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# ERYTHROMYCIN SYNERGISM WITH ESSENTIAL AND TRACE ELEMENTS

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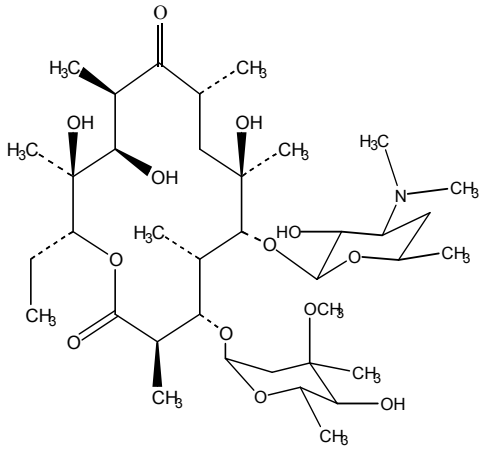
In order to establish the role of various essential and trace element complexation on the antibacterial activity of various macrolide antibiotics, the synergistic or antagonistic behavior of erythromycin metal complexes have been studied and compared with the parent drug. Metal complexes of erythromycin with magnesium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc and cadmium have been investigated for their antibacterial activity and compared with erythromycin by observing the changes in minimum inhibitory concentration (*MIC*) and by measuring the zone of inhibition of complexes against both Gram-negative and Gram-positive microorganisms. Various microorganisms used were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Shigella dysentary, Klebsiella pneumoniae* and *Staphylococcus epidermidis.* For *MIC* observation, serial dilution method was employed and zone sizes were determined by diffusion disk method.

Our investigations reveal that formation of erythromycin complexes result in synergistic effect i.e., antimicrobial activity of complexes of erythromycin increases with respect to parent erythromycin drug and *MIC* of drug metal complexes decreased.

**Keywords**: Erythromycin, macrolide antibiotic, drug interaction.

## INTRODUCTION

Erythromycin is an orally effective antibiotic, discovered in 1952 by McGuire and its co-workers, produced by a strain of *Streptomyces erythreus*, isolated from a soil sample collected in Philippine archiperago (British Pharmacopoeia 2002; Klaus 1979; Goodman & Gilman 1996; Martindale 1993; Merck Index 2000). Erythromycin or erythromycin A is (2R,3S,4S,5R,6R,8R,10R,11R,12S, 13R)-5-(3-amino-3,4,6-trideoxy-N,N-dimethyl--D-xylo-he-xopyranosyloxy)-3-(2,6-dideoxy-3-C,3-O-dimethyl--L-ribohexopyranosylo-xy)-13-ethyl-6,11,12-trihydroxy-2,4,6,8,10, 12-amethyl-9-oxopridecan-13-olide, $C_{37}H_{67}NO_{13}$, molecular weight 733.92 is a macrolide antibiotic consisting of the aglycone erythronolide A, the aminosugar desosamine and the neutral sugar cladinose having the following structure (Colin 1999).



Erythromycin A

Erythromycin is active against many penicillin resistant Gram-positive organisms such as *Staphylococci, Streptococci, Pneumococci* etc. It is usually bacteriostatic but it has been shown to be bactericidal in high concentrations against very susceptible organism. It is most effective *in vitro* against aerobic Gram-positive *cocci* and *bacill* (Sutter and Finegold 1976) and some Gram-negative bacteria, *Rickettsia* and *Protozoa* (John 1976). It is mainly used to treat pulmonary infections caused by *Mycoplasma legionella* and Gram-positive organisms in patients allergic to penicillin (Clark *et al*., 1992).

Gram-positive bacteria accumulate about 100 times more erythromycin than do Gram-negative microorganisms. The ionized form of the drug is considerably more permeable to the non-ionized form of the drug and this fact probably explains the increased antimicrobial activity that is observed at alkaline pH. Some *Staphylococci* are sensitive to erythromycin, the range of inhibitory concentration being very high; MIC for *Staphyllococcus epidermidis* 8 to >32 µg/ml and for *Staphylococcus aureus,* 0.12 to >128 µg/ml concentration (Weissbach 1977; Klaus 1979; Colin 1999 and Geo *et al.*, 1995). Erythromycin has been reported to cause clinically significant drug interactions involving inhibition of metabolism (Hansten 1996).

It is known to affect the cytochrome P-450. There are number of reported interactions of erythromycin with many drugs, like sulfonamides (Cloin 1999), ampicillin (Jawetz 1975; Igarashi *et al*., 1969), antacids like aluminum hydroxide, aluminum trisilicate, magnesium oxide, magnesium trisilicate and dimethylpolysiloxane (Hedrick

1983), warfarin (Arayne and Sultana 1993), cyclosporine (Rodin and Johnson 1988, Ben-ari 1988), digoxin (Sutton 1989; Honig 1992), phenytion (Al Humayyd 1997) and penicillins (Bach 1973).

Present studies comprise of antibacterial studies of metal complexes of erythromycin, with magnesium, calcium, chromium, manganese, ferric, cobalt, nickel, copper, zinc and cadmium and changes in microbiological activity of the parent erythromycin after complexation has been studied. These studies were carried out by observing the minimum inhibitory concentration (*MIC*) and by measuring the zone of inhibition of the complexes and compared with the parent erythromycin against both Gram-negative and Gram-positive microorganism. Various microorganisms used were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus Vulgaris, Shigella dysentery, Klebsiella pneumonia* and *Staphylococcus epidermidis*. For MIC observation, serial dilution method was employed and zone sizes were determined by diffusion disk method.

## EXPERIMENTAL

***Materials***

Erythromycin metal complexes (table 1) used for antibacterial studies were synthesized in lab-9 of the Department of Chemistry, University of Karachi. The synthesis and characterization of these complexes are reported elsewhere. The organisms used in the antimicrobial studies of erythromycin metal complexes were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Shigella dysentery, Klebsiella pneumonia* and *Staphylococcus epidermidis*. Media was Muller-Hinton agar and broth. Routine laboratory chemicals like barium chloride, sulfuric acid, sodium carbonate, mercuric chloride, sodium citrate and hydrochloric acid, organic polar and non-polar solvents, de-ionized water and pH 2 buffer solutions were used in these experiments.

**Table 1**
Erythromycin metal complexes

| S. No. | Complex | M.P.$^{o}$C |
|---|---|---|
| 1 | Erythromycin | 136 |
| 2 | Erythromycin magnesium | 182 |
| 3 | Erythromycin calcium | 176 |
| 4 | Erythromycin chromium | 146 |
| 5 | Erythromycin manganese | 126 |
| 6 | Erythromycin ferric | 160 |
| 7 | Erythromycin cobalt | 186 |
| 8 | Erythromycin nickel | 126 |
| 9 | Erythromycin copper | 240d |
| 10 | Erythromycin zinc | 172 |
| 11 | Erythromycin cadmium | 174d |

***Methods***

1 *Preparation of pH 2 buffer solution*

Citrate buffer of pH 2 was prepared by mixing 300 ml of 0.1M sodium citrate solution and 150 ml of 0.1M hydrochloric acid solution in a liter beaker and the final pH was adjusted by either of the two solutions. This buffer was sterilized by autoclaving at 121°C and at 15 psi pressure for 15 minutes.

2 *Preparation of erythromycin solutions*

The stock solution of erythromycin was prepared by dissolving 0.025 gram of drug in minimum volume of distilled ethanol in a 25 ml volumetric flask and the final volume was made up with buffer of pH 2. Aliquots were diluted between 0.25 μg/ml to 1 μg/ml to give the required concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 μg/ml with buffer of pH 2.

For subsequent dilutions of drug highest concentration was taken first i.e., 128 μg/ml was made by diluting 6.4 ml of stock solution in a 50ml volumetric flask up to the mark with the buffer of pH 2. Aliquots of 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/ml concentrations were prepared by serially diluting the later solution in the same solvent.

3 *Preparation of solutions of metals*

The stock and primary standard solutions of metal salts were prepared exactly in the same manner as those prepared for the antibiotics in the required concentrations (128, 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/ml). The stock and primary standard solutions of erythromycin metal complexes (as given in table 1) were also prepared in the same manner as those for erythromycin in the same concentrations.

*4 Preparation of Mueller–Hinton agar* (MHA), Mueller-Hinton broth (MHB), preparation of inoculums, controlling inoculum's density, MacFarland turbidity standards and agar dilution susceptibility tests were carried out according to standard procedures reported earlier (Sultana *et al.,* 2001).

5 *Preparation, inoculation and incubation of antimicrobial plates*

The agar medium prepared in conical flask was allowed to cool to 50°C on a water bath. Petri dishes were sterilized by placing them in an oven at 150°C for one and a half hour and labeled according to their concentrations (0.5 μg/ml to 128 μg/ml). Various dilutions of erythromycin and its metal complexes were prepared according to the procedures described above. These were added to the melted and cooled medium in a ratio of 1:9 (2 ml of dilution of each to 18 ml of agar for each petri dish). The medium was mixed by gently shaking the flask several times and the contents were poured into appropriate number of petri dishes marked, set

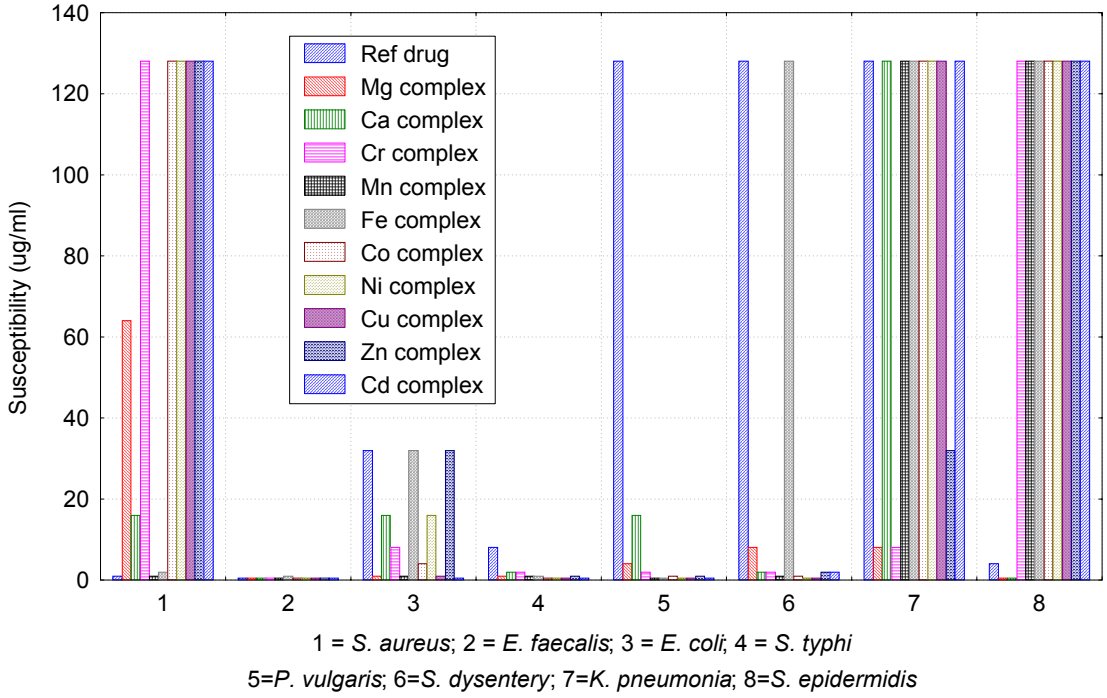



Fig. 1: Susceptibility of erythromycin metal complexes.

**Table 2**

Susceptibility (µg/ml) of erythromycin and its metal complexes against various organisms

| S. No. | Organism | Erythromycin-metal complexes with | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ref. drug | Mg | Ca | Cr | Mn | Fe | Co | Ni | Cu | Zn | Cd |
| 1 | *Staphylococcus aureus* | 1 | 64 | 16 | <128 | 1 | 2 | <128 | <128 | <128 | <128 | <128 |
| 2 | *Enterococcus faecalis* | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 3 | *Escherichia coli* | 32 | 1 | 16 | 8 | 1 | 32 | 4 | 16 | 1 | 32 | 0.5 |
| 4 | *Salmonella typhi* | 8 | 1 | 2 | 2 | 1 | 1 | 0.5 | 0.5 | 0.5 | 1 | 0.5 |
| 5 | *Proteus vulgaris* | <128 | 4 | 16 | 2 | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 1 | 0.5 |
| 6 | *Shigella dysentery* | <128 | 8 | 2 | 2 | 1 | <128 | 1 | 0.5 | 0.5 | 2 | 2 |
| 7 | *Klebsiella pneumonia* | <128 | 8 | <128 | 8 | <128 | <128 | <128 | <128 | <128 | 32 | <128 |
| 8 | *Staphylococcus epidermidis* | 4 | 0.5 | 0.5 | <128 | <128 | <128 | <128 | <128 | <128 | <128 | <128 |

aside on a flat horizontal surface and allowed to harden undisturbed till the contents solidified (Bertina 1987).

An inoculum (1-2 ml) of each organism was applied to the surface of each antimicrobial petri dish with the help of a sterilized wire loop. The inoculum was applied as a spot that made a circle [Bertina 1987; National Committee For Clinical Laboratory Standards 1990]. The inoculated petri dishes were not disturbed until the spot of inoculum was absorbed completely, after which they were then inverted and incubated at 37°C for 24 hours to obtain the growth of the test organism. Incubation under increased $CO_2$ atmosphere was avoided because of the resulting increase in surface pH, which might adversely affect some antimicrobial agents. The petri dishes were then examined for the presence or absence of growth. The lowest concentration of each antimicrobial that inhibited growth was considered the MIC (single colony or haze growth was ignored) (American Public Health Association 1987).

## RESULTS AND DISCUSSION

As the biological activity of erythromycin, as a result of interaction with minerals and trace elements is questionable,

a part of present studies deal with assessing and comparing the biological activity of these drug:metal complexes with their parent antibiotics.

Selection of an antibiotic for therapy of bacterial infection often depends on knowledge of the susceptibility of the infecting organism. (Gennaro 1985) Usually, it is possible to determine susceptibility by *in vitro* tests. When they are properly standardized, the result obtained correlate well with the response to therapy observed in clinical practice (Weissbach 1977). Some *Staphylococci* are sensitive to erythromycin, the range of MIC is very high for *Staphylococcus epidermidis*, 8 to > 32 μg/ml, and for *Staphylococcus aureus*, 0.12 to > 128 μg/ml.

Gram-positive bacteria accumulate about 100 times more erythromycin than do Gram-negative microorganisms. Cells are considerably more permeable to the non- ionized form of the drug, and this fact probably explains the increased antimicrobial activity that is observed at alkaline pH (Sabath *et al*., 1968).

During present *in vitro* studies of erythromycin reference standard (table 2), verified that it is active against both Gram-positive and Gram-negative strains of organisms. *Enterococcus faecalis* and *Staphylococcus epidermidis* were susceptible at 0.5 μg/ml concentration, while *Staphylococcus aureus* was at 1 μg/ml concentration, *Salmonella typhi* and *Escherichia coli* were susceptible at 8 and 32 μg/ml concentration respectively, whereas *Proteus vulgaris, Shigella dysentery and Klebsiella pneumonia* were susceptible at higher concentrations of 128 μg/ml. The antibacterial susceptibility of all the erythromycin metal complexes were compared with the parent drug in fig. 1.

I *Magnesium complex*
Antibacterial activity of erythromycin magnesium complex (table 2) reveal that *Enterococcus faecalis* and *Staphylococcus epidermidis* were susceptible at 0.5 μg/ml concentration. *Escherichia coli* and *Salmonella typhi* were susceptible at 1 μg/ml concentration and *Proteus vulgaris* at 4 μg/ml. On the other hand *Shigella dysentery* and *Klebsiella pneumonia* were susceptible at 8 μg/ml while *Staphylococcus aureus* was susceptible at 64 μg/ml concentration.

II *Calcium complex*
Calcium complex was susceptible to *Enterococcus faecalis* and *Staphylococcus epidermidis* at 0.5 μg/ml concentration whereas *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* were susceptible at 16 μg/ml concentration. *Shigella dysentery* and *Salmonella typhi* were susceptible at 2 μg/ml and *Klebsiella pneumonia* at concentration <128 μg/ml.

III *Chromium complex*
Chromium complex was susceptible against *Enterococcus faecalis, Salmonella typhi, Proteus vulgaris* and *Shigella dysentery* 0.5-2 μg/ml whereas *Escherichia coli* and *Klebsiella pneumonia* were susceptible at 8 μg/ml concentrations. *Staphylococcus aureus* and *Staphylococcus epidermidis* were susceptible at higher concentration of 128 μg/ml concentration.

IV *Manganese complex*
Erythromycin manganese complex was susceptible against *Enterococcus faecalis, Proteus vulgaris, Staphylococcus aureus, Escherichia coli* and *Shigella dysentery* in the range 0.5 to 1 μg/ml whereas *Kelebcilla pneumonia* and *Staphylococcus epidermidis* were susceptible at 128 μg/ml concentrations.

V *Iron complex*
Iron complex was susceptible against *Proteus vulgaris, Enterococcus faecalis, Salmonella typhi* and *Staphylococcus aureus* was at 0.5-2 μg/ml concentrations. *Escherichia coli Klebsiella pneumonia* and *Staphylococcus epidermidis* were susceptible at higher concentration of 128 μg/ml.

VI *Cobalt complex*
Erythromycin cobalt complex had shown susceptible against *Enterococcus faecalis, Salmonella typhi, Proteus vulgaris, Shigella dysentery* and *Escherichia coli* whereas *Staphylococcus aureus, Klebsiella pneumonia* and *Staphylococcus epidermidis* were resistant even at <128 μg/ml concentrations.

VII *Nickel complex*
Erythromycin nickel complex was susceptible at low concentration of 0.5 μg/ml against *Enterococcus faecalis, Salmonella typhi*, *Proteus vulgaris* and *Shigella dysentery* and at moderate concentration of 16 μg/ml concentration against *Escherichia coli*. *Staphylococcus aureus, Klesiella pneumonia* and *Staphylococcus epidermidis* were susceptible at higher than128 μg/ml.

VIII *Copper complex*
Erythromycin copper complex was susceptible against *Enterococcus faecalis, Salmonella typhi, Proteus vulgaris, Shigella dysentery* and *Escherichia coli* at 0.5-1 μg/ml concentrations. *Staphylococcus aureus, Klebsiella pneumonia* and *Staphylococcus epidermidis* were susceptible at higher than 128 μg/ml concentrations.

IX *Zinc complex*
Zinc complex was found susceptible at low concentrations against *Enterococcus faecalis, Salmonella typhi, Proteus vulgaris* and *Shigella dysentery* at 0.5-2 μg/ml, while *Escherichia coli* was at 32 μg/ml. *Staphylococcus aureus, Klebsiella pneumonia* and *Staphylococcus epidermidis* were susceptible at higher than 128 μg/ml.

X *Cadmium complex*

Antibacterial activity of erythromycin cadmium complex against *Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus vulgaris* showed the susceptibility in the range 0.5-2 µg/ml and against *Shigella dysentery*. *Staphylococcus aureus, Klebsiella pneumonia* and *Staphylococcus epidermidis* were susceptible at higher than 128 µg/ml concentrations.

Our investigations divulge that formation of erythromycin metal complexes results in synergistic effect i.e., antimicrobial activity of complexes of erythromycin increases with respect to parent erythromycin and MIC of drug metal complexes decreased.

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