Abstract
Usually we consider that the azithromycin capsules in Bangladesh maintain 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 minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of selected azithromycin capsule. The collected samples were analyzed according to USP specifications. 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 capsule 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.

Introduction
To evaluate the efficiency of antibiotic there are two factors, 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 capsule in Bangladesh.

Materials and methods
Collection of sample: The azithromycin capsule collected from the retail seller and standard sample collected from a pharmaceutical company in the Dhaka city.

Collection of organisms: Pseudomonas spp., Staphylococcus aureus, Shigella spp. and E. coli collected from the patient sample of Dhaka Medical College Hospital and Bacillus pumillus from Microbiology department, University of Dhaka.

Reagents
1. pH 6 sodium phosphate buffer
2. Hydrochloric acid
3. Trypsin
4. Sterile water etc.

Media
1. Mueller Hinton Broth (MHB)
2. Mueller Hinton Agar (MHA)
3. Nutrient Agar (NA)
4. Mennitol Salt Agar (MSA)
5. Cetrimite Agar (CA)
6. Blood Agar (BA)

Instruments and apparatus
1. Sterile 5 ml screw cap test tubes
2. 250 ml conical flask
3. 250 ml measuring cylinder
4. Inoculating loop
5. 1 ml and 0.1 ml micro pipette
6. 10 ml glass pipette
7. Beaker
8. Marker
9. Bunsen burner
10. Small and large (7” x 7”) petri plate
11. Borer
12. Voltex mixture machine (FISONS-11777)
13. Shaking or rotator machine (FISONS-200)
14. Electrical digital balance (AJ 150 L)
15. Spatula
16. pH meter (HANNA)
17. Laminar air flow (C-901)
18. Incubator adjuster at 370C. (SLI-600)
19. Spectrophotometer (Spectronic-20)
20. Freeze (MDF-U20806)
21. Micropepatte (GILSON)
22. Autoclave (HA-240M)

Preparation of azithromycin solution\(^5\)
128 mg equivalent 305x128 = 250 = 156.16 mg azithromycin di-hydrate capsule 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.\(^1\)

MIC and MBC determination procedure
Culture: Overnight Mueller Hinton broth cultures of *Staphylococcus aureus*, *E. coli*, *Bacillus pumillus*, *Shigella spp.* and *Pseudomonus spp.* at 37\(^0\)C were prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of MC, Farland 0.5 standard\(^3\), and then further diluted 1: 200 in Mueller Hinton broth. The inoculums thus prepared expected to obtain 105 to 106 C.F.U/ml.

Procedure\(^4\)
1. An appropriate amount (0.128 g antibiotic plus 1000 ml respective solvent) of azithromycin capsule was dissolved in respective solvent to prepare an antibiotic solution containing 128 mg/ml.
2. Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and describe below:
   (a) 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).
   (b) 1 ml of Mueller Hinton broth was added in each test tube.
   (c) 1 ml of antibiotic solution was added to test tube no 1 and A.C.
   (d) With a sterile micropipette and tips, after adequate mixture 1 ml was transferred from tube no. 1 to tube no. 2.
   (e) After a through mixing, 1 ml was transferred with a separate micro pipette from tube no 2 to tube no 3.
   (f) 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 1 ml 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.
3. 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.
4. The tubes were incubated at 37\(^0\)C for 24 hours.
5. The tubes were examined for growth and were determined 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 (-).
6. For determination of MBC, the concentration which was bactericidal, was then found by sub cultured the contents of selective tubes into a series of Mueller Hinton broth, which did not contain any antibiotic and started from last two non-visible tube to the lst two visible tube (direction tube no. 1 to tube no. 8). Then was inoculated into Mueller Hinton agar containing Petri plate by 0.1 sterile micropipette and separate 0.1 ml sterile tips in drop method.
7. The plates were incubated at 370C for 24 hours.

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>A.C</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>G.C</th>
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</thead>
<tbody>
<tr>
<td>i) Mueller Hinton broth 1ml</td>
<td>1</td>
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<td>ii) Antibiotic solution 1ml</td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>iii) Initial antibiotic concentration mg/ml</td>
<td>128</td>
<td>128</td>
<td>64</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<tr>
<td>iv) Bacterial suspension 1ml</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
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<td>0</td>
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<tr>
<td>v) Final Volume 2 ml</td>
<td>2</td>
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<td>2</td>
<td>2</td>
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<td>2</td>
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<td>2</td>
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<tr>
<td>vi) Final antibiotic concentration µg/ml</td>
<td>64</td>
<td>64</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
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</table>

A.C = Antibiotic Control, G.C = Growth Control
The aim of this research work was to evaluate the MIC and MBC of azithromycin capsule commercially available in Bangladesh. This study showed some important findings like higher MIC values of antibiotics tested. The study informs the doctors communities the important information about the the MIC and MBC of condition of the antibiotics.

Table-1 showed that MIC and MBC values of azithromycin capsule found highest against *Pseudomonas* spp. and *E. coli* was > 64.0 mg/ml and lowest against *B. pumillus* was 1 mg/ml. According to Table-1 MIC values of antibiotics tested against *Staphylococcus aureus* were 0.5 mg/ml to 8.0 mg/ml. Nadia (2005)\(^5\) showed that 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.

**Limitation of the research works**
The MIC and MBC values of selected azithromycin capsule evaluated in this study. Because of shortage of fund and Minimum research work result available regarding the MIC & MBC values of azithromycin in Bangladesh. For this reason it was difficult to obtain required amount of information for conducting this study. Even with all limitations, the research work provides useful information about the MIC and MBC values of selected azithromycin in Bangladesh.

**Recommendation**
To provide standard potent antibiotics in Bangladesh, suggestion and recommendations are as follows:

1. A complete study on biopotency of all antibiotics are essential.
2. A modern antibiotics testing laboratory have to be established.
3. Regular monitoring of the quality of antibiotics are essential.
4. Awareness must be created by using mass media about use and misuse of Antibiotics.

**References**