Determination of MIC and MBC of selected tetracycline capsule commercially available in Bangladesh
Kowser MM, Hoque MM, Fatema N

Abstract
Background: Usually we consider that the tetracycline capsules in Bangladesh maintain standard MIC and MBC. But how much is this assumption is true? this will be evaluated through this research work. Objective: This is a cross sectional study (January-2006 to December-2006) to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tetracycline capsule commercially available in Bangladesh. Method: The collected samples were analyzed according to BP specifications. The MIC was determined by broth dilution method. MBC were determined by the drop plate method from the tubes, where apparently no visible growth found. Results: The MIC value of tetracycline capsule against Bacillus pumillus, Staphylococcus aureus, Shigella spp. and E coli were found 1.0, 8.0, 0.5, 1.0 and > 64.0 µg/ml (micro gram per milliliter) respectively. The MBC value of tetracycline capsule against Bacillus pumillus, Pseudomonas spp. Staphylococcus aureus, Shigella spp. and E coli were found 2.0, 16.0, 1.0, 2.0 and > 64.0 µg/ml respectively. Conclusion: MIC and MBC values higher than that of the peak serum concentration must have chance of therapeutic failure and development of antibiotic tolerance and resistance to the bacteria.

Key words
Minimal inhibitory concentration (MIC), Minimal bactericidal concentration (MBC), Colony forming unit (C.F.U).

Introduction
To evaluate the efficiency of antibiotic there are two factors,¹ which influence potential utility of a antibiotic in a specific clinical situation. The first is the measure of potency of the antibiotic for the pathogen in question MIC and MBC. The second is relationship between the concentration time profile and potency of the antibiotic. This research work will play an important role to determine the MIC and MBC of selected tetracycline capsule in Bangladesh.

Materials and methods
1. Collection of sample: The tetracycline capsule collected from the retail seller and standard sample collected from the pharmaceutical company in the Dhaka city. 2. 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 the Microbiology Department of University of Dhaka.

Reagents
1. Sterile water 2. Hydrochloric acid 3. Potassium phosphate buffer (0.5M)

Media

Instruments and apparatus

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9. Bunsen burner
10. Small and large (7"x 7") petriplate
11. Voltex mixture machine (FISONS-11777) 
12. Shaking or rotator machine (FISONS-200)
13. Electrical digital balance (AJ 150 L)
14. Spatula
15. pH meter (HANNA)
16. Laminar air flow (C-901)
17. Incubator adjuster at 37°C. (SLI-600)
18. Spectrophotometer (Spectronic-20)
19. Freeze (MDF-U20806)
20. Micropipette (GILSON)
21. Autoclave (HA-240M)

Preparation of tetracycline solution^2
128 mg equivalent 330x128÷ 250=168.96 mg tetracycline hydrochloride (G-tetracycline) capsule was dissolved in 1000 ml sterile water and rotated at the rate of 75 rpm for 60 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 37°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 10^5 to 10^6 C.F.U/ml.

Procedure^3
1. An appropriate amount of tetracycline capsule was dissolved in respective solvent to prepare an antibiotic solution containing 128 mg/ml (0.128 µg drug plus 1000 ml respective solvent).
2. Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and described below:
   (a) Ten sterile tubes were placed in a rack and were labeled each 1 through 8 and first one labeled as A.C (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 upto 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°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 1st 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 37°C for 24 hours.
Table 1: MIC & MBC values of antibiotics tested against five organisms

<table>
<thead>
<tr>
<th>Antibiotics tested</th>
<th>Test organisms MIC / MBC in mg/ml</th>
<th>Bacillus pumillus</th>
<th>Pseudomonas Spp.</th>
<th>Staphylococcus aureus</th>
<th>Shigella spp</th>
<th>E. coli spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>1.0 / 0.5</td>
<td>1.0 / 2.0</td>
<td>0.5 / 1.0</td>
<td>1.0 / 2.0</td>
<td>&gt; 64.0</td>
<td></td>
</tr>
<tr>
<td>Cephradine</td>
<td>1.0 / 2.0</td>
<td>1.0 / 2.0</td>
<td>1.0 / 2.0</td>
<td>0.5 / 1.0</td>
<td>&gt; 64.0</td>
<td></td>
</tr>
<tr>
<td>Cefixime</td>
<td>1.0 / 2.0</td>
<td>&gt; 64.0</td>
<td>1.0 / 2.0</td>
<td>2.0 / 4.0</td>
<td>&gt; 64.0</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1.0 / 2.0</td>
<td>&gt; 64.0</td>
<td>2.0 / 4.0</td>
<td>&gt; 64.0</td>
<td>&gt; 64.0</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4.0 / 8.0</td>
<td>2.0 / 4.0</td>
<td>8.0 / 16.0</td>
<td>1.0 / 2.0</td>
<td>&gt; 64.0</td>
<td></td>
</tr>
</tbody>
</table>

Photograph represents that the MIC of tetracycline against Bacillus pumillus and staphylococcus aureus was found 1 µg/ml & respectively.

Table 1 showed that MIC values of tetracycline against Bacillus pumillus, Pseudomonas spp, Staphylococcus aureus, Shigella spp and E coli were found 1.0, 8.0, 0.5, 1.0 and > 64.0 µg /ml, respectively, and the MBC values of tetracycline against Bacillus pumillus, Pseudomonas spp, Staphylococcus aureus, Shigella spp and E coli were found 2.0, 16.0, 1.0, 2.0 and > 64.0 µg /ml, respectively.

Discussion

Oral doses of 500 mg every 6 hours of tetracycline hydrochloride produce peak blood level of 4-6 mg /ml. Intravenous injection of tetracycline give somewhat higher levels only temporary. Table 1 showed that MIC values of tetracycline against Bacillus pumillus, Pseudomonas spp, Staphylococcus aureus, Shigella spp and E coli were found 1.0, 8.0, 0.5, 1.0 and > 64.0 µg /ml, respectively. The MIC level of tetracycline for Pseudomonas spp. (8.0 mg /ml) and E. coli. (>64.0 µg /ml) were higher than the peak blood serum level (4-6 mg /ml). So, Tetracycline should not be the choice of antibiotic for these Pseudomonas spp. and E. coli induced infection due to high MIC level. Table 1 showed MBC values of tetracycline against Bacillus pumillus, Pseudomonas spp, Staphylococcus aureus, Shigella spp and E coli were found 2.0, 16.0, 1.0, 2.0 and > 64.0 µg /ml, respectively. Rashed et al. reported that 80% Shegilla spp. were resistant to tetracycline. The present study showed that Shegilla spp. was found sensitive to tetracycline. Maximum medical representative try to motivate the doctors to prescribe new and costly antibiotics. Some doctors think that more expensive and newer antibiotics will be more effective to treat infection.

Conclusion

The MIC and MBC values of selected tetracycline evaluated in this study. Because of shortage of fund, the study was performed only one type of sample antibiotic. No significant research work is available regarding the MIC & MBC of tetracycline in Bangladesh. For this reason it was difficult to
obtain required amount of information for conducting this study.

**Recommendation**
To provide standard potent antibiotics in Bangladesh, suggestion and recommendations are as follows:
1. A complete study on bio potency 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.

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**References**