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In vitro Effects of Magnesium-Aluminum Hydroxide (Maalox) on the Antibacterial Activity of Ciprofloxacin against Clinical Bacterial Isolates

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To investigate the *in vitro* effects of interactions between ciprofloxacin (CP) and magnesium-aluminium hydroxide (MA) combined against selected Gram-positive and Gram-negative bacteria. The interaction between CP and MA was accessed by agar diffusion method. Comparing the susceptibility of the isolates to CP alone with those of Ciprofloxacin-Magnesium-aluminum hydroxide (CPMA) combined showed that there were significant antagonistic and synergistic interactions *in vitro*. Antibacterial activities of ciprofloxacin were increased with 4.5-6.0 μg/ml of the MA but were drastically decreased with concentrations lower and higher than 4.5-6.0 μg/ml while development of resistant colonies within these zones of inhibitions was recorded. The susceptibility of the isolated resistant colonies was lesser than those obtained from the original isolates. The combination of ciprofloxacin (CP) and magnesium-aluminum hydroxide (Maalox) (MA) against bacterial isolates could result in development of resistant colonies. The roles of aluminum and magnesium in resistance development at the molecular level require further studies.

Keywords: Ciprofloxacin; ciprofloxacin-magnesium-aluminum hydroxide; antagonistic interactions; polyvalent metallic ions; resistance development.

Ciprofloxacin or 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl]-3-quinoline carboxylic acid is a fluorinated quinolone antibacterial agent extensively used worldwide[1] against both Gram-positive and Gram-negative microorganisms[2]. While ciprofloxacin is a fluorinated quinolone synthesized in late 1980s[3], it is a quinolone carboxylic acid derivative with an extensive antibacterial spectrum[4, 5] showing greater potency and lower toxicity than other fluoroquinolones[6, 7]. After oral administration, it has excellent tissue penetration, good bioavailability and it is relatively safe[8,9]. Though it is effective in the treatment of soft tissue infections[10-13], Neuhauser *et al*[14], Friedland *et al*[15] and MacDougall *et al*[16] reported that increasing incidence of ciprofloxacin resistance among Gram negative bacteria is associated with increased use of fluoroquinolones. While ciprofloxacin is well tolerated with low incidence of adverse effects[17], Wolfson and Hooper[18] and Petri[19] indicated few clinical reactions including gastrointestinal disturbances, central nervous systems toxicity and rash occurring in about 5–12 % of patients.

Fluoroquinolones, generally, are known to have numerous interactions with agents with local activity in the gastrointestinal system such as antacids [20-23] leading to loss of antibiotic activity.

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Several studies[24-26] reported that concomitant administration of aluminum, magnesium, zinc or iron with fluoroquinolones resulted in a significant decrease in the systemic availability of the drug indicating that widely used antacids markedly reduce the bioavailability of fluoroquinolone antimicrobial agents through chelation in the gastrointestinal tract[27,28].

While several studies[29-32] have reported that the bioavailability of ciprofloxacin was significantly impaired when orally co-administered with iron, aluminum- and magnesium- containing antacids, there is a dearth of information on the effect this combinations may have on the antimicrobial activities of ciprofloxacin against microorganisms *in vitro*. The study was, therefore, aimed at investigating *in vitro* interaction between ciprofloxacin and magnesium-aluminum hydroxide and the effects of this metallic ion on the antibacterial activity of ciprofloxacin against selected bacterial strains.

MATERIALS AND METHODS

Preparation of drug stock solutions

Stock solution of ciprofloxacin was prepared according to the NCCLS guidelines or manufacturer's recommendations[33]. Forty milligram of pure drug powder of ciprofloxacin, obtained from a pharmaceutical company in Lagos, Nigeria, dissolved in 3 ml of ethanol was made up to 100 ml in sterile distilled water to form the stock solution. Concentrations of 0.2 - 3.0 µg/ml of ciprofloxacin were prepared from stock solutions. Three antacid tablets, each containing magnesium trisilicate-250 mg and aluminum hydroxide-120 mg, were aseptically crushed into powdery form after obtaining the average weight. Ten milligram (10 mg) of the powdered antacid tablets was dissolved in 100 ml of sterile distilled water to form the initial stock solution. Concentrations ranging between 1.0 and 10.0 µg/ml of the magnesium-aluminum-hydroxide (Maalox) were prepared from the stock. Fresh drug solutions were daily prepared from stock solutions which were stored at -20°C for susceptibility studies.

Clinical isolates

Routine clinical Gram-negative and Gram-positive bacteria were used in this study. These bacterial isolates included *Bacillus brevis*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Staphylococcus albus*. The bacterial colonies were subjected to Gram staining, microscopic appearance, colony morphology and biochemical tests according to standard protocols for identification [34-36].

Antimicrobial susceptibility testing

The antibacterial activity was determined using agar diffusion assay technique according to the modified Kirby–Bauer diffusion technique[35] by swabbing the Mueller-Hinton agar (MHA) (Oxoids UK) plates with the resultant overnight culture of each of the test isolates. Multi-discs (Abtek) containing different antibiotics including Ofloxacin (5 µg), Ciprofloxacin (5 µg), Pefloxacin (5 µg), Streptomycin (10 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Amoxicillin (25 µg), Cefuroxime (30 µg) and Erythromycin (10 µg) were aseptically placed on the inoculated agar plates and incubated at 37°C for 24 h.

To determine the drug-drug interactions, prepared Mueller Hinton agar plates were swabbed with the resultant saline suspension of each bacterial strain. Wells were then bored into the agar medium with heat sterilized 6 mm cork borer. The wells were aseptically filled with 100 µl of freshly prepared different concentrations of ciprofloxacin (CP) alone and its combination with magnesium-aluminum-hydroxide (Maalox) preparations (CPMA) taking care not to allow spillage of the solutions onto the surface of the agar. The plates were allowed to stand for at least 30 min before being incubated at 37°C for 24 h[37]. The determinations were done in duplicate. After 24 h of incubation, all the plates were examined for zones of inhibition[38]. The diameter of the inhibition zones produced by the different antibiotic discs, ciprofloxacin alone and those of its combination with the Maalox were measured and interpreted using the CLSI zone diameter interpretative standards[39]. Resistant colonies (Rc) were isolated from within inhibition zones of plates containing drug combinations and were further identified and subjected to susceptibility testing. Each bacterial isolate was classified as susceptible (S), intermediate (I) and resistant (R) to antibiotics according to the zone diameter interpretation standard recommended by the Clinical Laboratory Standards Institute[40].

RESULTS

In this study, all the clinical strains were susceptible *in vitro* with the inhibition zones showing concentration-dependent activity of ciprofloxacin. The inhibition zones increases with increase in the concentration of ciprofloxacin as presented in Figures 1 – 7 showing antibacterial activity of ciprofloxacin (CP) alone and its combination with magnesium-aluminum hydroxide (CPMA) complex at different concentrations. Although, CP exhibited a significant activity against the different test strains of bacteria, the extent and activity observed for CPMA was significantly different from those of CP.

The interaction between CP and MA resulted in a complete lost or drastic reduction of antibacterial activities of CP as observed with the addition of 1.0 – 4.0 µg/ml and 8.0 – 10.0 µg/ml of the MA to the different concentrations of

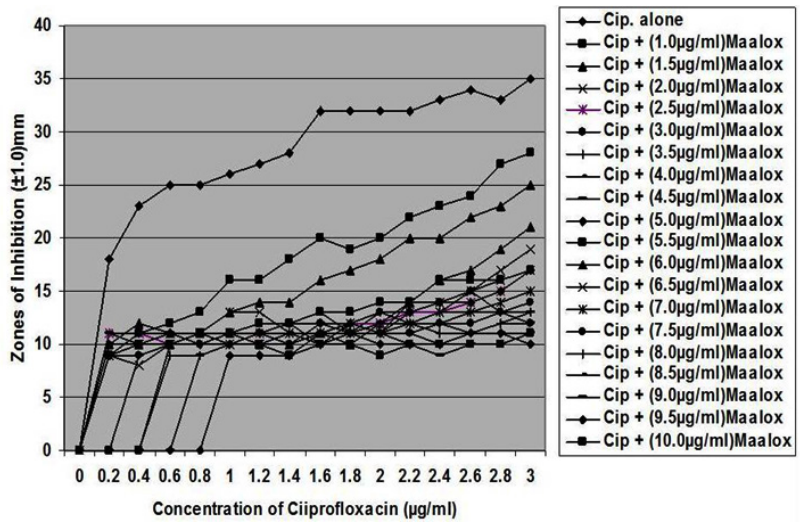

Fig. 1. *In vitro* susceptibility of *Staphylococcus aureus* to different concentrations of ciprofloxacin and its combination with magnesium/aluminum hydroxide

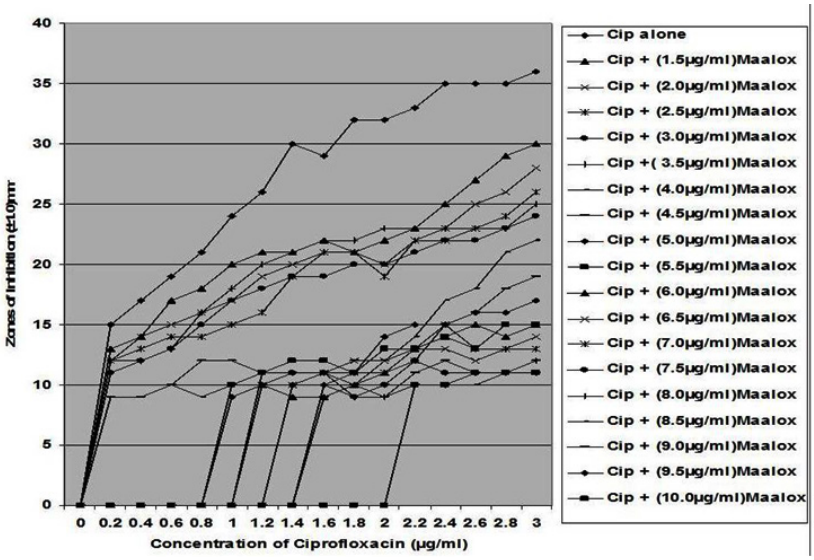

Fig. 2. *In vitro* susceptibility of *Salmonella typhi* to different concentrations of ciprofloxacin and its combination with magnesium-aluminum hydroxide

CP. However, there were increased antibacterial activities of CP in the presence of 4.5-6.0 µg/ml concentration of MA against the bacterial isolates. This increased antibacterial activities or synergy produced inhibition zones wider than those of CPMA at lower and higher concentration of the MA used. At lower concentrations (1 - 2 µg/ml) of MA combined with CP, more resistant colonies (Rc) were observed within the zones of inhibitions whereas at higher concentrations (8 - 10 µg/ml) of MA combined with CP, resistant colonies (Rc) within inhibition zones were drastically reduced or not observed within some inhibition zones. The resistant colonies (Rc) isolated from within the

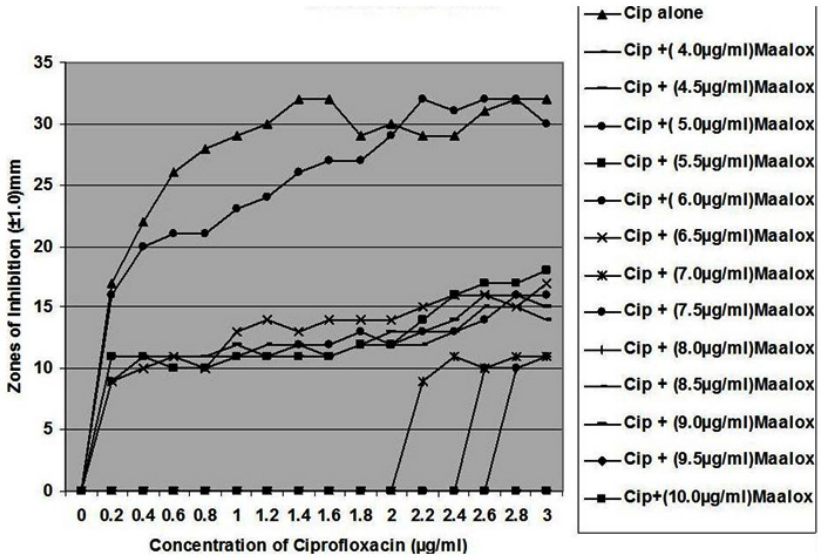

Fig. 3. *In vitro* susceptibility of *Pseudomonas aeruginosa* to different concentrations of ciprofloxacin and its combination with magnesium-aluminum hydroxide

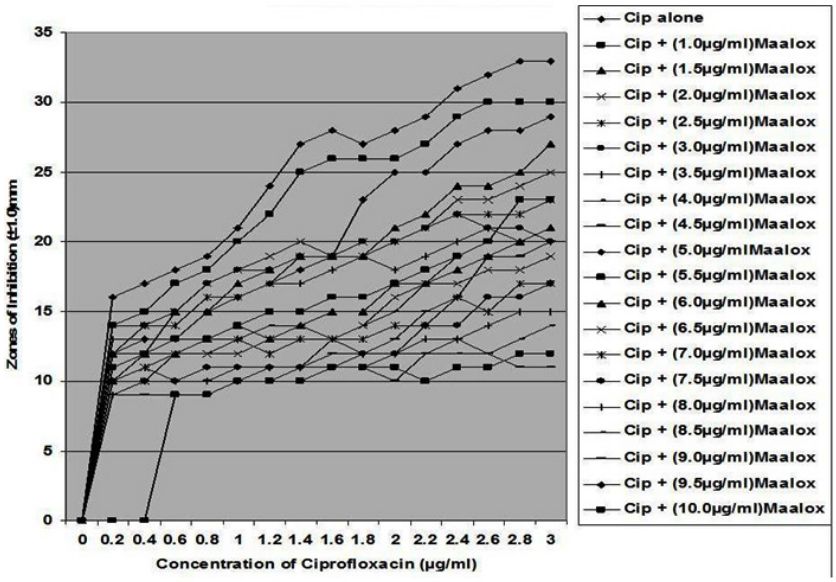

Fig. 4. *In vitro* susceptibility of *Bacillus brevis* to different concentrations of ciprofloxacin and its combination with magnesium-aluminum hydroxide

inhibition zones were characterized and further subjected to antibiotic susceptibility testing with standard antibiotic discs. These isolated resistant colonies (Rc) were less susceptible to the antibiotics when their inhibition zones were compared with those obtained from the original culture as shown in Table 1.

DISCUSSION

From this study, the susceptibility of bacteria to ciprofloxacin-magnesium-aluminum hydroxide (CPMA) indicated that synergistic and antagonistic interactions occurred *in vitro*. Synergy between CP and MA occur when concentrations of

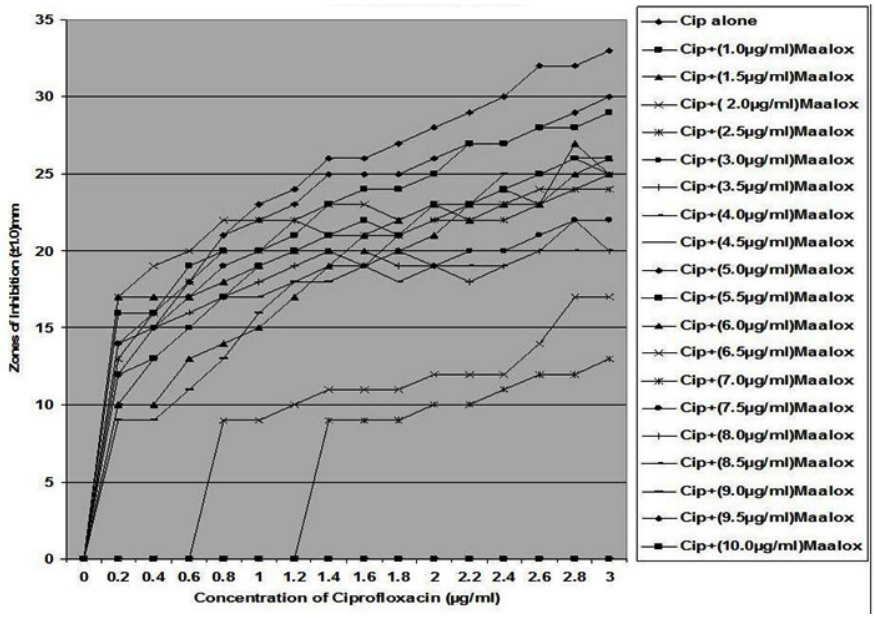

Fig. 5. *In vitro* susceptibility of *Escherichia coli* to different concentrations of ciprofloxacin and its combination with magnesium-aluminum hydroxide

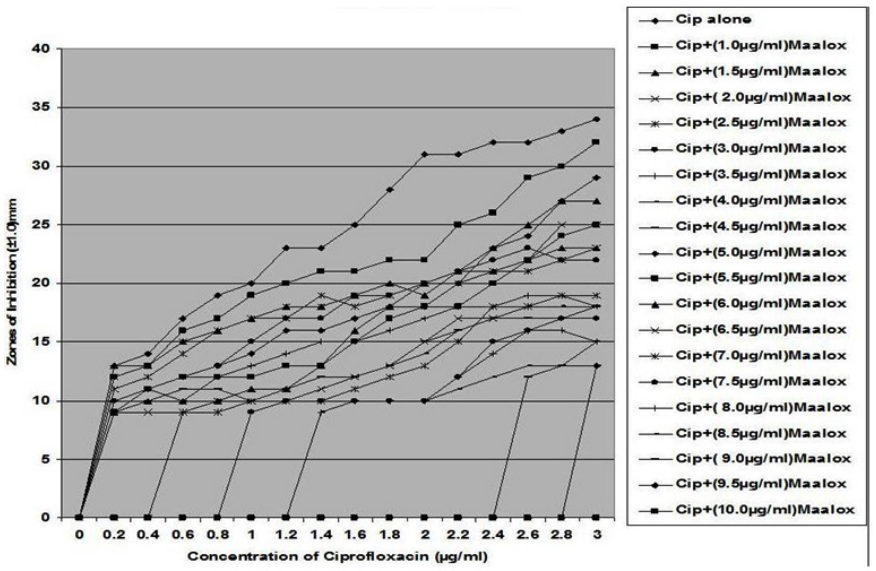

Fig. 6. *In vitro* susceptibility of *Staphylococcus albus* to different concentrations of ciprofloxacin and its combination with magnesium-aluminum hydroxide

4.5 – 6.0 µg/ml of MA was combined with CP and resulted in increase in the sizes of the inhibition zones as compared to those obtained from CP alone whereas interaction between CP and MA at other concentrations where there were resistant colonies within the inhibition zones indicated antagonism. This is contrary to previous study [41] that reported synergistic interaction at low

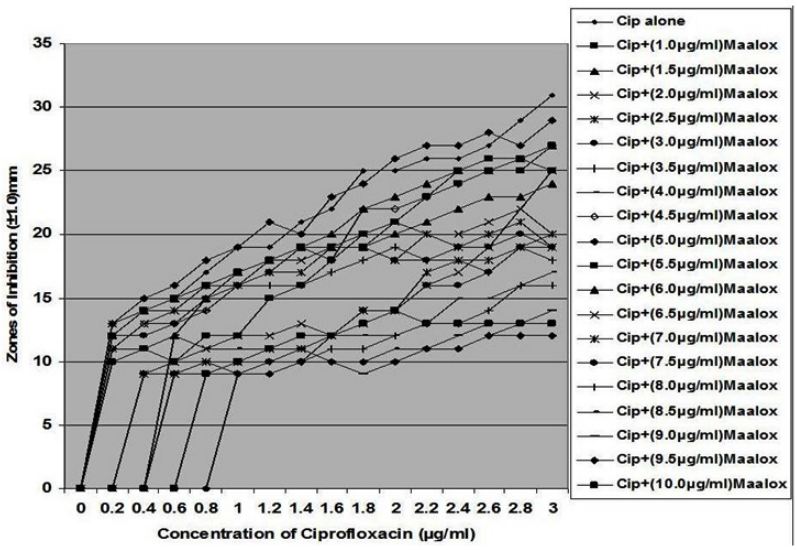

Fig. 7. *In vitro* susceptibility of *Staphylococcus epidermidis* to different concentrations of ciprofloxacin and its combination with magnesium/aluminum hydroxide

Table 1. *In vitro* susceptibility of bacterial isolates and their mutants to different antibiotic discs

	Ofl (5 µg)	Cip (5 µg)	Pfx (5 µg)	Str (10 µg)	Gen (10 µg)	Chl (30 µg)	Amx (25 µg)	Cef (30 µg)	Ery (10 µg)
	Inhibition zones produced by original broth culture (± 1.00 mm)								
Staphylococcus aureus	21	31	22	13	15	00	00	00	00
Salmonella typhi	21	33	20	00	00	00	00	00	00
Pseudomonas aeruginosa	32	30	16	00	00	00	00	00	00
Escherichia coli	30	32	32	16	00	00	00	00	00
Bacillus brevis	22	30	24	00	00	00	00	00	00
Staphylococcus albus	25	28	26	00	00	00	00	00	00
Staphylococcus epidermidis	25	32	30	00	00	00	00	00	00
	Ofl (5 µg)	Cip (5 µg)	Pfx (5 µg)	Str (10 µg)	Gen (10 µg)	Chl (30 µg)	Amx (25 µg)	Cef (30 µg)	Ery (10 µg)
	Inhibition zones produced by isolated mutant colonies (± 1.00 mm)								
Staphylococcus aureus	16	20	19	00	00	00	00	00	00
Salmonella typhi	20	22	00	00	00	00	00	00	00
Pseudomonas aeruginosa	20	22	00	00	00	00	00	00	00
Escherichia coli	15	25	16	00	00	00	00	00	00
Bacillus brevis	20	22	22	00	00	00	00	00	00
Staphylococcus albus	18	26	20	00	00	00	00	00	00
Staphylococcus epidermidis	19	20	15	00	00	00	00	00	00

Key: Ofl – Ofloxacin; Cip – Ciprofloxacin; Pfx – Pefloxacin; Str – Streptomycin; Gen – Gentamicin; Chl – Chloramphenicol; Amx – Amoxicillin; Cef - Cefuroxime; Ery - Erythromycin

concentrations of ciprofloxacin (0.78 to 1.85 µg/ml) and amphotericin B (0.24 to 0.39 µg/ml) and antagonistic interaction at higher concentrations of both ciprofloxacin (52.75 to 74.25 µg/ml) and amphotericin B (0.53 to 1.12 µg/ml). However, the synergy observed in vitro at lower concentrations is contrary to earlier in vivo reports that showed that aluminum-magnesium-containing antacids reduce the absorption of fluoroquinolone antibiotics[42-44].

Although it has been reported that hydrophobic quinolones chelate outer membrane-bound magnesium to become more hydrophobic in order to diffuse across the exposed lipid domains of the outer membrane[45], the combination of CP and MA may have prevented the CP from becoming more hydrophilic, thereby altering the outer membrane proteins and porin pathway to retard the accessibility of ciprofloxacin to their targets cells[46]. On the other hand, Palu *et al*[47], Willmott and Maxwell[48] and Bazile and Moreau[49] 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 a Mg^{2+} bridge to phosphates in the DNA backbone [50] and not a direct interaction of free quinolones with DNA[51]. This showed that CPMA could have caused the selection of resistant strains and enhanced alteration of the antibacterial activities of CP at the target site of action resulting in the development of resistant colonies. While the *in vitro* interaction between CP and MA is consistent with several *in vivo* studies showing that magnesium-aluminum-containing antacids reduce the absorption of fluoroquinolone antibiotics[42,43], this interaction is, possibly, due to chelation of the antibiotic by the metallic ions as reported *in vivo* by Höffken *et al.*[52], Lode,[53], and Randandt, *et al.,*[24] with aluminum forming a very stable complex which is not easily soluble with quinolones[54].

The biphasic activity observed with combinations of CP and MA, however, could not be explained with the simple complexes formed between CP and MA. While McCaffrey *et al*[55], Kaatz *et al*[56] and Piddock *et al*[57] reported mechanism of resistance to include a spontaneous single-step chromosomal mutation resulting in alterations in the A subunits of DNA gyrase, an active efflux system that prevents net drug accumulation mediated by the *norA* gene and low-level resistance mediated by the *cfx-ofx* locs, the 4.5–6.0 µg/ml concentrations of the MA could have suppressed this spontaneous single-step chromosomal mutation which might not have been possible with the lower concentrations of 1.0 – 4.0 µg/ml and higher concentrations of 6.5 – 10.0 µg/ml. While increasing molecular mass, hydrophobicity and negative charge hinder penetration of antibiotics through porin channels, the increased antibacterial activity resulting from combining CP with 4.5 - 6.0 µg/ml concentration of MA could have decreased the hydrophobicity of CP to cause an effective concentration of this antibiotic to get to the target sites. Thus, combining CP with MA at these concentrations could enhance the ability of CP to penetrate the cell envelope and reach its target to produce antibacterial effects almost equal to what was obtained with CP alone in some cases.

Although Aldred *et al*[58] indicated that quinolones convert gyrase and topoisemerase IV into toxic enzymes that fragment the bacterial chromosome, and mutations in gyrase or topoisomerase IV could lead to resistance[59-61], combining CP with MA could have incited resistance mechanisms such as altered protein interactions, drug metabolism and uptake and/or efflux of CP from the cells[62,63]. The development of resistance and the isolation of resistant colonies could be responsible for therapeutic failure observed when there is co-administration of metallic ions such as magnesium-aluminum hydroxide with ciprofloxacin *in vivo*. This is of significant medical importance during chemotherapy of bacterial infections as Noyes and Polk[64] had earlier reported failure of norfloxacin treatment resulting from concomitant treatment with an aluminum- and magnesium- containing antacid suspension while Preheim *et al*[65] reported that antacids interfere with the efficacy of ciprofloxacin, particularly, in patients infected with *Pseudomonas aeruginosa.* Also, because currently used antibiotics are not bactericidal against most strains of bacteria at concentrations readily achievable in the serum, combination of drugs for synergistic bactericidal activity becomes necessary[66]. However, while these agents are generally safe in the medical setting, their use can result in interactions leading to adverse clinical outcomes including sub-minimal doses of antibiotics and serious morbidity

in patients[67]. In addition to decrease in optimal plasma concentrations and urinary recoveries due to these interactions, there is also the potential for therapeutic failure, the development of antibiotic resistance and increases in health care costs[26].

In conclusion, combining CP and MA could result in both synergistic and antagonistic interactions and the *in vitro* effect of the antagonistic interaction could lead to development of bacterial resistance. While ineffective actualization of treatment of bacterial infections may result in development of resistance among bacteria, pharmacokinetic data from previous studies and data from this study suggested that combination of CP and MA preparations in antimicrobial therapy should be discouraged not only due to production of sub-therapeutic doses but also because of development of bacterial resistance that may necessitate the use of more expensive and probably more toxic antibiotics. Patients should be encouraged to avoid taking the antacid within two hours after a fluoroquinolone dose or six hours prior to the next antimicrobial dose. However, the mechanisms of interactions resulting in both antagonistic and synergistic effects *in vitro* between ciprofloxacin and magnesium-aluminum hydroxide (Maalox) require further studies.

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