



MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION OF AZITHROMYCIN TABLET IN BANGLADESH

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ABSTRACT

The azithromycin tablet in Bangladesh maintains standard MIC and MBC. But how much is this assumption is true; this will be evaluated through this research work. This is a cross sectional study to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of selected azithromycin tablet. The collected samples were analyzed according to USP specification. The MICs of azithromycin were determined by broth dilution method. MBCs were determined by the drop plate method from the tubes, where apparently no visible growth found. This study showed that MIC & MBC values of azithromycin tablet found highest against *Pseudomonas spp.*, *Shigella spp.* and *E. coli* were > 64.0 mg/ml (micro gram per milliliter) and lowest against *B. pumillus* was 1.0/2.0 mg/ml. MIC and MBC values higher than that of the peak serum concentration of azithromycin must have chance of therapeutic failure and development of azithromycin tolerance and resistance to the bacteria tested. To evaluate the efficiency of antibiotic there are two factors². Which influence potential utility of a antibiotics in a specific clinical situation. The first is the measure of potency of the antibiotic for the pathogen in question minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The second is relationship between the concentration time profile and potency of the antibiotics. This research work will play an important role to determine the MIC and MBC of selected azithromycin tablet in Bangladesh.

Keywords: Minimum Inhibitory, Bacteriocidal concentration of azithromycin tablet

INTRODUCTION

Azithromycin Tablets contain the active ingredient azithromycin, an azalide, a subclass of macrolide antibiotics, for oral administration. Azithromycin has the chemical name (2R, 3S, 4R, 5R, 8R, 10R, 11R, 12S, 13S, 14R) - 13 - [(2,6 - dideoxy - 3 - C - methyl - 3 - O - methyl - α - L - ribo - hexopyranosyl)oxy] - 2 - ethyl - 3,4,10 - trihydroxy - 3,5,6,8,10,12,14 - heptamethyl - 11[[3,4,6 - trideoxy - 3 - (dimethylamino) - β - D - xylo - hexopyranosyl]oxy] - 1 - oxa - 6 - azacyclopentadecan - 15 - one. Azithromycin is derived from erythromycin; however, it differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring. Azithromycin Tablets 250 mg and 500 mg are supplied for oral administration as mottled pink, unscored, film-coated, modified-oval-shaped tablets containing

azithromycin monohydrate equivalent to 250 mg or 500 mg azithromycin and the following inactive ingredients: butylated hydroxytoluene, calcium phosphate dibasic anhydrous, carmine, colloidal silicon dioxide, FD&C red # 40 lake, FD&C yellow # 6 lake, hypromellose (2910, 15cP), lactose monohydrate, magnesium stearate, pregelatinized starch, sodium lauryl sulfate, talc, titanium dioxide and triacetin. To reduce the development of drug-resistant bacteria and maintain the effectiveness of Azithromycin Tablets and other antibacterial drugs, Azithromycin Tablets should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

METHODS AND MATERIALS

Collection of sample: The azithromycin tablet collected from the retail seller and standard sample

collected from Orion Pharma Ltd, Bangladesh.

Collection of organisms: *Pseudomonas spp.*, *Staphylococcus aureus*, *Shigella spp.* and *E. coli* collected from the patient sample of ICDDR, Bangladesh.

Reagents: pH 6 sodium phosphate buffer, Hydrochloric acid, Trypcin, and Sterile water etc.

Media: Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), Nutrient Agar (NA), Mennitol Salt Agar (MSA), Cetrimite Agar (CA), Blood Agar (BA).

Instruments and apparatus: Sterile 5 ml screw cap test tubes, 250 ml conical flask, 250 ml measuring cylinder, Inoculating loop, 1 ml and 0.1 ml micro pipette, 10 ml glass pipette, Beaker, Marker, Bunsen burner, Small and large (7" x 7") petri plate, Borer, Voltex mixture machine, (FISONS-11777), Shaking or rotator machine (FISONS-200), Electrical digital balance (AJ 150 L), Spatula, pH meter (HANNA), Laminar air flow (C-901), Incubator adjuster at 370C. (SLI-600), Spectrophotometer (Spectronic-20), Freeze (MDF-U20806), Micropepatte (GILSON) and Autoclave (HA-240M).

Preparation of azithromycin solution: 128 mg equivalent $305 \times 128 \div 250 = 156.16$ mg azithromycin tablet was dissolve in 1000 ml pH 6 sodium phosphate buffer prepare 100 ml of 0.1M dibasic sodium phosphate adjust with hydrochloric acid to pH 6 and at 0.10 mg trypcin and mix. rotated at the rate of 100 rpm for 45 minute at room temperature.

MIC and MBC determination procedure:

Culture: Overnight Mueller Hinton broth cultures of *Staphylococcus aureus*, *E. coli*, *Bacillus pumillus*, *Shigella spp.* and *Pseudomonas spp.* at 370 °C were prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of MC, Farland 0.5 standard and then further diluted 1: 200 in Mueller Hinton broth. The inoculums thus prepared expected to obtain 105 to 106 C.F.U/ml.

Procedures: An appropriate amount (0.128 g antibiotic plus 1000 ml respective solvent) of azithromycin tablet was dissolved in respective solvent to prepare an antibiotic solution containing 128 mg/ml. Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and describe below: Ten sterile tubes were placed in a rack and were labeled each 1 through 8 and first one labeled as antibiotic control) and last one was labeled

as G.C (growth control). 1 ml of Mueller Hinton broth was added in each test tube. 1 ml of antibiotic solution was added to test tube no 1 and A.C. With a sterile micropipette and tips, after adequate mixture 1 ml was transferred from tube no. 1 to tube no. 2. After a through mixing, 1 ml was transferred with a separate micro pipette from tube no 2 to tube no 3.

This procedure was repeated through the next-to-next up to the tube no.8. Except tube no G.C (using fresh pipette for each dilution). From tube no 8 1ml was removed and discarded. The last tube (tube G.C) received no antimicrobial agent and was served as a growth control. First A.C labeled test tube was served as a antibiotic control. Each tube was inoculated (including the growth control except antibiotic control) with 1 ml of the culture of respective organism. The final concentration of antimicrobial agent in this test tube was half of the initial dilution series because of the addition of an equal concentration of inoculums in Mueller Hinton broth. The tubes were incubated at 37 ° C for 24 hours. The tubes were examined for growth and were determine the MIC of tested antibiotics, which is bacteriostatic for the test organism.

The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-). For determination of MBC, the concentration which was bactericidal was then found by sub cultured the content of selective tubes into a series of Muller Hinton broth, which did not contain any antibiotic and started from last two non-visible tubes to the 1st two visible tubes (direction tube no. 1 to tube no.8). Then was inoculated into Muller Hinton agar containing Petri plate by 0.1 sterile micropipette and separate 0.1 ml sterile tips in drop method. The plates were incubated at 370° C for 24 hours.

RESULT AND DISCUSSIONS

The aim of this research work was to evaluate the MIC and MBC of azithromycin tablet commercially available in Bangladesh. This study showed some important findings like higher MIC values of antibiotics tested.

The study informs the doctor's communities the important information about the MIC and MBC of condition of the antibiotics. Table-3 showed that MIC and MBC values of azithromycin tablet found highest against *pseudomonas spp.* And *E.coli* was > 64.0 mg/ml and lowest against *B.pumillius* was 1 mg/ml. According to Table-3 MIC values of antibiotics tested against *Staphylococcus aureus* were 0.5 mg/ml

to 8.0 mg/ml. The MIC range of tetracycline, ciprofloxacin and azithromycin for same organism

were 0.12 mg/ml to 32.0 mg/ml. significant variation of MIC values seen in this study.

Table 1: List of companies in Bangladesh are producing azithromycin tablet

Brand Name	Company Name
ASIZITH TABLET	ASIATIC
AVALON TABLET	TECHNO DRUGS
AZALID TABLET	ORION LABORATORIES
AZIMEX TABLET	DRUG INTERNATIONAL
AZIMON TABLET	MONICO PHARMA
AZIN TABLET	ACME
AZINIL TABLET	APEX PHARMA
AZIROX TABLET	NAVANA
AZITHROCIN TABLET	BEXIMCO PHARMA
AZITHROMAX TABLET	ZISKA PHARMA
AZOMAC TABLET	GENERAL PHARMA
AZYTH TABLET	SANDOZ NOVARTIS
CINALID TABLET	JAYSON PHARMA
MACZITH TABLET	BIO-PHARMA
ODAZYTH TABLET	ACI
TRIDOSIL TABLET	INCEPTA PHARMA
ZIMAX TABLET	SQUARE PHARMA
ZITHRIN TABLET	RENETA
ZITHROX TABLET	SK+F

Table 2: MIC & MBC determination

Tube No	A.C	1	2	3	4	5	6	7	8	G.C
1. Mueller Hinton broth 1 ml	1	1	1	1	1	1	1	1	1	1
2. Antibiotic solution 1 ml	1	1	1	1	1	1	1	1	1	0
3. Initial antibiotic concentration mg/ml	128	128	64	32	16	8	4	2	1	0
4. Bacterial suspension 1 ml	0	1	1	1	1	1	1	1	1	0
5. Final volume 2 ml	2	2	2	2	2	2	2	2	2	2
6. Final antibiotic concentration ug/ml	64	64	32	16	8	4	2	1	0.5	0

A.C=Antibiotic Control, G.C=Growth Control.

Table 3: MIC & MBC values of antibiotics tested against five organisms

Antibiotics tested	Test organism MIC/MBC in ug/ml				
	<i>Bacillus pumillus</i>	<i>Pseudomonas</i>	<i>Staphylococcus aureus</i>	<i>Shigella spp</i>	<i>E.coli spp</i>
Tetracycline	1.0/2.0	8.0/16.0	0.5/1.0	1.0/2.0	>64.0

Cephadrine	1.0/2.0	8.0/16.0	0.5/1.0	4.0/8.0	>64.0
Cefixime	1.0/2.0	>64.0	1.0/2.0	2.0/4.0	>64.0
Azithromycin	1.0/2.0	>64.0	2.0/4.0	>64.0	>64.0
Ciproflaxacin	4.0/8.0	2.0/4.0	8.0/16.0	1.0/2.0	>64.0

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