

IN VITRO SYNERGY OF FUSIDIC ACID AND AMIKACIN AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Objective: To test in vitro combination of fusidic acid and amikacin against infections caused by methicillin resistant *Staphylococcus aureus* (MRSA).

Methodology: In vitro study conducted in Department of Pharmacology and Microbiology, Dr. Ziauddin Medical University. The duration of study was March 2004- February 2005. Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method and by minimum inhibitory concentration (MIC) by broth macrodilution and checkerboard technique for synergy. FIC (fractional inhibitory concentrations) were calculated.

Results: MIC of fusidic acid was 0.03-1µg/ml and amikacin 0.5-16µg/ml respectively. The combination of these demonstrated synergy. Evidence of synergy correlated directly with the MICs of fusidic acid and amikacin.

Conclusion: Combination therapy with fusidic acid and amikacin may be a reasonable alternative in the treatment of infections caused by MRSA isolates and encourages clinical evaluation.

KEY WORDS: Vancomycin, Synergism, MRSA, Fusidic Acid, Amikacin.

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INTRODUCTION

Vancomycin is universally accepted as the drug of choice for treatment of infections caused by MRSA.¹ It is a glycopeptide and it inhibits a late step in bacterial cell wall synthesis by forming hydrogen bonds with the D-Ala-D-Ala terminus of the bacterial peptidoglycan

side chain. The resistance to vancomycin is by replacement of D-Ala by D-lactate. It is an expensive drug and clinicians are experiencing the emergence of strains with reduced susceptibility to vancomycin (MICs 8-16 µg/ml) are vancomycin intermediate *S.aureus* (VISA) and the strains fully resistant to vancomycin (MICs $\geq 32.0\mu\text{g/ml}$) are vancomycin resistance *S.aureus* (VRSA).²

Recent reports from all over the world have shown increased prevalence of MRSA and cases showing resistance or reduced susceptibility to vancomycin have been a serious concern for the clinicians.³⁻⁵ Therefore, there is clearly a need for new antibiotic regimes with strong bactericidal activity against MRSA. An alternate to develop new agents would be use of well-known antistaphylococcal compounds in combination.⁶

MIC is considered the 'gold standard' of determining the susceptibility of organisms to antimicrobials.⁶ MIC is defined as the lowest concentration of a drug that will inhibit the growth of an organism after overnight

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incubation.^{6,7} The antibiotic synergy in this study was done by the checkerboard technique.⁸ The interpretation of these results is done by calculation of fractional index.⁹

MATERIALS AND METHODS

Antimicrobial susceptibility testing was performed by modified Kirby Bauer method on Mueller- Hinton agar plates for eleven antibiotics, those chosen were oxacillin(1µg), cephalixin (10µg)s, ofloxacin(5µg), fusidic acid (10µg), penicillin(10µg), vancomycin (30µg), erythromycin (15µg), gentamicin (10µg), teicoplanin(30µg), amikacin (30µg) and clindamycin (2µg). ATCC *S.aureus* 25923 was used as control strain.

Determination of Minimal Inhibitory Concentration by Broth Macrodilution: MICs were determined by broth macrodilution in brain heart infusion (BHI) according to standards of National committee for clinical laboratory standard (NCCLS).

The MICs of various antibiotics were tested in the beginning of this project.

Preparation of antibiotic stock solution.

1. Fusidic acid (Leo pharmaceutical) 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015µg/ml.
2. Amikacin (Bristol Myer Squibb) 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125µg/ml.

Inoculum was prepared having 0.5 McFarland's turbidity with a standardized number of organisms and was diluted 1/100, then tubes were inoculated. The MIC was the lowest concentration of antibiotic that yielded no visible growth after incubation at 37°C for 24 hours.

Inclusion Criteria: Fifty oxacillin resistant *S.aureus* isolates samples from the inpatient and outpatient department of Dr. Ziauddin Hospital, North campus Karachi, 2004-2005, were included.

Exclusion Criteria: All oxacillin sensitive *S.aureus* were excluded.

B. Synergism of Antibiotics by Checkerboard Method: One ml antibiotic dilution (0.5ml of amikacin and 0.5 ml of fusidic acid) were taken in 36 tubes and one ml organism inocula were

added to them. These tubes were incubated at 37°C for 24 hours tubes showing turbidity indicated growth and those that were clear indicated inhibition. FIC index was calculated by FIC (MIC of drug A in combination with drug B/MIC of drug B alone). FIC of <0.5 was defined as synergy, an FIC index of > 0.5 to 1 was defined as additive or indifferent, and FIC of > 4.0 was defined as antagonism.

RESULTS

Results of 50 isolates of MRSA were tested for antimicrobial susceptibility by MIC and checkerboard test for synergy. Antimicrobial susceptibility of isolated strains of *S.aureus* were tested against the following antibiotics:

oxacillin, cephalixin, ofloxacin, fusidic acid, vancomycin, penicillin, erythromycin, gentamicin, teicoplanin, amikacin and clindamycin. Majority of these MRSA strains were resistant to all antibiotics except for Vancomycin (100%), teicoplanin (100%), fusidic aid (98%) and amikacin (46%).

In Table-I the antibiotic sensitivity against fusidic acid and amikacin is compared. All of the 50 samples were found to be sensitive to fusidic acid by Kirby Bauer's disk diffusion method, and the organisms are categorized into three groups according to their varying responses in the two techniques.

The MIC of fusidic acid was found to be in range of 0.03-1µg/ml whereas that of amikacin in range of 0.5-16µg/ml when they were used

Table-I: Zone size and MIC

MRSA (No.)	Fusidic Acid		Amikacin	
	Disc Diffusion	MIC (µg/ml)	Disc Diffusion	MIC (µg/ml)
22	19S - 40S	0.03 - 1	17S	0.5 -2
24	19S - 40S	0.03 - 1	8R - 16R	8 - 16
4	19S - 40S	0.03 - 1	8R - 16R	8 - 16
Control	30S	0.03	17S	1

MIC: Minimum Inhibitory Concentration.

R: Resistant I: Intermediate S: Sensitive

Zone Size:

Fusidic acid Resistant= 18 Intermediate= 18 Sensitive=19

Amikacin Resistant= 14 Intermediate= 16 Sensitive=17

MIC Range:

Fusidic acid: 0.03 - 1µg/ml Amikacin: 16-32 µg/ml

alone. (Table II & III) as shown from lower left to upper right both drugs were present in increasing concentration. The lower left shaded area indicated lowest concentration of both antibiotic and turbidity. The clear area with higher concentration of both antibiotics showed no growth.

In this study according to calculations FIC index for fusidic acid and amikacin, 46 strains (92%) showed synergistic and four strains (8%) additive response (Table-IV). The study does not show any FIC index above 4.0. Hence the combination of antibiotics showed a significant synergistic effect and this combination could be suggested for trial in vivo studies. FIC for 46 strains tested by the above combination remained below 0.5 (synergistic), four strains had index between 0.5 and 1 (additive) and no antagonism was seen.

DISCUSSION

This study investigated the comparisons of the MICs of antibiotic used alone or in combination to treat MRSA clinical isolates from patients of Dr. Ziauddin Hospital. In combination we have found a synergistic or additive effect of fusidic acid and amikacin in vitro against MRSA.

Resistance of many strains of *S.aureus* to many antibiotics except vancomycin and also the emergence of decreased sensitivity to vancomycin in a number of cases of MRSA is making it difficult to treat this pathogen which is major cause of hospital associated and community acquired infections worldwide.^{10,11} Several new strategies to treat MRSA have been considered including the use of antibiotic combinations.¹²⁻¹⁴

Table-II: Checker Board Technique (Amikacin MIC = 0.5µg/ml, FD MIC= 1µg/ml)

FD	0.06/4	0.125/4	0.25/4	0.5/4	1/4	2/4
	0.06/1	0.125/2	0.25/2	0.5/2	1/2	2/2
	0.06/0.5	0.125/1	0.25/1	0.5/1	1/1	2/1
	0.06/0.25	0.125/0.25	0.25/0.25	0.5/0.25	1/0.25	2/0.25
	0.06/	0.125/	0.25/	0.5/	1/	2/
	0.125	0.125	0.125	0.125	0.125	0.125
			AK			

AK= Amikacin.
FD = Fusidic acid

Table-III: Checker Board Technique (Amikacin MIC = 8i g/ml, FD MIC= 1i g/ml)

FD	1(4)	2(4)	4(4)	8(4)	16(4)	32(4)
	1(2)	2(2)	4(2)	8(2)	16(2)	32(2)
	1(1)	2(1)	4(1)	8(1)	16(1)	32(1)
	1(0.5)	2(0.5)	4(0.5)	8(0.5)	16(0.5)	32(0.5)
	1(0.25)	2(0.25)	4(0.25)	8(0.25)	16(0.25)	32(0.25)
	1(0.125)	2(0.125)	4(0.125)	8(0.125)	16(0.125)	32(0.125)
				AK		

AK= Amikacin.
FD = Fusidic acid

The combination therapy, reduces adverse effects and could prevent the emergence of mutant strains against antistaphylococcal agents.¹⁵ Fusidic acid is one of the few oral antibiotics that is still effective against MRSA. Many strains of MRSA are sensitive to fusidic acid but rapid resistance develops when given for a prolonged period. Recommendations are for its use in combination with another antistaphylococcal drug.¹⁶ Fusidic acid is a narrow spectrum agent that acts by inhibiting bacterial protein synthesis by interference with elongation factor G (EF-G). EF-G is an essential bacterial protein that promotes translocation on the ribosome after peptide bond formation. Fusidic acid binds to the EF-G ribosome complex in combination with either GTP or GDP and stabilizes EF-G GDP on the ribosome, preventing further elongation by inhibiting the GTPase function of EF-G. In prokaryotes there is one type of elongation factor, EF-2. However the eukaryotic cells have other elongation factors, EF-Tu and EF-1, which can perform the functions of EF-2.¹⁷

Published studies do not support fusidic acid use as monotherapy.^{18,19} Interaction studies of fusidic acid with other antibiotics give varying results depending on methodology. However, interaction with beta-lactams is generally indifferent, as it is with rifampicin, while aminoglycosides and macrolides appear

Table IV: FIC Index

No. of MRSA ISOLATES	FIC	
46	< 0.5	Synergistic
4	>0.5 - < 1	Additive
0	>1, < 2	Indifferent
0	>4	Antagonism

to be synergistic and fluoroquinolones antagonistic.¹⁷

In our study synergism in 96% clinical isolates of MRSA was observed and in 8% isolates additive effect was seen but no antagonism was observed. Therefore a combination of fusidic acid and amikacin could be a very good alternate therapy for MRSA. Our finding is in line with published data.¹⁷⁻¹⁹

CONCLUSION

In vitro synergy and additive effect of combination of fusidic acid and amikacin was seen against 50 clinical isolates of MRSA. Clinical studies should be performed to determine the efficacy of synergistic effect of this combination against different clinical isolates of MRSA. The FIC calculated in this study for the above combination against 46 isolates was synergistic, 4 was additive while no antagonism was seen.

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