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Research Article

In vitro antimicrobial activity of cefsulodin and kanamycin in combinations

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ABSTRACT

Background: Infectious diseases are the greatest challenge of the world. The main failure in the treatment of infectious diseases is development of antibiotic resistance by the infective agents. Combination drug therapy is proposed to be more successful to contain diseases. But before the selection of combination of antibiotics, it is important to determine interaction of such antibiotics. Two antibiotics may have either synergistic or antagonistic action. In this study it was designed to find out the Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC), which is usually used for the quantitative assessment of bacterial sensitivity to antibiotics.
Methods: Checkerboard titration in microtitre trays used for this assay, and Fractional Inhibitory Concentration (FIC) and Fractional Bactericidal Concentration (FBC) measured to identify the type of interaction between the two antibiotics. Cefsulodin (Cef) and Kanamycin (Kan) were used against Escherechia coli (Esch. coli) and Staphylococcus aureus (Staph. aureus) to determine the efficacy of these antibiotics in combination.
Results: MIC of cefsulodin and kanamycin against Staph. aureus was 3.125 and 3.125 respectively. MIC of Cef for Esch. coli was 6.25 and for Kan 50. FIC for Staph. aureus was 1. FIC for Esch. coli was different in different antibiotic concentrations and the least value was 0.37. There was no bactericidal effect of these antibiotics in combination against these organisms.
Conclusion: Combination of two drugs cefsulodin and kanamycin showed synergistic action against Esch. coli and additive against Staph. aureus. So combined drug therapy can be used for better treatment with low toxicity, broad spectrum activity, and prevent emergence of drug resistance organism.

Keywords: Combination drug therapy, Cefsulodin, Kanamycin, Staph. aureus, Esch. coli

INTRODUCTION

Infectious diseases are worldwide problems caused by pathogenic organisms.[1] Introduction of antimicrobial agents in therapeutic armamentarium made possible to control this diseases.[2] Treatment of infection by chemical agents/antibiotics has selective killing action on pathogenic organisms and also have toxic side effect in humans. Medicines having high Chemotherapeutic Index are advisable for therapy.[3] Antibiotics used to treat the bacterial infection may act as bacteriostatic or bactericidal. Some antibiotics act at bacterial cell wall synthesis, and others act on protein synthesis, as well as on bacterial cell membrane.[3-5] Treatment of infections is often initiated empirically, determination of bacterial susceptibility to an antimicrobial agent is essential because of widespread resistance to all classes of antimicrobial agents.[6,7] It is necessary to use another antibiotic or derivative of the previous antibiotic against particular resistant bacteria, or combination of two drugs

for the successful treatment of infectious diseases. Combination drug therapy is proposed as a successful treatment for diseases, providing broad-spectrum coverage, toxicity reduction, improving efficacy, preventing the emergence of drug resistant mutants. Combination therapy is widely used in treating dreadful infectious diseases, like Tuberculosis and AIDS.[8-11] In combination drug therapy, it is necessary to evaluate these drugs interaction. Some combination drugs react as synergistic, and on the other hand some may act as a antagonistics.[12] Synergistic combinations of two or more agents can overcome toxicity and other side effects otherwise associated with high doses of single drugs.[8,13,14] It was observed in our setup that often the treatment of infections were not up to desirable level which might be because of various factors, of them, resistance by organisms could be one. Therefore in this study, it was desired and designed to know effect of combination of cefsulodin and kanamycin against Esch. coli and Staph. aureus. For this purpose the Fractional Inhibitory Concentration (FIC) index was adopted which is a mathematical expression used to represent the interaction of drugs.

METHODS

Staphylococcus aureus and Escherichia coli were the organisms used for antimicrobial susceptibility by using cefsulodin and kanamycin in combination. The standard strains used for this study Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922, and the antibiotics were procured from HiMedia Pvt. Ltd.

The dilutions of the antibiotics were prepared by two fold serial dilution from 400 µg/ml to 0.39 µg/ml in nutrient broth. Microtitre plate was used for testing the MIC by adding 100µl each antibiotic solution in each microtitre wells to attain final volume of 200µl in each well except row H wells and column 12 wells of which contain only single antibiotic of 100 µl with 100µl of nutrient broth acting as control (Figure 1 and Figure 2). Thus row H determined the MIC of Cefsulodin; whereas column 12 determined MIC of Kanamycin. Microtitre tray was allowed to stay at room temperature for 30 minutes for interaction/mixing of antibiotics. Bacterial concentration of Esch. coli and Staph. aureus were prepared to get 10^7CFU/ml as per McFarland 0.5 turbidity comparator. MBC was studied by using nutrient agar plates divided into 12 portions and to each portion the 10µl test organisms were inoculated. To those inocula 10µl of combined solution of different concentration of antibiotics were placed. MIC was studied using same concentration of organisms i.e. 10^7CFU/ml which were inoculated into all the wells of microtitre plates containing different concentration of antibiotics except H12 well. Trays and plates were incubated at 37°C overnight.[15,16] Reading were observed after 18-24 hrs. FIC value was derived by using following formula:

$$\text{FIC} = \frac{\text{Concentration of antibiotics 1 in this well}}{\text{MIC of antibiotic 1}} + \frac{\text{Concentration of antibiotics 2 in this well}}{\text{MIC of antibiotic 2}}$$

RESULTS

Growth of bacteria in microtitre wells and on nutrient agar plates were recorded as G for growth, NG for no growth on a 96 well template sheet. MIC and MBC of cefsulodin calculated from row H and for kanamycin from column 12 of microtitre plate. The growth on nutrient agar was recorded on 96 well template sheet. Results on MIC, MBC, FIC, FBC value for Escherichia coli and Staphylococcus aureus with cefsulodin and kanamycin combined antibiotic activity are shown in Table 1 and Table 2. The results indicate the MIC value of cefsulodin and kanamycin on Escherichia coli were 6.25µg/ml and 50µg/ml respectively. Antibiotic concentration of cefsulodin and kanamycin, at which organisms were inhibited were 1.56µg/ml and 6.25µg/ml respectively. The FIC was found to be 0.37. MIC value for cefsulodin and kanamycin on Staphylococcus aureus were 3.125 µg/ml. Antibiotic concentration of Cef and Kan at which organisms were inhibited, were 1.56µg/ml. The FIC was found to be 1. FIC of Escherichia coli and Staphylococcus aureus indicated synergy and additive respectively. There was no bactericidal effect of Cef and Kan against Esch. coli and Staph. aureus (Table 1, Figure 1 & Table 2, Figure 2).

Table 1: MIC, MBC, FIC values for Esch. coli with cefsulodin and kanamycin combined antibiotic activity.

Clear Well	Cefsulodin Concn. (µg/ml)	Kanamycin Concn. (µg/ml)	FIC	Interpr-etations	MBC
H7	6.25 (MIC)	0			
G7	6.25	0.78	1.01	AD	
F8	3.125	1.56	0.53	PSYN	
E8	3.125	3.125	0.56	AD	NE
D9	1.56	6.25	0.37	SYN	
C10	0.78	12.5	0.37	SYN	
B11	0.39	25	0.56	AD	
A11	0.39	50 (MIC)			

*Abbreviations: Synergism (SYN); Partial Synergism (PSYN); Additive (AD); No Effect (NE).

Table 2: MIC, MBC, FIC values for Staph. aureus with cefsulodin and kanamycin combined antibiotic activity.

Clear Well	Cefsulodin Concn. (µg/ml)	Kanamycin Concn. (µg/ml)	FIC	Interpr-etations	MBC
H8	3.125 (MIC)	0			NE
G8	3.125	0.78	1.24	AUT	
F9	1.56	1.56	1.0	AD	
E8		3.125 (MIC)			

*Abbreviations: Additive (AD); Autonomy (AUT); No Effect (NE).

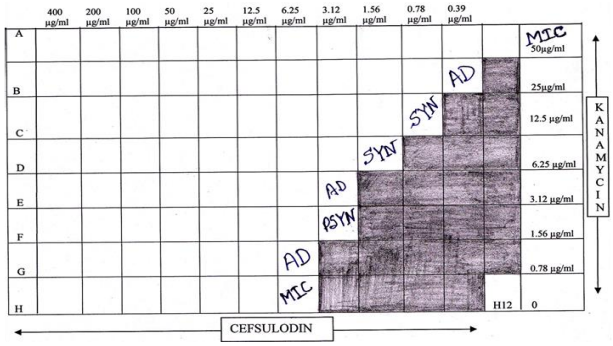

Figure 1: Checkerboard microtitre template sheet for determining MIC and MBC of cefsulodin, kanamycin against Esch. coli.

*H12: The well with only antibiotics no inoculation of organism.
*Row H wells contain single antibiotic, cefsulodin.
*Column 12 wells contain single antibiotic, kanamycin.
*Abbreviations: Synergism (SYN); Partial Synergism (PSYN); Additive (AD).

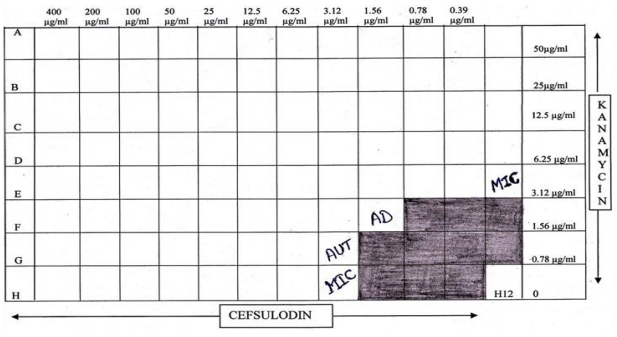

Figure 2: Checkerboard microtitre template sheet for determining MIC and MBC of cefsulodin, kanamycin against Staph. aureus.

*H12: The well with only antibiotics no inoculation of organism.
*Row H wells contain single antibiotic, Cefsulodin.
*Column 12 wells contain single antibiotic, Kanamycin.
*Abbreviations: Additive (AD); Autonomy (AUT).

Isobologram graph showed synergy for Cef and Kan combination against Esch. coli (Figure 3).

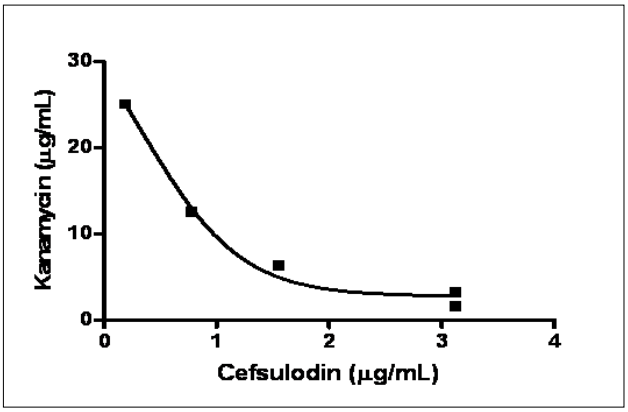

Figure 3: Isobologram showing combined effect of kanamycin and cefsulodin on Esch. coli.

DISCUSSION

Infectious diseases caused by pathogenic organism, may be transmissible or communicable diseases, cause serious health problems in human population. Antimicrobial agents, vaccines are used for cure and prophylaxis of these diseases. Control/cure of these diseases due to resistant organism against antimicrobial agents is serious problem. Organism may develop resistance to antibiotic mainly by mutation. Usage of single antibiotic, on many occasions, proved to be futile due to resistance developed by organism. So it is important to create some effective antibiotic therapy against these organisms. Combination drug therapy has been tried against resistant organism.

In this study we performed combination drug activity against Esch. coli and Staph. aureus as organisms and cefsulodin and kanamycin as drugs. MIC and MBC, was determined which indicated sensitivity of organisms used against these drugs. Also we found FIC, which indicates type of interaction between two drugs. FIC value, 0.5 indicates synergy, 0.5-1 means additive, 1-2 autonomy, >2 indicates antagonism. According to our results Cef and Kan combination showed synergy for Esch. coli (Table 1 and Figure 1), and additive for Staph. aureus (Table 2 and Figure 2). From the result it was found that Cef concentration 1.56 and Kan concentration 6.25 can work better against Esch. coli. Isobologram graph showed synergy for Cef and Kan combination against Esch. coli (Figure 3). Saito et al. according to their study, combination of cefsulodin, cefotaxime, latamoxef, cefotaten, dibekacin, sisomicin showed synergistic action against Esch. coli, and FIC values were 0.26-0.5.[17] Another study by A. L. Baltch et al. combination of enoxacin and cefsulodin, amikacin and others, showed no synergy against Staph. aureus, and also they didn't find any antagonism against Staph.aureus.[18] By determining MIC, MBC, and FIC, appropriate antibiotic may be selected. By this method even if the bacterial growth can be arrested, rest of the process of eradication/ elimination

of infecting agent can be taken care by the host defence mechanisms.

In conclusion; cefsulodin in combination with kanamycin at FIC value 0.37, against Esch. coli has synergistic action. Same combination against Staph. aureus showed additive. Hence, use of combined drug therapy can be adopted to overcome drug resistance in organism and also for better treatment and to reduce the cost of the treatment and avert side effects of the medicine.

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REFERENCES

1. Vito R. Iacoviello, Stephen H. Zinner. Principles of anti-infective therapy. In: Jonathan Cohen, William G. Powderly, eds. Mosby Infectious Diseases. 2nd ed. Spain: Elsevier Limited; 2004: 1705-1715.
2. Philip S. Brachman. Infectious diseases-past, present, and future. Int J Epidemiol. 2003;8:684-6.
3. Franz-Josef Schmitz, Ad C. Fluit. Mechanisms of antibacterial resistance. In: Jonathan Cohen, William G. Powderly, eds. Mosby Infectious Diseases. 2nd ed. Spain: Elsevier Limited; 2004: 1733-1748.
4. JL Avorn, JF Barrett, PG Davey, SA McEwen, TF O'Brien, SB Levy. Antibiotic resistance: synthesis of recommendations by expert policy groups. In: WHO and Allience for the Prudent Use of Antibiotics. Boston, MA, United States of America: WHO; 2000: 1-155.
5. Harry W. Lampiris, Daniel S. Maddix. Clinical use of antimicrobial agents. In: Bertram G. Katzung, Susan B. Masters, Anthony J. Trevor, eds. Lange Basic & Clinical Pharmacology. 12th ed. New Delhi, ND: Tata McGraw-Hill; 2012: 901-913.
6. Lorenzo Drago, Elena De Vecchi, Lucia Nicola, Maria Rita Gismondo. *In vitro* evaluation of antibiotics' combinations for empirical therapy of suspected methicillin resistant Staphylococcus aureus severe respiratory infections. BMC Infect Dis. 2007;7:111.
7. Stuart B. Levy. Factors impacting on the problem of problem of antibiotic resistance. J Antimicrob Chemother. 2002;49:25-30.
8. Keith CT, Borisy AA, Stockwell BR. Multicomponent therapeutics for networked systems. Nat Rev Drug Discov. 2005;4:71-8.
9. Ting-Chao Chou. Drug combination studies and their synergy quantification using the Chou-Talalay method. Am Associ Cancer Res. 2010;70:440-6.
10. Graeme N. Forrest, Kimberly Tamura. Rifampin combination therapy for non-mycobaterial infections. Clin Microbiol Rev. 2010;23(1):14-34.
11. Ellsworth M. Campbell and Lin Chao. A population model evaluating the consequences of the evolution of double-resistance and tradeoffs on the benefits of two-drug antibiotic treatments. PLos One. 2014;9(1):e86971.
12. Peter Torella Joseph, Chait Remy, Kishony Roy. Optimal drug synergy in antimicrobial treatments. PLoS Comput Biol. 2010;6(6):e1000796.
13. Sharom JR, Bellows DS, Tyers M. From large networks to small molecules. Curr Opin Chem Biol. 2004;8:81-90.
14. Kaelin WG Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Drug Discov. 2005;4:71-8.
15. H. Hsieh Michael, M. Yu Chen, L. Yu Victor, W. Chow Joseph. Synergy assessed by checkerboard. A critical analysis. Diagn Microbiol Infect Dis. 1993;16:343-9.
16. ASM Science. synergism testing: broth microdilution checkerboard and broth macrodilution methods, 2012. Available at: http://www.asmpress.org/asmppress/files/ccLibrary Files/Filename/ 000000000260/05.12.pdf. Accessed 12 May 2012.
17. Saito M, Azuma K, Nishino T, Tanino T. Combination action of sisomicin, dibekacin and cefotetan, cefotaxime, latamoxef, and cefsulodin against Escherichia coli, Serratia marcescens, and Pseudomonas aeruginosa. Jpn J Antibiot. 1983;36(10):2833-43.
18. AL Baltch, C Bassey, G Fanciullo, RP Smith. *In vitro* antimicrobial activity of enoxacin in combination with eight other antibiotics against Pseudomonas aeruginosa, Enterobacteriaceae and Staphylococcus aureus. J Antimicrob Chemother. 1987;19(1):45-8.

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