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In vitro microbial efficacy analysis of tobracef, a fixed dose combination of ceftazidime and tobramycin against *Acinetobacter lwoffii, Morganella morganii, Enterobacter cloacae, Hafnia alvei, Citrobacter freundii & Serratia grimesii*

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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. In present study is an attempt to determine efficacy of ceftazidime, tobramycin and Tobracef, their Fixed Dose Combination (FDC) on some microorganisms. Efficacy was evaluated on the basis of *Minimum Inhibitory Concentration (MIC) and Antibiotic Susceptibility Test (AST). In A*cinetobacter lwoffii, *Morganella morganii, Enterobacter cloacae, Hafnia alvei, Citrobacter freundii* and Serratia *grimesii.* MIC were found to be 8µg/l, 4µg/l, 16µg/l, 4 µg/l, 2µg/l and 1µg/l for ceftazidime respectively. In a tobramycin alone the MIC were found to be 4 µg/l, 8 µg/l, 8 µg/l, 8 µg/l, 4 µg/l and 8 µg/l respectively whereas in tobracef MIC were found to be 2 µg/l, 2 µg/l, 4 µg/l, 2 µg/l, 1 µg/l and 0.5 µg/l. Results of AST also showed more lytic zone by Tobracef in all organisms when compared with ceftazidime and tobramycin alone. These results indicate that the Tobracef has better bactericidal activity in comparison to ceftazidime and tobramycin alone in organisms under study.

Key words : *Minimum Inhibitory Concentration (*MIC), AST, Ceftazidime, Tobramycin, Tobracef.

INTRODUCTION

Ceftazidime belongs to cephalosporin class of antibiotics with broad spectrum activity.[1, 2] It is stable to both plasmid and chromosomal β-lactamase resistance then other cephalosporins.[3, 4] 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 β-lactam has showed increased efficacy.

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[5-8].

Ceftazidime and tobramycin combination therapy is considered by some clinicians to be the clinical standard[9]. 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[10, 11].

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[12, 13] 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.

MATERIALS 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 lwoffii (MTCC No. 496), Morganella morganii (MTCC No. 662), Enterobacter cloacae (MTCC No. 509), Hafnia alvei (MTCC No. 1426), Citrobacter freundii (MTCC No. 1658) and Serratia grimesii (MTCC No. 1887).

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. lwoffii, M. morganii, E. cloacae, H. alvei, C. freundii* and *S. grimesii* were determined by broth micro dilution method as per the standard National Committee for Clinical Laboratory Standards.[14] Overnight MH broth cultures were used to prepare inocula of 10^5 CFU/ml. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37 °C.

RESULTS

MIC studies

In case of *A. lwoffii, M. morganii, E. cloacae, H. alvei, C. freundii* and *S. grimesii* MIC were found to be 8 µg/l, 4 µg/l, 16 µg/l 4 µg/l 2 µg/l and 1 µg/l for ceftazidime respectively. In a tobramycin alone the MIC was found to be 4 µg/l, 8 µg/l, 8 µg/l 8 µg/l 4 µg/l and 8 µg/l respectively and Tobracef MIC was found to be 2 µg/l, 2 µg/l, 4 µg/l, 2 µg/l, 1 µg/l and 0.5 µg/l.

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

Susceptibility studies

MIC of all microbial strain under study resulted in reduction in Tobracef when compared with ceftazidime and tobramycin alone (Table 2).

Table 1: Minimum Inhibitory Concentrations determination of ceftazidime, tobramycin and *Tobracef* with *A. lwoffii, M. morganii, E. cloacae, H. alvei, C. freundii* and *S. grimesii*

S. No	Microorganism	Drug [mg/L]	MIC Concentration (mg/L)
1	*A. lwoffii*	Ceftazidime	8
		Tobramycin	4
		Tobracef	2
2	*M. morganii.*	Ceftazidime	4
		Tobramycin	8
		Tobracef	2
3	*E. cloacae.*	Ceftazidime	16
		Tobramycin	8
		Tobracef	4
4	*H. alvei.*	Ceftazidime	4
		Tobramycin	8
		Tobracef	2
5	*C. freundii.*	Ceftazidime	2
		Tobramycin	4
		Tobracef	1
6	*S. grimesii*	Ceftazidime	1
		Tobramycin	8
		Tobracef	0.5

Table 2: Results of comparative antimicrobial susceptibility test studies of ceftazidime, tobramycin and tobracef

S. No.	Microorganism	Zone diameter Inhibition (mm)		
		Ceftazidime (30 µg)Avg.± S.D.	Tobramycin (10µg)Avg.± S.D.	Tobracef (40µg)Avg.± S.D.
1	*A. lwoffii*	23.80 ± 0.58	26.74 ± 0.87	28.07 ± 0.34
2	*M. morganii*	27.14 ± 0.40	23.30 ± 0.99	28.34± 0.89
3	*E. cloacae*	27.83 ± 0.12	22.08 ± 1.06	23.24 ± 0.65
4	*H. alvei*	25.82 ± 0.19	25.12 ± 0.43	29.77± 0.63
5	*C. freundii*	27.71 ± 0.22	26.80 ± 0.40	29.41 ± 1.48
6	*S. grimesii*	33.68 ± 0.39	22.74 ± 0.10	35.86 ± 0.05

Average ± standard deviation

DISCUSSION

A synergistic interaction between the two antibiotics is one reason for using this combination.[15] A further indication for antibiotic combinations is to prevent emergence of resistance.[16] 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.[17] Aminoglycoside class antibiotic exert a killing effect by binding to bacterial ribosomes and inhibiting bacterial protein synthesis.

Useful antibiotic classes based on a β - lactam structure include broad penicillins, cephalosporins, carbapenems, all of which inhibit bacterial cell wall synthesis. β - lactamase enzymes that rapidly degrade the cephalosporins β-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 β- lactamase inhibitor in order to increase the drug's effectiveness. Cephalosporins also contain a β- lactam ring, but are structurally more resistance to β-lactamase degradation.

Ceftazidime is a third generation cephalosporins, with good antibacterial activity.[2, 18] 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.

Tobracef has lower MIC than ceftazidime and tobramycin alone against *A. lwoffii, M. morganii, E. cloacae, H. alvei, C. freundii* and *S. grimesii.* AST studies also showed that bacterial lytic zone was more under influence of Tobracef than ceftazidime and tobramycin alone.

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

REFERENCES

1. Seibert G., Limbert M., Winkler I. and Dick T., The Antibacterial activity *in – vitro* and β – lactamase stability of the new cephalosporin HR 810 in comparison with five other cephalosporins and two aminoglycosides, Infection., **11**(5): 275 - 279 (1983).
2. Jone R.N., Pfaller M.A., Allen S.D., Gerlach E.H., Fuchs P.C. and Aldridge K.E., Antimicrobial activity of Ceftazidime : an update compared to five third - generation cephalosporins against nearly 6000 recent clinical isolates from five medical centers, *Diagn. Microbiol. Infect. Dis.,* **14**: 361-364 (1991).
3. Jacoby G.A. and Carreras I., Activities of β - lactam antibiotics against *Escherichia coli* strains producing extended spectrum β - lactamases, Antimicrob. Agents. Chemother., **34**(5): 858-862 (1990).
4. Stobberingh E.E. and Houbesn A.W., Including capacity and selection of resistance varients of Ceftazidime [HR 810] in comparison with other β – lactam compounds, *Chemother.*, **34**: 490-496 (1988).
5. Baltch A.L., Bassey C., Hammer M.C., Smith R.P., Conroy J.V. and Michelsen P.B., Synergy with cefsulodin or piperacillin and three aminoglycosides or aztreonam against aminoglycoside resistant strains of *Pseudomonas aeruginosa,* J. of Antimicrob. Chemother., **27**: 801-808 (1991).
6. *Cappelletty D.M. and* Rybak M.J., Comparison of methodologies for synergism testing of drug combinations against resistant strains of *Pseudomonas aeruginosa, Antimicrob. Agents. Chemother.*, **40**: 677-683 (1996).
7. Chin N.X. and Neu H.C., Synergy of new C-3 substituted cephalosporinsand Tobramycin against *Pseudomonas aeruginosa* and *Pseudomonas cepacia,* Diagn. Microbiol. Infect. Dis., **12**: 343-349 (1989).
8. Den Hollander J.G., Horrevorts A.M., Van Goor M.L., Verbrugh H.A. and Mouton J.W., Synergism between Tobramycin and Ceftazidime against a resistant *Pseudomonas aeruginosa* strain, tested in an *in - vitro* pharmacokinetic model, Antimicrob. Agents. Chemother., **41**: 95-100 (1997).
9. Master V., Roberts G.W., Coulthard K.P., Baghurst P.A., Martin A., Roberts M.E., Onishko C.R., Martin A.J., Linke R.J., Holmes M., Jarvinen A., Kennedy D., Colebatch K.A., Hansman D. and Parsons D.W., Efficacy of once-daily tobramycin monotherapy for acute pulmonary exacerbations of cystic fibrosis : a preliminary study, Pediatr. Pulmonol., **31**(5): 367-376 (2001).
10. Dancer S.J., How antibiotics can make us sick: the less obvious adverse effects of antimicrobial chemotherapy, Lancet. Infect. Dis., **4**(10): 611- 9 (2004).
11. Gleckman R.A. and Czachor J.S., Antibiotic side effects, *Semin. respir. Crit. care. Med.*, **21**: 53-60 (2000).
12. French G.L., Clinical impect and relevance of antibiotic resistance, *Adv. Drug. Deliv. Rev.,* **57**: 1514-27 (2005).
13. Harbarth S., Nobre V. and Pittet D., Dose antibiotic selection impact patient outcome?, Clin. Infect. Dis., **44**: 87-93 (2007).
14. National Committee for Clinical Laboratory Standards, *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7 - A4., National Committee for Clinical Laboratory Standards* (1997).
15. Farrell W., Wilks M. and Drasar F.A., Synergy between aminoglycosides and semi-synthetic penicillins against gentamicin-resistant gram negative rods*, J. of Antimicro. Chemother.*, 523-9 (1979).
16. Moellering R.C., Allsop A., Bush K., Quinn J., Baquero F. and Philipps I., Antibiotic resistance: lessons for the future, Clin.Infectious Dis., **27**: Suppl 1, S, 135-40 (1998).
17. Zhanel G.G., Hoban D.J., Schurek K., & Karlowsky J.A., Role of efflux mechanisms on fluoroquinolone resistance in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa,*Inter. *J. of Antimicro. Agent.,* **24**(6): 259-535 (2004).
18. Banerjee D. and Stableforth D., The Treatment of Respiratory *Pseudomonas* Infection in Cystic Fibrosis : What Drug and & Which Way?, Drugs., **60**(5): 1053-1064 (2000).