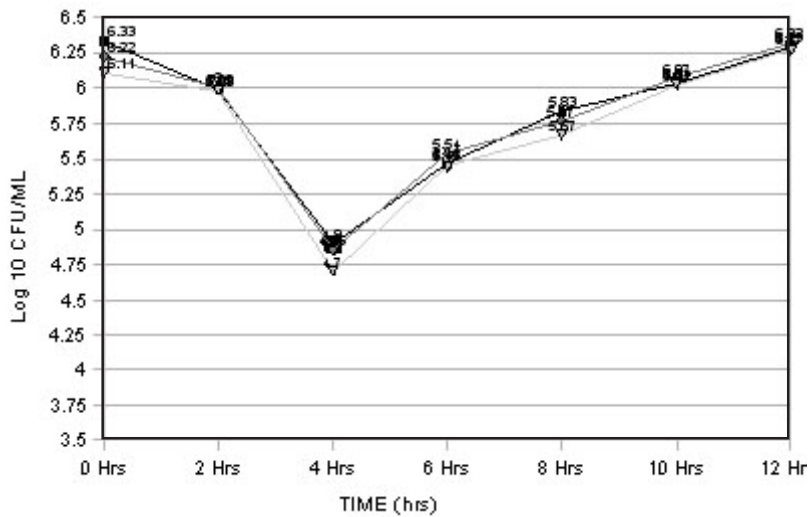


Fig. 3: Time Kill Curve of *C. braakii*

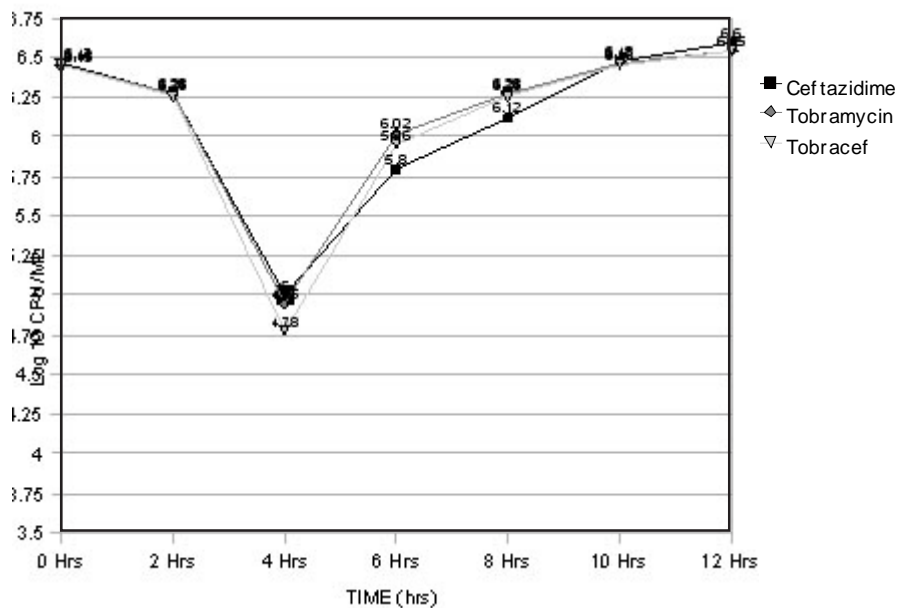


Fig. 4: Time Kill Curve of *P. aeruginosa*

ml receptively and tobramycin alone 6.45 log<sub>10</sub>CFU/ml, 6.27 log<sub>10</sub>CFU/ml, 4.95 log<sub>10</sub>CFU/ml, 6.02 log<sub>10</sub>CFU/ml, 6.28 log<sub>10</sub>CFU/ml, 6.47 log<sub>10</sub>CFU/ml and 6.55 log<sub>10</sub>CFU/ml. (Fig - 4)

## DISCUSSION

A further indication for antibiotic combinations is to prevent emergence of resistance<sup>15</sup>. A synergistic interaction between the two antibiotics is one reason for using this combination<sup>16</sup>. Antibiotic combinations have long been used to provide antibacterial activity against multiple potential pathogen for initial empirical treatment of critically ill patients. Several studies of antibiotic combination therapy for gram negative infection conducted from the 1970s to the 1990s. The consensus is that combination therapy is probably more effective than mono therapy only for infections. Gram negative bacterial species typically have a higher degree of antibiotic resistance than gram positive bacteria. This is largely in part due to the presence of a selectively permeable outer membrane which restricts the entrance of small hydrophobic molecules, including many available antibiotics<sup>17</sup>. Aminoglycoside class antibiotic exert a killing effect by binding to bacterial ribosomes and inhibiting bacterial protein synthesis.

Ceftazidime is a third generation cephalosporins, with good antibacterial activity.<sup>2, 18</sup> Clinically administration of two or more antibiotics in the treatment of infections is usually rationalized with the knowledge that multiple antibiotics often exert additive or synergistic effects, increasing the likelihood of pathogen eradication. In comparison

with older cephalosporins, it crosses the bacterial outer cell membrane faster and has advantages of rapid penetration in periplasmic space as well as of extended spectrum of the activity that include gram positive and gram negative organisms.

Useful antibiotic classes based on a  $\beta$ -lactam structure include broad penicillins, cephalosporins, carbapenems, all of which inhibit bacterial cell wall synthesis.  $\beta$ -lactamase enzymes that rapidly degrade the cephalosporins  $\beta$ -lactam ring have a primary bacterial resistance mechanism against this class of drug since the commencement of clinical cephalosporins use in the 1940s. Because of this, most cephalosporins derived antibiotics still clinically used are formulated to include a  $\beta$ -lactamase inhibitor in order to increase the drug's effectiveness. Cephalosporins also contain a  $\beta$ -lactam ring, but are structurally more resistance to  $\beta$ -lactamase degradation.

Tobrcef has lower MIC than ceftazidime and tobramycin alone against *A. baumannii*, *M. smegmatis*, *C. braaki* and *P. aeruginosa*.

This investigation indicated that Tobrcef has better efficacy as compared to ceftazidime and tobramycin alone in organisms under study.

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