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acta SATECH **3**(1): 98 - 109 (2009)

Antibiotic interaction of Cotrimoxazole and Magnesium - Aluminum ions against clinical isolates

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ABSTRACT

This study was designed to determine the effects of magnesium-aluminum preparation on the antibacterial activity of Cotrimoxazole, a combination of Trimethoprim (TMP) and Sulfamethoxazole (SMX) in the ratio 1:5, on clinical isolates. Different concentrations of TMP and SMX were combined and tested against the clinical isolates using the agar diffusion method. Each of the combined concentrations of TMP:SMX was then combined with different concentrations of magnesium-aluminum hydroxide and tested against clinical isolates of Proteus morganella, Citrobacter freundii, Klebsiella edwardsii, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus and zones of inhibition were measured where present. The effects of magnesium-aluminum preparations on the antibacterial activities of cotrimoxazole were observed to be antagonistic in nature. One-way ANOVA indicated that the interaction between cotrimoxazole and magnesium-aluminum preparation is significant ($p<0.05$). The study indicated that magnesium-aluminum preparation significantly reduced the effects of the antibacterial activity of cotrimoxazole on the clinical isolates. However, resistant colonies were not observed within the zones of inhibition.

INTRODUCTION

Cotrimoxazole is a broad spectrum antibiotic combination of trimethoprim and sulfamethoxazole in the ratio of 1 to 5, used in the treatment of bacterial infections (Ilupeju *et al*., 2004). Cotrimoxazole is more efficient than either of its components individually in treating bacterial infections, though it is associated with a greater incidence of adverse effects, including allergic responses (Naisbitt *et al.*, 2001). Its widespread use has been restricted in many countries to specific circumstances like upper and lower respiratory tract infection (Doern *et al.*, 1999), renal and urinary tract infections (Libecco and Powell, 2004, Ruxer *et al.,* 2007), skin and wound infections (Smilack, 1999), gastrointestinal tract infections (Kielhofner, 1990), septicaemia and infections caused by sensitive organisms (Kielhofner, 1990; Libecco and Powell, 2004). It has broad spectrum activity against Gram-positive and Gram-negative aerobic bacteria, *Nocardia* (Torre *et al.*, 1998), *Legionella* (Libecco and Powell, 2004), *Chlamydia* (Kielhofner, 1990), nontuberculous mycobacteria (*Mycobacterium kansasii*, *M. marinum*, *M. fortuitum*, *M. scrofulaceum* and *M. avium-intracellulaire*), and various protozoa (*Pneumocystis carinii*, *Plasmodium* species and *Toxoplasma gondii*) (Lewin *et al.*, 1993; Torre *et al.*, 1998; Ribera *et al.*, 2001).

Notably, cotrimoxazole has good antimicrobial activity against aerobic Gram-negative bacilli such as *Flavobacterium meningosepticum* (Kielhofner, 1990), *Brucella species*, *Pseudomonas pseudomallei*, *Pseudomonas maltophilia* (Lewin *et al.*, 1993) and *Acetinobacter species* which are usually resistant to 3rd generation cephalosporins. It is bactericidal for such Gram-negative organisms as *Escherichia coli*, *Klebsiella, Enterobacter* (Nicolle *et al.*, 1994), *Salmonella* (Ibezim *et al*., 2006; Carlos, 1998) and *Shigella* (Ilupeju *et al*., 2004). It is also active against many strains of *Serratia* (Dudley *et al.*, 1984), *Providencia, Stenotrophomonas maltophilia*,

Burkholderia pseudomallei, Pseudomonas cepacia (Lewin *et al.*, 1993) but not against *Pseudomonas aeruginosa* (Libecco and Powell, 2004). It is inactive against anaerobes and enterococci (Crider and Colby, 1985) but inhibits most *Nocardia* and *Staphylococcus aureus* (Elwell *et al.*, 1986) while about 50% *Staphylocuccus epidermidis*, *Moraxella catarrhalis*, *Haemophilus influenzae* (Neu and Labthavikul, 1982), *H. ducreyi*, *Listeria monocytogenes* and some atypical mycobacteria are inhibited by this drug combination.

Specific indications for its use include treatment and prophylaxis of pneumonia caused by *Pneumocystis jiroveci* (*P. carinii*) (CDCP, 1999; Wininger and Fass, 2002), infections caused by *Listeria monocytogenes* (Minkowski *et al*., 2001), *Nocardia spp*, *Stenotrophomonas maltophilia* (*Zanthomonas maltophilia*) and *Toxoplasma gondii* (Torre *et al.*, 1998), shigellosis (Gotuzzo *et al.*, 1989), traveller's diarrhea (Erricson *et al.*, 1988), prophylaxis ofcerebral toxoplasmosis in HIV patients (Torre *et al.*, 1998) as well as Whipple's disease and Meloidosis (Smilack, 1999; Libecco and Powell, 2004). TMP:SMX is an alternative choice for beta-lactam-allergic adults and children having *Listeria monocytogenes* meningitis (Minkowski *et al.*, 2001), mild acute sinusitis or otitis media that has not been treated with another antimicrobial in the last 4 to 6 weeks (Feldman *et al.,* 1990; Ibezim *et al.*, 2006).

Many bacteria are obligate folic acid synthesizers, and humans use folic acid from their diets (Marvistavet, 2006).

Cotrimoxazole is an antimicrobial agent that acts as DNA inhibitor (Minkowski *et al.,* 2001). It inhibits folic acid production in bacteria thereby blocking the production of nucleotides and in turn, bacterial DNA (Smilack, 1999). TMP:SMX acts by preventing the production of folic acid first by sulphamethoxazole and then by trimethoprim (Ibezim *et al.*, 2006). Sulphamethoxazole, a structural analog of P-aminobenzoic acid (PABA), binds to the enzyme dihydropteroate sythetase competitively inhibiting the conversion of PABA to dihydrofolic acid (Minkowski, *et al.,* 2001). Trimethoprim acts on the next step in the pathway. It is a dihydrofolic acid analog which binds to the enzyme dihydrofolate reductase and hinders the production of tetrahydrofolic acid (Kielhofner, 1990). The drug's overall synergistic effect is based on a blockade of the various steps involved in microbial folate synthesis which is necessary for the formation of purines and ultimately, of deoxyribonucleic acid (Marvistavet, 2006). The key action of TMP-SMX appears to be inhibition of thymidine synthesis, since small concentrations of this nucleoside are capable of reversing the drug's action *in vitro*.

Pharmacokinetically, TMP/SMX follows first order kinetics for absorption, distribution, metabolism and excretion (Tartaglione *et al.*, 1988). After oral administration, 15% of the drug is absorbed readily regardless of other medications and reaches peak serum concentrations within 2 to 4 hours. Peak serum concentrations are reached 1 to 2 hours after intravenous administration with equilibrium being reached on the third day of therapy (Dudley *et al.*, 1984). TMP:SMX is distributed throughout all body tissues including cerebrospinal, middle ear, vaginal and synovial fluids, lung parenchyma, placenta and breast milk (Libecco and Powell, 2004). The volume of distribution of TMP exceeds that of SMX due to its lipophilic properties. Tissue concentrations of TMP and SMX are 30% to 50% and 20% respectively, of serum concentrations. Optimal synergy of the two agents against susceptible bacteria *in vivo* is achieved with a TMP:SMX serum concentration ratio of 1:20 (Dudley *et al.*, 1984). Administration of TMP/SMX at a fixed combination of 1:5 in oral and intravenous preparations results in serum concentrations of approximately 1:20 because of increased tissue distribution of TMP compared with SMX (Kielhofner, 1990). Sixty to 80% of TMP and 20% to 30% of SMX is excreted in the urine within 24 hours, either as unchanged drug or as biologically inactive urinary metabolites (Canas *et al.*, 1996). The relatively long half life of both drugs (11 hours for TMP and 9 hours for SMX) permits doses to be given every 8 to 12 hours (Morgan and Raymond, 1990; Stevens *et al.*, 1991). While some studies have reported interactions between chloramphenicol, doxycycline and Cotrimoxazole (Dance *et al.,* 1989), Coumarin anticoagulant and Cotrimoxazole (Schalekamp *et al.,* 2007) as well as Rifampin and Cotrimoxazole (Ribera *et al.*, 2001), there is a dearth of information on the effect of antacids on the antibacterial activities of cotrimoxazole against microorganisms.

MATERIALS AND METHODS

Clinical isolates

Routine clinical isolates of Gram-negative and Gram-positive bacteria were used in this study. Clinical isolates of the microorganisms were obtained from Obafemi Awolowo University Teaching Hospital Complex (O.A.U.T.H.C.), Ile-Ife, University College Hospital, University of Ibadan, Ibadan and Federal Medical Centre, Abeokuta. They were *Citrobacter freundii* (2), *Proteus morganella* (2), *Staphylococcus*

aureus (6), *Eshcerichia coli* (2), *Klebsiella pneumoniae* (1) and *Klebsiella edwardsii* (1).

Maintenance of organisms

The test isolates were subcultured weekly on fresh nutrient agar slants, stored at 4°C for 24hr and later incubated at 37°C for 24hr prior to each study.

Standardization of microbial cultures

The surface of each colony on nutrient agar slant was touched with a loop, and the growth was transferred into a tube containing 10ml of Mueller Hinton broth medium. The broth culture was incubated at 35°C for 6 hours until it achieved or exceeded the turbidity of the 0.5 McFarland standards. The turbidity of each actively growing broth culture was adjusted with sterile broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard using a spectrophotometer OD625 of 0.08 - 0.1 (1 cm light path) to give a suspension containing approximately 1 to 2 x 10^8 CFU/ml for each test organism.

Preparation of drug stock solution

The stock solution of SMX was prepared by dissolving 100mg of SMX in 10ml of acetone. By serially diluting the stock solution, different concentrations of (250, 500, 750, 1000, 1250, 1500, 2000 and 2500)µg/ml were prepared. Also, the stock solution of TMP was prepared by dissolving 20mg of TMP in 10ml of absolute acetone while different concentrations of (50, 100, 150, 200, 250, 300, 400 and 500)µg/ml were prepared. Both stock and different concentrations of these aliquots were stored in a freezer at -20°C until use. Different TMP:SMX concentrations were combined in ratio 1:5 according to Cotrimoxazole formulation for antimicrobial susceptibility studies. Antacid tablets containing magnesium trisilicate-250mg and aluminum hydroxide-120mg (Dana Pharmaceuticals PVT. Ltd, Ambernath, India) were crushed into powder form. One milligram of the powder was dissolved in 10ml of sterile distilled water to form the initial stock solution. Different concentrations containing (0.5, 2.5, 5.0, 7.5 and 10.0)µg/ml of the magnesium-aluminum hydroxide were prepared from the stock. Aliquots of stock solutions and the different concentrations were stored in the freezer at -20°C till used.

Susceptibility Tests

Susceptibility testing was performed by a standard agar dilution technique (Washington & Sutter, 1980) using Mueller Hinton agar (Lab. M; International Diagnostic Group Plc., Lancashire, UK). Exactly 100µl volume of the standardized inoculums of each test organism was dispersed into 20ml volumes of molten Mueller Hinton Agar prepared according to the manufacturer's instruction, poured into sterile petri dishes and allowed to set. Labeled wells were made with heat-sterilized 5mm cork borer while few drops of each freshly prepared different concentration of cotrimoxazole alone and its combination with magnesium-aluminum preparations were respectively and aseptically dispensed into each well, allowed standing for 1h before incubating at 35°C for 24h. The determinations were done in duplicates. The zones of inhibition were measured after incubation. All activities were carried out under a sterile inoculating hood.

The level of significance between the zones of inhibitions produced by TMP:SMX alone and its combination with magnesium-aluminum hydroxide was determined using Analyses of variance (ANOVA). $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

All the clinical strains were initially susceptible with the zones of inhibition showing dose dependency on cotrimoxazole. Conversely, a significant decrease in the antibacterial activities of cotrimoxazole was observed when different concentrations of magnesium-aluminum preparations were combined with different concentrations of cotrimoxazole as shown in Tables 1-12. Statistically, a comparison of the differences between means of different treatment indicated that there are significant differences between the activities of cotrimoxazole alone and its combinations with different concentrations of magnesium-aluminum hydroxide preparation. Also, a comparison of the effect of a concentration of magnesium-aluminum on the antibacterial activities of cotrimoxazole with other different concentrations of magnesium-aluminum hydroxide preparations combined with other different concentrations of cotrimoxazole indicated that the antagonistic effect of magnesium-aluminum preparations increased with increase in the concentrations of magnesium-aluminum hydroxide preparations. Hence, the mean difference between one combination of different concentrations of cotrimoxazole with a concentration of magnesium-aluminum preparation and another combination of different concentration of cotrimoxazole with a concentration of magnesium-aluminum preparation are significant ($P<0.05$).

In this study, it was observed that different

concentrations of cotrimoxazole used alone have higher antibacterial activities against clinical isolates of bacteria than when it was combined with different concentrations of magnesium-aluminum hydroxide. This was expressed in the sizes of the zones of inhibitions obtained. This observed result indicated that there was antagonism between magnesium-aluminum hydroxide and cotrimoxazole resulting in reduction in the antibacterial activities of cotrimoxazole.

In previous *in vivo* study on interaction between ciprofloxacin and magnesium-aluminum hydroxide preparations, Palu *et al.* (1992), Willmott and Maxwell (1993) and Bazile and Moreau (1994) suggested that quinolone-Mg^{2+} complex, formed as a result of interaction between ciprofloxacin and magnesium-aluminum preparations, interact with DNA and gyrase, possibly by forming Mg^{2+} bridge to phosphates in the DNA backbone (Palumbo *et al.,* 1993) and not a direct interaction of free quinolones with DNA (Shen & Pernet, 1985).

The implication of the observed interaction between cotrimoxazole and magnesium-aluminum hydroxide preparation is that cotrimoxazole which targets the synthesis of folic acid, a precursor for DNA, was impeded from getting to its target site of action. A most probable reason for this could be chelation between the magnesium-aluminum hydroxide and cotrimoxazole as observed in the *in vivo* interactions between magnesium-aluminum hydroxide and the fluoroquinolones (Deppermann & Lode, 1993; Schmidt & Dalhoff, 2002) with the formation of metallic ion-complexes (Campbell& Hasinoff, 1991). However, a further study on the mechanism of interaction between magnesium-aluminum hydroxide and cotrimoxazole will elucidate the chemistry of the interaction between them.

It is, therefore, concluded that magnesium-aluminum hydroxide and cotrimoxazole of cannot be combined together during the treatment of gastroenteritis and other infections caused by the test organisms in this study as this can ultimately result in resistance.

Table 1: *In vitro* susceptibility of *Citrobacter freundii* 2A to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination

Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(µg/ml)	3.250	2.781	P > 0.05	-0.4320 to 6.932
Cot alone vs Cot+2.5(µg/ml)	3.750	3.208	P < 0.05	0.06803 to 7.432
Cot alone vs Cot+5.0(µg/ml)	6.250	5.347	P < 0.001	2.568 to 9.932
Cot alone vs Cot+7.5(µg/ml)	6.375	5.454	P < 0.001	2.693 to 10.06
Cot alone vs Cot+10.0(µg/ml)	7.875	6.738	P < 0.001	4.193 to 11.56
Cot+0.5(µg/ml) vs Cot+2.5(µg/ml)	0.5000	0.4278	P > 0.05	-3.182 to 4.182
Cot+0.5(µg/ml) vs Cot+5.0(µg/ml)	3.000	2.567	P > 0.05	-0.6820 to 6.682
Cot+0.5(µg/ml) vs Cot+7.5(µg/ml)	3.125	2.674	P > 0.05	-0.5570 to 6.807
Cot+0.5(µg/ml) vs Cot+10.0(µg/ml)	4.625	3.957	P < 0.01	0.9430 to 8.307
Cot+2.5(µg/ml) vs Cot+5.0(µg/ml)	2.500	2.139	P > 0.05	-1.182 to 6.182
Cot+2.5(µg/ml) vs Cot+7.5(µg/ml)	2.625	2.246	P > 0.05	-1.057 to 6.307
Cot+2.5(µg/ml) vs Cot+10.0(µg/ml)	4.125	3.529	P < 0.05	0.4430 to 7.807
Cot+5.0(µg/ml) vs Cot+7.5(µg/ml)	0.1250	0.1069	P > 0.05	-3.557 to 3.807
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	322.4	5	64.48	
Individual (between rows)	490.0	7	70.00	
Residual (random)	191.3	35	5.464	
Total	1004	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 2: *In vitro* susceptibility of *Citrobacter freundii* 22C to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	2.000	3.187	P < 0.05	0.02287 to 3.977
Cot alone vs Cot+2.5(g/ml)	4.125	6.573	P < 0.001	2.148 to 6.102
Cot alone vs Cot+5.0(g/ml)	5.250	8.365	P < 0.001	3.273 to 7.227
Cot alone vs Cot+7.5(g/ml)	3.625	5.776	P < 0.001	1.648 to 5.602
Cot alone vs Cot+10.0(g/ml)	5.375	8.564	P < 0.001	3.398 to 7.352
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	2.125	3.386	P < 0.05	0.1479 to 4.102
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	3.250	5.178	P < 0.001	1.273 to 5.227
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	1.625	2.589	P > 0.05	-0.3521 to 3.602
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	3.375	5.378	P < 0.001	1.398 to 5.352
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	1.125	1.793	P > 0.05	-0.8521 to 3.102
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	-0.5000	0.7967	P > 0.05	-2.477 to 1.477
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	1.250	1.992	P > 0.05	-0.7271 to 3.227
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	-1.625	2.589	P > 0.05	-3.602 to 0.3521
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	0.1250	0.1992	P > 0.05	-1.852 to 2.102
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	1.750	2.788	P > 0.05	-0.2271 to 3.727
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	171.4	5	34.27	
Individual (between rows)	177.0	7	25.28	
Residual (random)	55.15	35	1.576	
Total	403.5	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 3: *In vitro* susceptibility of *E. coli* 7 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	-0.6250	0.7542	P > 0.05	-3.236 to 1.986
Cot alone vs Cot+2.5(g/ml)	3.000	3.620	P < 0.05	0.3894 to 5.611
Cot alone vs Cot+5.0(g/ml)	4.125	4.978	P < 0.001	1.514 to 6.736
Cot alone vs Cot+7.5(g/ml)	2.500	3.017	P > 0.05	-0.1106 to 5.111
Cot alone vs Cot+10.0(g/ml)	3.875	4.676	P < 0.001	1.264 to 6.486
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	3.625	4.374	P < 0.01	1.014 to 6.236
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	4.750	5.732	P < 0.001	2.139 to 7.361
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	3.125	3.771	P < 0.01	0.5144 to 5.736
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	4.500	5.430	P < 0.001	1.889 to 7.111
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	1.125	1.358	P > 0.05	-1.486 to 3.736
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	-0.5000	0.6033	P > 0.05	-3.111 to 2.111
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	0.8750	1.056	P > 0.05	-1.736 to 3.486
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	-1.625	1.961	P > 0.05	-4.236 to 0.9856
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	-0.2500	0.3017	P > 0.05	-2.861 to 2.361
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	1.375	1.659	P > 0.05	-1.236 to 3.986
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	160.4	5	32.07	
Individual (between rows)	322.0	7	46.00	
Residual (random)	96.15	35	2.747	
Total	578.5	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 4: *In vitro* susceptibility of *Klebsiella edwardsii* 292A to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	4.125	4.817	P < 0.001	1.428 to 6.822
Cot alone vs Cot+2.5(g/ml)	5.375	6.277	P < 0.001	2.678 to 8.072
Cot alone vs Cot+5.0(g/ml)	6.500	7.591	P < 0.001	3.803 to 9.197
Cot alone vs Cot+7.5(g/ml)	6.875	8.029	P < 0.001	4.178 to 9.572
Cot alone vs Cot+10.0(g/ml)	8.250	9.635	P < 0.001	5.553 to 10.95
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	1.250	1.460	P > 0.05	-1.447 to 3.947
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	2.375	2.774	P > 0.05	-0.3224 to 5.072
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	2.750	3.212	P < 0.05	0.05257 to 5.447
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	4.125	4.817	P < 0.001	1.428 to 6.822
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	1.125	1.314	P > 0.05	-1.572 to 3.822
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	1.500	1.752	P > 0.05	-1.197 to 4.197
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	2.875	3.358	P < 0.05	0.1776 to 5.572
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	0.3750	0.4380	P > 0.05	-2.322 to 3.072
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	1.750	2.044	P > 0.05	-0.9474 to 4.447
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	1.375	1.606	P > 0.05	-1.322 to 4.072
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	336.2	5	67.24	
Individual (between rows)	296.0	7	42.28	
Residual (random)	102.6	35	2.933	
Total	734.8	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 5: *In vitro* susceptibility of *Klebsiella pneumoniae* 3 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	6.750	6.972	P < 0.001	3.700 to 9.800
Cot alone vs Cot+2.5(g/ml)	6.500	6.714	P < 0.001	3.450 to 9.550
Cot alone vs Cot+5.0(g/ml)	4.250	4.390	P < 0.01	1.200 to 7.300
Cot alone vs Cot+7.5(g/ml)	7.000	7.230	P < 0.001	3.950 to 10.05
Cot alone vs Cot+10.0(g/ml)	8.875	9.167	P < 0.001	5.825 to 11.92
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	-0.2500	0.2582	P > 0.05	-3.300 to 2.800
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	-2.500	2.582	P > 0.05	-5.550 to 0.5500
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	0.2500	0.2582	P > 0.05	-2.800 to 3.300
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	2.125	2.195	P > 0.05	-0.9250 to 5.175
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	-2.250	2.324	P > 0.05	-5.300 to 0.8000
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	0.5000	0.5164	P > 0.05	-2.550 to 3.550
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	2.375	2.453	P > 0.05	-0.6750 to 5.425
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	2.750	2.840	P > 0.05	-0.3000 to 5.800
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	4.625	4.777	P < 0.001	1.575 to 7.675
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	1.875	1.937	P > 0.05	-1.175 to 4.925
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	383.9	5	76.79	
Individual (between rows)	113.6	7	16.24	
Residual (random)	131.2	35	3.749	
Total	628.8	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0015

Table 6: *In vitro* susceptibility of *Klebsiella pneumoniae* 11 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	3.875	3.881	P < 0.01	0.7297 to 7.020
Cot alone vs Cot+2.5(g/ml)	5.750	5.759	P < 0.001	2.605 to 8.895
Cot alone vs Cot+5.0(g/ml)	6.750	6.761	P < 0.001	3.605 to 9.895
Cot alone vs Cot+7.5(g/ml)	2.500	2.504	P > 0.05	-0.6453 to 5.645
Cot alone vs Cot+10.0(g/ml)	2.250	2.254	P > 0.05	-0.8953 to 5.395
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	1.875	1.878	P > 0.05	-1.270 to 5.020
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	2.875	2.880	P > 0.05	-0.2703 to 6.020
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	-1.375	1.377	P > 0.05	-4.520 to 1.770
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	-1.625	1.628	P > 0.05	-4.770 to 1.520
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	1.000	1.002	P > 0.05	-2.145 to 4.145
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	-3.250	3.255	P < 0.05	-6.395 to -0.1047
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	-3.500	3.505	P < 0.05	-6.645 to -0.3547
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	-4.250	4.257	P < 0.01	-7.395 to -1.105
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	-4.500	4.507	P < 0.01	-7.645 to -1.355
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	-0.2500	0.2504	P > 0.05	-3.395 to 2.895
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	244.6	5	48.92	
Individual (between rows)	83.31	7	11.90	
Residual (random)	139.6	35	3.988	
Total	467.5	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0145

Table 7: *In vitro* susceptibility of *Proteus morganella* 18A to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	3.250	2.781	P > 0.05	-0.4320 to 6.932
Cot alone vs Cot+2.5(g/ml)	3.750	3.208	P < 0.05	0.06803 to 7.432
Cot alone vs Cot+5.0(g/ml)	6.250	5.347	P < 0.001	2.568 to 9.932
Cot alone vs Cot+7.5(g/ml)	6.375	5.454	P < 0.001	2.693 to 10.06
Cot alone vs Cot+10.0(g/ml)	7.875	6.738	P < 0.001	4.193 to 11.56
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	0.5000	0.4278	P > 0.05	-3.182 to 4.182
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	3.000	2.567	P > 0.05	-0.6820 to 6.682
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	3.125	2.674	P > 0.05	-0.5570 to 6.807
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	4.625	3.957	P < 0.01	0.9430 to 8.307
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	2.500	2.139	P > 0.05	-1.182 to 6.182
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	2.625	2.246	P > 0.05	-1.057 to 6.307
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	4.125	3.529	P < 0.05	0.4430 to 7.807
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	0.1250	0.1069	P > 0.05	-3.557 to 3.807
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	1.625	1.390	P > 0.05	-2.057 to 5.307
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	1.500	1.283	P > 0.05	-2.182 to 5.182
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	322.4	5	64.48	
Individual (between rows)	490.0	7	70.00	
Residual (random)	191.3	35	5.464	
Total	1004	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 8: *In vitro* susceptibility of *Staph. aureus* 107 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	3.500	2.741	P > 0.05	-0.5222 to 7.522
Cot alone vs Cot+2.5(g/ml)	3.000	2.350	P > 0.05	-1.022 to 7.022
Cot alone vs Cot+5.0(g/ml)	7.625	5.972	P < 0.001	3.603 to 11.65
Cot alone vs Cot+7.5(g/ml)	7.750	6.070	P < 0.001	3.728 to 11.77
Cot alone vs Cot+10.0(g/ml)	11.00	8.615	P < 0.001	6.978 to 15.02
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	-0.5000	0.3916	P > 0.05	-4.522 to 3.522
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	4.125	3.231	P < 0.05	0.1028 to 8.147
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	4.250	3.329	P < 0.05	0.2278 to 8.272
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	7.500	5.874	P < 0.001	3.478 to 11.52
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	4.625	3.622	P < 0.05	0.6028 to 8.647
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	4.750	3.720	P < 0.05	0.7278 to 8.772
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	8.000	6.266	P < 0.001	3.978 to 12.02
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	0.1250	0.09790	P > 0.05	-3.897 to 4.147
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	3.375	2.643	P > 0.05	-0.6472 to 7.397
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	3.250	2.545	P > 0.05	-0.7722 to 7.272
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	642.6	5	128.5	
Individual (between rows)	401.1	7	57.31	
Residual (random)	228.2	35	6.521	
Total	1272	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 9: *In vitro* susceptibility of *Staph. aureus* 253 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	8.125	7.430	P < 0.001	4.680 to 11.57
Cot alone vs Cot+2.5(g/ml)	9.500	8.687	P < 0.001	6.055 to 12.94
Cot alone vs Cot+5.0(g/ml)	12.00	10.97	P < 0.001	8.555 to 15.44
Cot alone vs Cot+7.5(g/ml)	10.50	9.602	P < 0.001	7.055 to 13.94
Cot alone vs Cot+10.0(g/ml)	9.875	9.030	P < 0.001	6.430 to 13.32
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	1.375	1.257	P > 0.05	-2.070 to 4.820
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	3.875	3.544	P < 0.05	0.4301 to 7.320
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	2.375	2.172	P > 0.05	-1.070 to 5.820
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	1.750	1.600	P > 0.05	-1.695 to 5.195
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	2.500	2.286	P > 0.05	-0.9449 to 5.945
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	1.000	0.9145	P > 0.05	-2.445 to 4.445
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	0.3750	0.3429	P > 0.05	-3.070 to 3.820
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	-1.500	1.372	P > 0.05	-4.945 to 1.945
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	-2.125	1.943	P > 0.05	-5.570 to 1.320
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	-0.6250	0.5715	P > 0.05	-4.070 to 2.820
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	730.9	5	146.2	
Individual (between rows)	635.6	7	90.80	
Residual (random)	167.4	35	4.783	
Total	1534	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 10: *In vitro* susceptibility of Staph. aureus 810 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	4.500	2.355	P > 0.05	-1.520 to 10.52
Cot alone vs Cot+2.5(g/ml)	6.625	3.467	P < 0.05	0.6051 to 12.64
Cot alone vs Cot+5.0(g/ml)	8.000	4.186	P < 0.01	1.980 to 14.02
Cot alone vs Cot+7.5(g/ml)	11.00	5.756	P < 0.001	4.980 to 17.02
Cot alone vs Cot+10.0(g/ml)	14.75	7.719	P < 0.001	8.730 to 20.77
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	2.125	1.112	P > 0.05	-3.895 to 8.145
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	3.500	1.832	P > 0.05	-2.520 to 9.520
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	6.500	3.401	P < 0.05	0.4801 to 12.52
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	10.25	5.364	P < 0.001	4.230 to 16.27
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	1.375	0.7195	P > 0.05	-4.645 to 7.395
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	4.375	2.289	P > 0.05	-1.645 to 10.39
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	8.125	4.252	P < 0.01	2.105 to 14.14
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	3.000	1.570	P > 0.05	-3.020 to 9.020
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	6.750	3.532	P < 0.05	0.7301 to 12.77
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	3.750	1.962	P > 0.05	-2.270 to 9.770
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	1049	5	209.7	
Individual (between rows)	615.1	7	87.88	
Residual (random)	511.2	35	14.61	
Total	2175	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	Yes			P<0.0001

Table 11: *In vitro* susceptibility of Staph. aureus 997 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	22.25	3.111	P > 0.05	-0.2821 to 44.78
Cot alone vs Cot+2.5(g/ml)	21.88	3.058	P > 0.05	-0.6571 to 44.41
Cot alone vs Cot+5.0(g/ml)	21.50	3.006	P > 0.05	-1.032 to 44.03
Cot alone vs Cot+7.5(g/ml)	21.63	3.023	P > 0.05	-0.9071 to 44.16
Cot alone vs Cot+10.0(g/ml)	20.13	2.814	P > 0.05	-2.407 to 42.66
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	-0.3750	0.05243	P > 0.05	-22.91 to 22.16
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	-0.7500	0.1049	P > 0.05	-23.28 to 21.78
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	-0.6250	0.08738	P > 0.05	-23.16 to 21.91
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	-2.125	0.2971	P > 0.05	-24.66 to 20.41
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	-0.3750	0.05243	P > 0.05	-22.91 to 22.16
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	-0.2500	0.03495	P > 0.05	-22.78 to 22.28
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	-1.750	0.2447	P > 0.05	-24.28 to 20.78
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	0.1250	0.01748	P > 0.05	-22.41 to 22.66
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	-1.375	0.1922	P > 0.05	-23.91 to 21.16
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	-1.500	0.2097	P > 0.05	-24.03 to 21.03
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	3095	5	619.1	
Individual (between rows)	1004	7	143.4	
Residual (random)	7162	35	204.6	
Total	11260	47		
Are means signif. different? (P < 0.05)	Yes			P<0.6710
Is there significant matching? (P < 0.05)	No			

Table 12: *In vitro* susceptibility of Staph. aureus 77 to Cotrimoxazole alone and Cotrimoxazole-Magnesium/Aluminum combination				
Bonferroni's Multiple Comparison Test	Mean Diff.	t	P value	95% CI of diff
Cot alone vs Cot+0.5(g/ml)	1.750	1.953	P > 0.05	-1.073 to 4.573
Cot alone vs Cot+2.5(g/ml)	2.875	3.208	P < 0.05	0.05211 to 5.698
Cot alone vs Cot+5.0(g/ml)	1.375	1.534	P > 0.05	-1.448 to 4.198
Cot alone vs Cot+7.5(g/ml)	2.625	2.929	P > 0.05	-0.1979 to 5.448
Cot alone vs Cot+10.0(g/ml)	1.625	1.813	P > 0.05	-1.198 to 4.448
Cot+0.5(g/ml) vs Cot+2.5(g/ml)	1.125	1.255	P > 0.05	-1.698 to 3.948
Cot+0.5(g/ml) vs Cot+5.0(g/ml)	-0.3750	0.4185	P > 0.05	-3.198 to 2.448
Cot+0.5(g/ml) vs Cot+7.5(g/ml)	0.8750	0.9765	P > 0.05	-1.948 to 3.698
Cot+0.5(g/ml) vs Cot+10.0(g/ml)	-0.1250	0.1395	P > 0.05	-2.948 to 2.698
Cot+2.5(g/ml) vs Cot+5.0(g/ml)	-1.500	1.674	P > 0.05	-4.323 to 1.323
Cot+2.5(g/ml) vs Cot+7.5(g/ml)	-0.2500	0.2790	P > 0.05	-3.073 to 2.573
Cot+2.5(g/ml) vs Cot+10.0(g/ml)	-1.250	1.395	P > 0.05	-4.073 to 1.573
Cot+5.0(g/ml) vs Cot+7.5(g/ml)	1.250	1.395	P > 0.05	-1.573 to 4.073
Cot+5.0(g/ml) vs Cot+10.0(g/ml)	0.2500	0.2790	P > 0.05	-2.573 to 3.073
Cot+7.5(g/ml) vs Cot+10.0(g/ml)	-1.000	1.116	P > 0.05	-3.823 to 1.823
ANOVA Table	SS	df	MS	P value
Treatment (between columns)	41.92	5	8.383	
Individual (between rows)	247.6	7	35.37	
Residual (random)	112.4	35	3.212	
Total	401.9	47		
Are means signif. different? (P < 0.05)	Yes			P<0.0001
Is there significant matching? (P < 0.05)	No			P<0.0001

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