

MACROLIDES RESISTANCE IN GRAM POSITIVE AND GRAM NEGATIVE CLINICAL ISOLATES IN KARACHI

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ABSTRACT

Develop' lent of surveillance programs at national level is one of the most effective ways to control antibiotic resistance. To accomplish this task seventy clinical isolates of each of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species* and *Pseudomonas aeruginosa* were collected from different hospitals and pathological laboratories in Karachi. An in vitro study of these isolates was carried out by Agar dilution method using different group of Macrolides.

No satisfactory results observed in Macrolide group. This group was moderately active against *Staph. aureus*. Gram negative isolates showed high level of resistance against this group.

INTRODUCTION

Antimicrobial resistance is a serious and ever increasing problem At one end of the spectrum it affects the individual patient where resistance lead to failed therapy, and possibly increased hospitalization, morbidity or even death. At the other end of the spectrum there are adverse consequences internationally challenging the ability of the countries to control diseases of major public health interest such as tuberculosis and to contain the ever spiraling cost of antimicrobial therapy.

Widespread use of the older agents of Macrolides has led to the emergence of resistance notably in *Staph. aureus* and later in group A *Streptococci*. Resistance to Erythromycin A is of both chromosomal and plasmid origin, and can be inducible or constitutive. Intrinsic resistance of Gram negative bacilli to macrolides is probably due to the relative impermeability of the cellular outer membrane to the hydrophobic compounds. However, in Azithromycin, the nitrogen inserted into the lactone ring contributes to improved activity against Gram negative bacteria in particular *Haemophilus*

species. (Bryskier and Butzler 1997). Resistance to macrolides results from at least three types of plasmid mediated alterations: a decrease in the permeation of the drug through the cell envelope, as occur with *Staph. epidermidis* (Lampson et al., 1986); inducible or constitutive production of a methylase enzyme that methylate specific adenine residues in 23S rRNA and modifies the ribosomal target, resulting in reduced ribosomal binding of macrolides and lincosamides (Lecberq et al 1988; Weisblum, 1995); and hydrolysis of macrolides, by esterases produced by *Enterobacteriaceae* (Bathelemy et al., 1984). Bactrial resistance to macrolides, lincosamide, and Streptomycin antibiotics by target modification also reported to occur. Chromosomal mutations that alter a 50S ribosomal protein also may confir resistance (Kapusni k-Uneret et al., 1995).

EXPERIMENTAL

Agar Dilution Susceptibility Test:

To determine the MIC for one or more bacterial isolates, the study drug may be incorporated into a liquefied agar medium (45-50°C), which is then mixed, poured into

petri dishes and allowed to solidify (Barry, 1976; Snyder *et al.*, 1976). A series of petri plates are prepared with increasing concentration of the drug and with the aid of a multiple inoculum replicator (Steers *et al.*, 1959) as many as 11 different strains can be spot inoculated on to each plate. After overnight incubation, the MIC end point is read as the lowest concentration that completely inhibits growth, disregarding a single colony or faint haze or growth (Barry, 1976; Ericson, 1971; Washington, 1985).

Preparation of Antimicrobial Plates:

- Dilutions of antimicrobial agents are prepared in sterile double distilled water or other appropriate diluents at a concentration 10 times that desired in the final test (Barry, 1976; Washington, 1985).
- The Agar medium is then prepared in flask or tubes and allowed to cool in a 50°C water bath.
- Sufficient volumes are prepared to fill each 9 cm petri plates with 20 to 25 ml of Agar.
- The diluted antimicrobial solutions are added to the melted and cooled medium in a ratio of 1 part antimicrobial agent to 9 part medium (2 ml of drug to 18 ml of Agar for each petri plate).
- The medium is then mixed by gently inverting the tube or flask several times. The contents are then poured into the appropriate number of petri plates.
- The plates are then set aside on a flat horizontal surface and allow to harden undisturbed.
- For reference the Agar plates should be prepared on the same day that the tests are to be performed. However for most other purposes, the antimicrobial plates can be refrigerated in a sealed plastic bag for at least 1 week without a significant loss of antimicrobial activity (Ryan *et al.*, 1970).

Inoculation of Test Plates:

Apply an inoculum (1-2 ml) of each organism to the surface of each antimicrobial plates with the help of a replicating device containing 11 wire loops, one for the standard and 10 for the clinical isolates. The inoculum should be applied as a spot that covers a circle about 5-8 mm in a diameter and each spot should contain about 10^4 viable cells (Ericson, 1971; Barry, 1976; NCCLS, 1990).

Incubation of Test Plates:

The inoculated plates are allowed to stand undisturbed until the spot of inoculum have absorbed completely. The plates are then inverted and allowed to incubate at 37°C for 16 to 24 hours.

Examine plate for the presence or absence of growth. The lowest concentration of each antimicrobials that inhibit growth (ignore single colony or faint inoculum haze) is considered the MIC (Wentworth, 1987).

RESULT AND DISCUSSION

The increase in antibiotic resistance has led to predictions of doom in the international press and to depression in the medical community. It has focused to attention upon measure for fighting resistance, for most of which is susceptibility resistance.

Present research work consists of 3 antimicrobial agents of Macrolide group which are tested against 70 isolates of each pathogen i.e. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species* and *Pseudomonas aeruginosa*.

Macrolide group of antibiotics is generally active against Gram positive cocci and bacilli. These antibiotics are not active against most enteric Gram negative bacilli. However, it has modest activity in vitro against other Gram negative organisms (Kapusnik-uner *et al.*, 1997).

Erythromycin, Clarithromycin and Roxithromycin:

All these three macrolide antibiotics have satisfactory activity against *staphylococcus aureus*, including beta lactamase producing strains, but Methicillin resistant strains are usually resistant to these antibiotics (Alvis Kucers 1997). Drabu Y.J. et al in 1987 reported that MIC_{90s} of Erythromycin against *Staph. aureus* was 1.0 ug/ml and MIC_{90s} of Roxithromycin was ranged from 0.03 to greater than or equal to 16.0 ug/ml. Soussy C. J., et al in 1988 noted that Erythromycin sensitive *Staph. aureus* were inhibited by Roxithromycin at MIC of 0.06 — 4.0 ug/ml but Erythromycin resistant strains were resistant to Roxithromycin. Ritchie D.J. et al in 1993 reported that Methicillin susceptible *Staph. aureus* exhibited resistance to both Clarithromycin and Erythromycin. Chang et al in 1995 noted that resistance rates to macrolides were 80% or more in Methicillin resistant *Staph. aureus* and about 30% in

Methicillin sensitive *Staph. aureus*. The data of the present work shows that Erythromycin, Clarithromycin and Roxithromycin inhibit about 83%, 93% and 86% of the tested strains of *Staph. aureus* at MIC of 8.0 ug/ml (Table 1 and 2). The MIC_{50s} and MIC_{90s} for these antibiotics are 1.0 ug/ml and >8.0 ug/ml (Table 2). Ritchie D. J., et al in 1993 reported that *Klebsiella pneumoniae* and *E. coli* were resistant to Clarithromycin and Erythromycin. The present research work comply with the results reported by Ritchie et al and it is observed that *E. coli*, *Klebsiella spp.* and *Ps. aeruginosa* showed very high resistance against Erythromycin, Clarithromycin and Roxithromycin. The resistance rates are (73%, 83% and 71%) against *E. coli*; (86%, 83% and 86%) for *Klebsiella spp.* and (97%, 94% and 96%) against *Ps. aeruginosa* at MIC of 64.0 ug/ml (Table 1 and 2). The MIC_{50s} and MIC_{90s} of these three antibiotics for all the tested Gram negative organisms are >64.0 ug/ml (Table 2).

Table 1Population Distribution of MIC of Macrolides Antibiotics for 70 *S. aureus* Isolates

ERYTHROMYCIN								
Conc	0.25	0.5	1	2	4	8	>8	% RESISTANT
Sensitive strains	16	10	9	9	8	6	12	25.71
CLARITHROMYCIN								
Conc	0.25	0.5	1	2	4	8	>8	% RESISTANT
Sensitive strains	23	9	12	6	9	6	5	15.71
ROXITHROMYCIN								
Conc	0.25	0.5	1	2	4	8	>8	% RESISTANT
Sensitive strains	20	9	8	10	8	5	10	21.43

Population distribution of MIC of Macrolides antibiotics for 70 *E.coli* isolates

ERYTHROMYCIN								
Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	2	5	4	4	2	2	51	72.86
CLARITHROMYCIN								
Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	2	1	1	2	4	2	58	82.86
ROXITHROMYCIN								
Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	3	4	5	3	3	5	50	71.43

Population distribution of MIC of Macrolides antibiotics
for 70 *Klebsiella* isolates

ERYTHROMYCIN

Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	1	0	3	1	5	0	60	85.71

CLARITHROMYCIN

Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	2	1	1	2	4	2	58	82.86

ROXITHROMYCIN

Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	0	0	2	1	5	2	60	85.71

Population distribution of MIC of Macrolides antibiotics
for 70 *Ps. Aeruginosa* isolates

ERYTHROMYCIN

Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	0	0	0	2	0	0	68	97.14

CLARITHROMYCIN

Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	0	1	0	2	1	0	66	94.29

ROXITHROMYCIN

Conc	2	4	8	16	32	64	>64	% RESISTANT
Sensitive strains	0	0	0	1	2	0	67	90

Table-2

Minimum Inhibitory Concentration (MIC50 and MIC90) of Macrolide Antibiotics
against *S. aureus*, *E. coli*, *Klebsiella spp.* and *Ps. aeruginosa*

Name of antibiotics	<i>S. aureus</i>		<i>E. coli</i>		<i>Klebsiella spp.</i>		<i>Ps. aeruginosa</i>	
	MIC50 (ug/ml)	MIC90 (ug/ml)	MIC50 (ug/ml)	MIC90 (ug/ml)	MIC50 (ug/ml)	MIC90 (ug/ml)	MIC50 (ug/ml)	MIC90 (ug/ml)
Erythromyci	1	>8.0	>64.0	>64.0	64	64	64	64
Clarithromyci	1	>8.0	>64.0	>64.0	64	64	64	64
Roxthromycin	1	>8.0	>64.0	>64.0	64	64	64	64

CONCLUSION

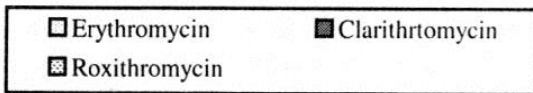
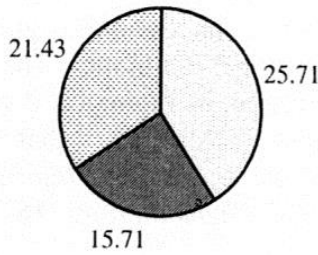
It is expected that the present work will be a mile stone in the antibacterial treatment and will bring new idea in the field of antimicrobial chemotherapy that will enable the Physicians, Doctors and Pharmacists for the treatment of problem bacterial infections and to select the most appropriate antibiotic for patient.

Further it is expected that the antibiotic guideline (WHO) must be followed by the healthcare persons.

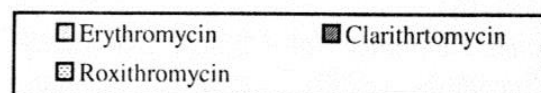
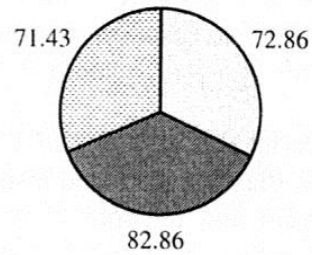
Finally it is suggested that suboptimal use of antibiotic and self-medication should be prohibited by the Health Ministry.

Graphical Representation of Different Macrolides against Gram Positive and Gram Negative isolates

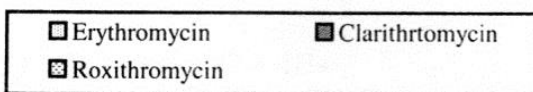
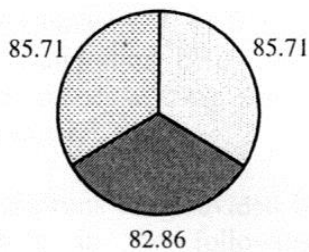
Percentage *Staphylococcus aureus* resistance against different macrolides



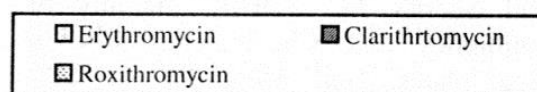
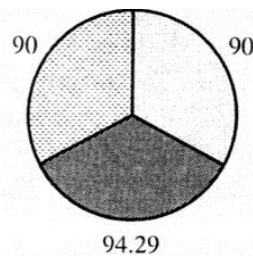
Percentage *E. con* resistance against different Macrolides



Percentage *Klebsiella* spp. resistance against different macrolides



Percentage *Pseudomonas aeruginosa* resistance against different Macrolides



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