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Research Article

ANTIMICROBIAL STUDIES OF SELECTED ANTIBIOTICS AND THEIR COMBINATION WITH ENZYMES

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ABSTRACT

The aim of the present study was to determine the MICs (Minimum inhibitory concentrations) of selected nine antibiotics by cup plate method by agar diffusion method and then the combination effect of few antibiotics with enzymes like lysozyme and serratiopeptidase was observed. The combination shows effective against six cultures when compared to antibiotic alone. Lysozyme and Serratiopeptidase were tried along with varying concentrations of gatifloxacin, rifampicin, streptomycin & ampicillin. The results indicated that some combination of serratiopeptidase with specified antibiotics were effective partially on selected cultures.

Key words: Antibiotics, Cup plate method, Lysozyme, Minimum inhibitory concentrations, Serratiopeptidase.

INTRODUCTION

Antimicrobials are the substances which inhibit the growth or kill the microorganisms. Antibiotics are a class of antimicrobial agents. Antibiotics act by inhibition of cell wall synthesis, inhibition of folate metabolism, binding to ribosomes to prevent translation and interference with nucleic acid synthesis. The testing of antimicrobials is done by MICs. It is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after over night incubation. Among all the antibiotics only few are very effective because most of the antibiotics show side effects. And some antibiotics show adverse reactions when they are used in high doses. To reduce these side effects combination of drugs were discovered. The combination effect shows the more activity than the single drug. When two drugs combined they give either additive effect or synergistic effect.

Some enzymes present in our body, and they act against bacteria, in infections. The localized delivery of the enzyme along with antibiotic may provide better relief than antibiotic alone. But proteolytic enzymes may reduce bioadhesivity because of their mucolytic action. Most of the enzymes are stable at neutral pH and room temperature. By increasing the temperature readily denatured. These enzymes lack side effects. They are stable up to 60°C . Lysozyme, serratiopeptidase, chitinases, endoglucanases, urease, lipase, etc. are some of the antibacterial enzymes[1]. In clinical trials, for the efficient combinations, the following antibiotics methicillin, lincomycin, erythromycin, benzylpenicillin, ampiox, morphocycline, gentamicin, tobramycin and tetracycline with lysozyme were studied experimentally by some Russian groups[2] and these studies proved their high efficacy of combination. In combination therapy, they observed remission in about 81% of the cases against 56.4% with antibiotics alone[2,3]. The antibiotic ofloxacin was found to be effective with serratiopeptidase[1].

MATERIALS AND METHODS

Drugs and Enzymes

Rifampicin (Lykalaboratories, Ahmedabad), Linezolid (Matrixdrugs, Hyderabad), Clarithromycin (Alembic ltd, Vadodara), Streptomycin (Sarabhai chemicals, Baroda), Gatifloxacin, Sparfloxacin (Dr. Reddy's, Hyderabad), Cloxacillin (Ranboxy, Delhi), Amoxacillin, Ampicillin, Serratiopeptidase (Surya pharmaceutical ltd, Chandigarh), Lysozyme (Fluka, Germany)

Micro organisms

Bacillus subtilis (MTCC – 441), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-722), *Proteus vulgaris* (MTCC-426), *Klebsiella Pneumonia* (MTCC-109) [IMTECH, Chandigarh] Methicillin resistant *Staphylococcus aureus* (ATCC-43300) [CMC, Vellore].

Determination of antimicrobial activity of antibiotics

Nine antibiotics were tested against six different bacterial cultures for the minimum inhibitory concentrations determination. The antibiotic solutions were prepared with solvents and prepared serial dilutions. Finally, plates with different concentrations of various antibiotics were prepared. The overnight cultures were streaked on to the plates and incubated at 37^{0}C for 18 hours. The growth or no growth was found.

Determination of effect of combination of antibiotics and enzymes

Gatifloxacin was found to be more active as compared to other antibiotics on all the cultures. Gatifloxacin (concentrations below MIC) and Lysozyme (below MIC) were taken directly in to LB agar medium. All the six cultures were streaked on the plates and then incubated at 37^{0}C for overnight. Growth or no growth was observed.

Another enzyme, serratiopeptidase was also tried along with varying concentrations of gatifloxacin, rifampicin, streptomycin and ampicillin (below MIC). All the combinations were taken directly into LB agar medium. All the six cultures were streaked on the plates and then incubated at 37^{0}C for 18 hrs. Growth or no growth was observed.

RESULTS AND DISCUSSION

Nine antibiotics were tested against six different bacterial cultures for the MICs determination. The antibiotic solutions were prepared with solvents and prepared serial dilutions. Finally, plates with different concentrations of various antibiotics were prepared. The overnight cultures were streaked on to the plates and incubated at 37^{0}C for 18 hours. The growth or no growth was found. Among nine antibiotics, gatifloxacin shows more activity against six cultures followed by sparfloxacin. The rank order was gatifloxacin> sparfloxacin> rifampicin > clarithromycin > linezolid > streptomycin > ampicillin > cloxacillin > amoxicillin.

The study of different combinational effect of lysozyme and gatifloxacin shows less effect. But the different combinational effect of serratiopeptidase with gatifloxacin, rifampicin, streptomycin and ampicillin shows the synergistic effect. Ampicillin and serratiopeptidase combination was effective on all other combinations.

Table 1: Comparison of minimum inhibitory concentrations (µg/ml) of selected antibiotics against various cultures

S.No.	Antibiotic	B.S	S.A	E.C	P.V	K.P	MRSA
1	Gatifloxacin	0.05	0.1	0.1	0.05	0.05	0.1
2	Sparfloxacin	3	0.5	7	7	0.05	7
3	Rifampicin	0.5	0.5	0.3	0.3	12	22
4	Clarithromycin	0.5	0.5	0.5	130	50	130
5	Linezolid	25	25	0.7	0.7	25	25
6	Streptomycin	27	27	25	27	30	27
7	Ampicillin	10	10	10	10	10	10
8	Cloxacillin	15	15	15	15	15	15
9	Amoxacillin	15	15	15	15	15	15

B.S- *Bacillus subtilis* , S.A- *Staphylococcus aureus*, E.C- *Escherichia coli*, P.V- *Proteus vulgari*, K.P- *Klebsiella pneumonia*, MRSA- Methicillin resistant *Staphylococcus aureus*

Table 2: Combinational effect of antibiotics and enzymes

Combinations	Conc. of antibiotic	Conc. of enzyme	Inference
G & L (I,II.III,IV)	0.01-0.03µg/ml	1 - 5 mg/ml	ineffective
G & S (I,II,III)	0.03-0.05µg/ml	10-100µg/ml	slight influence on inhibition
R & S (I,II,III)	0.2-0.3µg/ml	10-100µg/ml	combn II inhibited the growth of *S.aureus* &combn III inhibited the growth of *S.aureus & K.pneumonia*
Str & S (I,II,III)	22-25µg/ml	10-100µg/ml	combn II inhibited the growth of S.aureus & *K.pneumonia* and combn III inhibited the growth of S.aureus
A & S (I,III,III)	5-8µg/ml	10-100µg/ml	combn I & II inhibited growth of *S.aureus & K.pneumonia* and combn III inhibited the growth of all the 6 cultures

G – gatifloxacin, L – lysozyme, S – serratiopeptidase, R – rifampicin, Str – streptomycin, A - ampicillin

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