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Full Length Research Paper

A study of the *in vitro* interaction of cotrimoxazole and ampicillin using the checkerboard method

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In this study, the *in vitro* interaction of two standard antibiotics – cotrimoxazole and ampicillin trihydrate were studied by the checkerboard technique, using clinical isolates of *P. aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. The organisms were exposed to the individual antibiotics as well as different combination ratios of the same, and the zones of inhibition as well as the minimum inhibitory concentrations (MICs) measured. Synergistic interactions were recorded by the antibiotics against *Staph. aureus* and *S. typhi* while indifferent interaction occurred with *P. aeruginosa. P. aeruginosa* however, showed resistance to the two antibiotics when they were used alone. The implication is that cotrimoxazole and ampicillin can be used in combination as a superior treatment of infections caused by *Staph. aureus* and *S. typhi*.

Key words: *In vitro* interaction, cotrimoxazole, ampicillin, *Peudomonas aeruginosa, Staphylococcus aureus*, *Salmonella typhi*.

INTRODUCTION

Therapy with antimicrobial combinations has been in use for a long time and is often applied to take advantage of different mechanisms of action and/or toxicity profiles of the agents involved (Ryback et al., 1996). The combination of two different compounds may result in a biocidal (bactericidal, fungicidal or virucidal) activity, which is significantly greater than the sum of the activities of the agents individually. When this phenomenon is exhibited, the combination is said to be synergistic. If the combination results to a worsening effect, it is said to be antagonistic. A result which is less than synergistic but not antagonistic is said to be indifferent or additive (Lorian, 1991).

Synergistic combinations are often sought in infections where the development of resistance and/or subsequent failure to monotherapy is prevalent, in empirical treatment of life threatening infections prior to identification of the responsible organism, prevention of emergence of bacterial resistance and treatment of polymicrobial infections. Combination antimicrobial therapy should be considered for the treatment of serious Gram-negative infections caused by *Enterobacter cloacae, Peudomonas aeruginosa* and *Serratia marcesceis* and certain gram-positive infections by *Enterococcus* spp. and *Staphylococcus* spp. (Rybak et al., 1996). Selection of agents should be dependent on local susceptibility patterns, clinical experience, site of infection, potential activities and cost.

Among the techniques employed in the evaluation of the combination of two antimicrobials potentially exhibiting synergism, are the checkerboard technique and the time-killing curve method. The checkerboard or fractional inhibitory concentration (FIC) technique employs a methodology similar to that utilized for the

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determination of the minimum inhibitory concentration (MIC). The combination is said to have synergistic effect if there is a 4-fold reduction in the MIC of each antimicrobial agent tested alone (Rybak et al., 1996). In the time-killing curve method on the other hand, the reduction of a fixed inoculum over 24 h exposure of combination antimicrobials is compared with the effect of each agent used alone (Vinh et al., 1980).

Cotrimoxazole, a combination of a sulphonamide with trimetoprim, is a synergistic combination, which, just like ampicillin, has been used in treating many bacterial infections (Stolz et al., 1977; The Pharmaceutical Codex, 1979; Robert, 1980). However, some bacteria have been known to develop resistance to both antibiotics. For instance, in a survey of children with otitis media in Memphis, Tennessee, 29% of isolates of *Staphylococcus aureus* were penicillin resistant and 25% of these were also resistant to cotrimoxazole. Consequently, the study of the ampicillin-cotrimoxazole combination becomes logical, as frequency of development of microbial resistance to the combination is likely to be lower than it would be to either agent used alone.

In terms of mechanism of action, cotrimoxazole acts by inhibiting the bacterial nucleic acid synthesis while ampicillin acts by inhibiting cell wall synthesis or activation of enzymes that disrupt bacterial cell walls to cause loss of viability and often cell lysis (Henry et al., 1986; Mandell et al., 1996; Norden, 1992; Okore, 1990; Jawetz, 1995).

The *in vitro* susceptibility patterns of several other antimicrobial combinations against varying microorganisms have also been demonstrated. For example, striking synergistic bactericidal activity of glycine-carbenicllin, EDTA combinations against a particular strain of *Pseudomonas aeruginosa* has been observed which none of the agents used alone was capable of (Gerberick et al., 1980). Chinwuba et al. (1991) have also shown that carbenicillin, netilmicin, ceftriaxone and ampicillin produced inter-isolate variations in the synergy of their combinations with gentamicin against *P. aeruginosa.*

The purpose of the present study is to investigate the occurrence of interaction between ampicillin and cotrimoxazole, using the checkerboard technique.

MATERIALS AND METHODS

Materials

The culture media used in the study included agar, McConkey agar and deoxycholate citrate agar all from Oxoid, USA and nutrient broth, mannitol salt agar and cetrimide agar all from Merck, Germany. Ampicillin was obtained from Lyka Labs, India, cotrimoxazole from Juhel, Enugu and acetone from BDH, England. Clinical isolates of the microorganisms were obtained from Kenol Laboratories, Nsukka and included *Escherichia coli, Salmonella typhi, Staph. aureus* and *P. aeruginosa.*

Preparation of culture media

All the culture media were formulated according to the manufacturers' specifications. Basically, they were weighed, dissolved in the required amount of water with the aid of heat, distributed in Bijou bottles (10 ml capacities) or test tubes (2 ml volumes) and then sterilized in the autoclave (Gallenkamp, England) at 121°C for 15 min.

Isolation and purification of test organisms

Gram staining was carried out on all the isolated microorganisms. Further characterization, isolation and purification were carried out by streaking the inculum on sterile agar plate and subsequent culturing in selective media for each microorganism. *Staph. aureus* was culture on mannitol salt agar, *P. aeruginosa* on cetrimide agar and *S. typhi* on deoxycholate citrate agar.

Activation and maintenance of organisms

The isolated, purified and characterized test isolates were subcultured weekly on fresh nutrient agar slants, stored at 4°C for 24 h and later incubated at 37°C for 24 h prior to each study. The organism was successively subcultured in 10 ml sterile nutrient agar slant for 3 days.

Standardization of microbial cultures

Five milliliter (5 ml) of sterile water was added to 5 ml of agar slant containing 24 h old culture of the particular microorganism and shaken carefully for a thorough harvest of the organism. The microbial suspension containing 10^9 colony forming units (cfu)/ml was serially diluted to obtain a population of 10^3 cfu/ml, which was the standard inoculum size for each test.

Preparation of drug stock solution

The stock solution of ampicillin was prepared by weighing and subsequently dissolving appropriate quantities of the drug in 100 ml of double strength nutrient broth. Serial dilutions (2-fold) of the this stock solution were undertaken to get the appropriate drug concentrations in 2 ml single strength sterile nutrient broth in a test tube.

For the cotrimoxazole, appropriate quantity was solubilised with acetone and thereafter distributed in suitable quantity of double strength sterile nutrient broth to obtain the target drug concentration. Two-fold serial dilutions of this stock solution were made with the nutrient broth to obtain the desired graded drug concentrations in single strength nutrient broth.

Susceptibility test

A 0.02 ml volume of the standardized inoculum of each test organism was introduced into the respective dilutions of each drug in the test tubes. The tubes were incubated at 37°C for 20 h. Minimum inhibitory concentration was taken as the lowest concentration of each drug, which prevented the visible growth of each test isolate.

Test for synergism

Stock solutions of ampicillin containing 2000, 19.7 and 10 µg/ml,

Table 1. Minimum inhibitory concentrations (MIC) of ampicillin and cotrimoxazole against various test organisms.

Drugs	Minimum inhibitory concentration (MIC) (µg/ml)			
	P. Aeruginos	***Escherichia coli***	***S. typhi***	***S. aureus***
Ampicillin	>2000	1.953	1.231	0.156
Cotrimoxazole	1704.55	1000	500	500

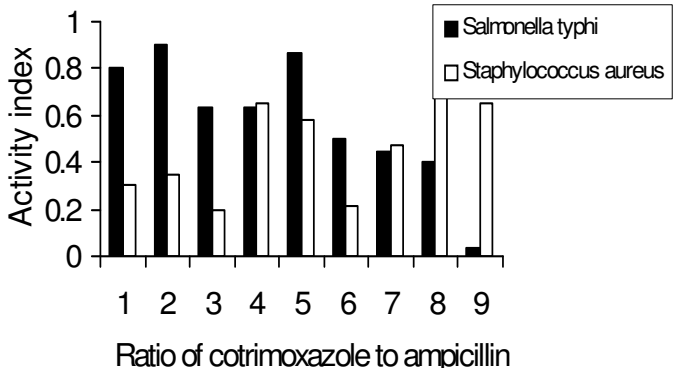

Figure 1. Activity of different combination ratios cotrimoxazole and ampicilin against designated organisms (1=9:2; 2=8:2; 3:73; 4=6:4; 5=5:5; 6=4:6 7=3:7; 8=2:8 9=1 9).

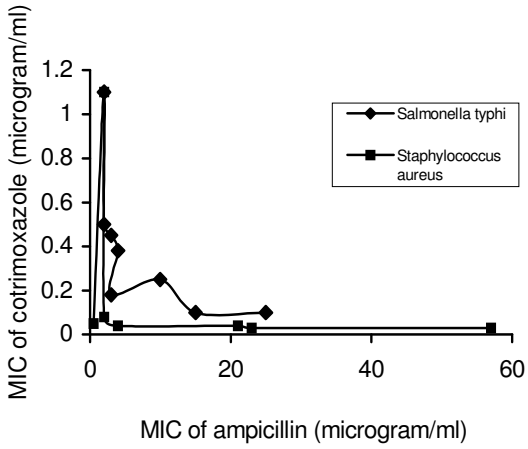

Figure 2. Isobologram showing synergy of cotrimoxazole and ampicilin combination sagainst the test organisms

and cotrimoxazole containing 3409.1, 1000 and 500 µg/ml were suitably prepared in double strength nutrient broth. Varying proportions of the drugs were combined in ratios ranging from 0:10 to 10:0 of ampicillin and cotrimoxazole, according to the continuous variation checkerboard method (Okore, 1990). Each sample was serially diluted (2-fold) with 2 ml of single strength sterile nutrient broth in ten test tubes followed by addition of 0.02 ml of standardized inoculum of a given microorganism. These tubes were incubated at 37°C for 20 h.

The combined activities of the drugs against the organisms were determined on the basis of the FIC index expressed as:

$$\text{FIC Index} = A^1/A + B^1/B$$

Where A^1 and B^1 are the respective concentrations of ampicillin and cotrimoxazole producing the combined MICs, while A and B are the MICs of the single drugs.

RESULTS AND DISCUSSION

The results of the MIC determination for ampicillin and cotrimoxazole when used singly against the test organisms are presented in Table 1, while Figure 1 shows the results of the combinations. Table 2 shows the MICs and FICs of the various combinations of ampicillin and cotrimoxazole against *P. aeruginosa* and *S. typhi*, while Table 3 shows the same parameters against *S. aureus*.

The results obtained show that the test isolates, *S. typhi* and *S. aureus* were susceptible to ampicillin while *P. aeruginosa* was resistant to both ampicillin (MIC > 2000 µg/ml) and cotrimoxazole (MIC = 1704.55 µg/ml). *S. typhi* and *Staph. aureus* were both resistant to cotrimoxazole, with each exhibiting an MIC of 500 µg/ml.

From Tables 2 and 3, indifferent interaction was obtained in the combination of the two drugs against *P. aeruginosa,* while synergistic effects resulted in the combinations against *S. aureus* and *S. typhi.* The combination of cotrimoxazole and ampicillin at the ratios of 9:1, 8:2 and 5:5 showed the greatest synergistic effects against *S. typhi*. Conversely, *S. aureus* was most sensitive to the combination ratios 6:4, 5:5, 2:8, and 1:9. The FIC index values of >1 obtained for the combined drugs against *P. aeruginosa* indicate an indifferent activity, while the values of <1 obtained for the combinations against *S. typhi* and *Staph. aureus* indicate synergism (Elion et al., 1954). Figure 2 shows the isobolograms of the interactions of cotrimoxazole and ampicillin against *S. typhi* and *Staph. aureus.* The concavity of the isobolograms confirms synergism of the interaction (Vinh et al., 1980).

The implication of this study is that combinations of ampicillin and cotrimoxazole can be employed in the superior treatment of salmonellosis and other infections caused by *S. typhi* as well as in infections caused by *Staph. aureus.* In conclusion, a synergistic interaction

Table 2. MICs and FICs of varying combinations of ampicillin and cotrimoxazole against *P. aeruginosa* and *S. typhi.*

Drug combination ratio Cotrimoxazole:Ampicillin)	MIC (µg/ml)(Cotrim:Ampic)	FIC (µg/ml) (Cotrim:Ampic)	FIC Index	Effect
Pseudomonas aeruginosa				
10:0	1704.55: -	-	-	-
9:1	3068.19:200	1.8: 0.0125	1.812	Indifferent
8:2	2727.28:400	1.6:0.025	1.625	Indifferent
7:3	2386.37:600	1.4: 0.036	1.436	Indifferent
6:4	2045.16: 800	1.2 : 0.050	1.250	Indifferent
5:5	1704.55:1000	1.0 : 0.063	1.063	Indifferent
4:6	No inhibition	-	-	-
3:7	No inhibition	-	-	-
2:8	No inhibition	-	-	-
1:9	No inhibition	-	-	-
10:0	No inhibition	-	-	-
Salmonella typhi				
10:0	500: -	-	-	-
9:1	28.13:0.123	0.056: 0.100	0.156	Synergism
8:2	12.50:0.123	0.025: 0.100	0.125	Synergism
7:3	21.88: 0.231	0.044: 0.188	0.232	Synergism
6:4	9.38:0.246	0.019: 0.110	0.219	Synergism
5:5	3.91:0.154	0.008: 0.125	0.134	Synergism
4:6	6.25:0.370	0.013: 0.300	0.313	Synergism
3:7	4.69:0.431	0.009: 0.350	0.359	Synergism
2:8	3.13:0.493	0.006: 0.400	0.407	Synergism
1:9	3.13: 1.106	0.006: 0.899	0.905	Synergism
10:0	- : 1.231	-	-	-

Table 3. MICs and FICs of varying combinations of ampicillin and cotrimoxazole against *Staphylococcus aureus*

Drug combination ratio Cotrim/Ampicillin	MIC (µg/ml) (Cotrim:Ampic)	FIC(µg/ml) (Cotrim:Ampic)	FIC Index	Effect
10:0	500: -	-	-	-
9:1	56.25: 0.063	0.11: 0.401	0.513	Synergism
8:2	25.00: 0.063	0.050: 0.401	0.451	Synergism
7:3	21.88: 0.094	0.044: 0.601	0.645	Synergism
6:4	4.69: 0.031	0.009: 0.200	0.210	Synergism
5:5	3.91: 0.039	0.008: 0.250	0.258	Synergism
4:6	6.25: 0.094	0.013: 0.601	0.013	Synergism
3:7	2.34: 0.055	0.005: 0.350	0.355	Synergism
2:8	0.78: 0.031	0.002: 0.200	0.202	Synergism
1:9	0.39: 0.035	0.001: 0.225	0.226	Synergism
10:0	- : 0.156	-	-	-

occurred between ampicillin and cotrimoxazole against clinical isolates of *S. typhi* and *Staph. aureus.* An indifferent interaction however occurred between the two drugs against *P. aeruginosa*.

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