In vitro antimicrobial susceptibility test of two Doxycycline (DOXACIL®& MEGADOX®) brands of Bangladesh

A Thesis paper submitted to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy

Pharmacy Department

East West University

Submitted by

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CERTIFICATE

This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Aftabnagar, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Shuvo Banarjee (ID-2008-1-70-015).

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CERTIFICATE

This is to certify that the thesis in vitro antimicrobial susceptibitity test of Doxacil® and Megadox® of Bangladesh submitted to the Department of Pharmacy, East West University, Aftabnagar, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Shuvo Banarjee (ID-2008-1-70-015) under my guidance and supervision and that no part of the thesis has been submitted for any other degree. I further certify that all the sources of information, laboratory facilities availed of this connection is dully acknowledged.

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Abstract

Antibiotic resistance has become a major clinical and public health problem affecting both current and future generations. Doxycycline plays a vital role to protect the microorganisms. It acts against various types of bacteria. It's clinical usefulness has been declining because of the appearance of an increasing number of doxycycline-resistant isolates of clinically important bacteria. The major objective of this study was to find out quality and safety of different brands of doxycycline available in Bangladesh. Two different brands (Doxacil® and Megadox®) doxycycline were collected from the local market of Bangladesh. In five different concentrations antibiotic test according to the disc diffusion method was used to determine zone of inhibition and this test was performed with four different microorganisms (*Staphylococcus aureus, E.coli, Sreptococcus pneumonia, Haemophilus influenzae*) for the two brands. Results obtained after performing the test, from the comparison with standard it was concluded that, all the brands have similar antimicrobial susceptibility like the standard. The variation found in the sensitivity pattern to these commonly used drugs in present study could be attributed to the prevailing usage and abuse of the drugs in the area under study. All the brands having values within acceptance range. Under antimicrobial susceptibility test, the two brands of doxycycline capsule and reference standard gave similar results against four microorganisms.

CHAPTER 1: Introduction

1.1 Overview

Antibiotics are considered the most important invention in the history of modern medicine.The impact of the use ofantibiotics for reducing human morbidity, mortality and economic losses.Antibiotics are important drugs, perhaps the most important. Antibiotics are effective against bacterial infections, certain fungal infections and some kinds of parasites but don't work against viruses. There are different types of antibiotics or antimicrobial drugs in the world to treat diseases. Doxycycline plays a vital role to protect the microorganisms. It acts against various types of bacteria. They have the ability to inhibit the growth or kill invading microorganisms without harming the cells of the host(like human).It should be noted that in instances, the selective toxicity is relative rather than absolute which requires that the concentration of the drug be carefully controlled toattack the microorganism while still being tolerated by the host.Moreover, there are also risks of developing antibiotic resistance by the microorganisms which will ultimately makes the drugs useless or inactive to treat infections. Moreover, antibiotic therapy is not viable solution because of the rapid increase of antibiotic resistance, particularly in endemic areas (Chen J., et al, 2009).

Antibiotic resistance occurs due to drug adulteration and abuse of the related drugs. But antibiotics are frequently misused, overprescribed or incorrectly taken by patients and recklessly fed to farm animals. As a result, lifesaving antibacterial drugs lose effectiveness faster than new ones are developed to replace them. Resistance to antimicrobials as the result of unnecessary and inadequate use of antibiotics has become a global health problem. It is estimated that up to 50% of antimicrobials are used unnecessarily, and that they are the cause of approximately 25% of adverse drug reactions. Antibiotic resistance occurs when antibiotics no longer work against disease-causing bacteria. These infections are difficult to treat and can mean longer lasting illnesses, some resistant infections can even cause death. So, there is a need to ensure a controlled use of antibiotics, which is the key strategy against development of resistance to antimicrobials (Erdeljić V., et al, 2004).

1.2 Doxycycline

Doxycycline is a semi-synthetic structural isomer of the tetracycline family. It exhibits good intra-cellular penetration, with bacteriostatic activity on many bacteria.It is active against a wide range of gram-positive and gram-negative bacteria; used also as doxycycline calcium and doxycycline hyclate, administered orally or intravenously. Itis mainly used in the treatment of respiratory and urinary tract infections and some other disease; its efficacy may be affected by the crystal form. It is prescribed in the treatment of infections caused by susceptible bacterial strains. The advantages of doxycycline are as following:

- Excellent safety record
- Among currently available tetracycline's, doxycycline has the least affinity for calcium
- Long half-life permits once- or twice-daily dosing
- Blood and tissue levels are equivalent whether doxycycline is administered orally or intravenously
- Highly effective against all of the common pathogens that cause upper respiratory tract infections
- Quite active against Streptococcus pneumoniae, the most important respiratory tract pathogen in otitis, sinusitis, bronchitis, and community-acquired pneumonia.
- Active against penicillin-resistant pneumococci.
- Active against all common typical (Streptococcus pneumoniae, Haemophilusinfluenzae) and atypical (Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella species) pathogens that cause pneumonia.
- Anti-inflammatory effects (Legendre A.O., et al, 2012).

1.3 Chemistry

Doxycycline is a semi-synthetic structural isomer of the tetracycline family. Its chemical name is (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.At sub antimicrobial doses, doxycycline is an inhibitor of matrix metalloproteinase.It exhibits good intra-cellular penetration, with bacteriostatic activity on many bacteria. The basic structure of doxycycline contains a napthacene ring structure with a number of different groups attached at various sites. In doxycycline, replacement of the 6-hydroxyl group with hydrogen, allowing for better stability in both acidic and basic conditions, as well a better absorption profile and a longer half-life. The temperature dependence of the thermal degradation values was 28 ± 2 °C. The thermal degradation values of doxycycline were approximately 1.5 and 3 times higher than those of tetracycline respectively. Changes in the treatment medium pH (7.0–4.0) and water activity (0.99–0.93) scarcely varied the antibiotics' thermal stability. Only when doxycycline was heat-treated at pH 4.0 did its thermal resistance increase by 3 times (Showalter H., et al, 2012).

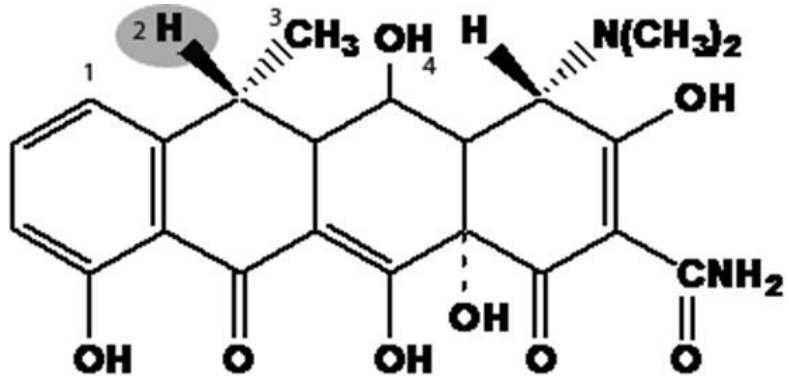

Fig1.1:Molecular structure of doxycycline

Formula: $C_{22}H_{24}N_2O_8 \bullet H_20$

1.4 Therapeutic uses

Doxycycline is bacteriostatic against a wide variety of organisms, both gram positive and negative bacteria. Doxycycline has been used to treat many infectious diseases but has been particularly useful in the treatment of chlamydial infections, community-acquired respiratory tract infections, and zoonotic infections, e.g., Lyme disease and ehrlichiosis. It is frequently used to treat vancomycin-resistant enterococci. Doxycycline has also been used extensively to treat patients with community-acquired pneumonia due to both typical and atypical pathogens .In fact, doxycycline remains highly active against S. pneumoniae and is even active against highly penicillin-resistant strains. Doxycycline's usefulness will continue as new pathogens emerge that are susceptible to this antibiotic (Cunha B.A., et al, 1999).

1.5 Spectrum of Activity

The tetracyclines are active against Gram-positive and Gram-negative bacteria. Doxycycline is typically active against *Bacillus anthracis, Listeria monocytogenes*, and *S. aureus,* although tetracyclines are not the drug of choice in the treatment of any type of staphylococcal infection. Thetetracyclines are unreliable against streptococcal infections, as resistance rates havebeen reported to be 50 percent. Use of any tetracycline for a streptococcal infection should be guided

by culture and sensitivity data. Doxycycline is the most widely used of the tetracycline today, although it is commonly applied to the treatment of acne vulgaris. Doxycycline is frequently used in a first-line therapy, such as in the treatment of uncomplicated genital Chlamydia

trachomatis infections or acute fever. In addition, doxycycline may be used after initial therapy has failed due to decreased antibiotic sensitivity or antibiotic resistance, as in the case of infections with penicillin-resistant Streptococcus pneumoniae.

The drug is active *in vitro* against-

- *Escherichia coli*
- *Chlamydia trachomatis*
- *Enterobacteraerogenes*
- *Staphylococcus aureus*
- *Lyme disease*
- *Rocky mountain spotted fever*
- *Folliculitis*
- *Acne*
- *Hidradenitissuppurativa*
- *Shigella*
- *Acinetobacter*
- *Haemophilusinfluenzae*
- *Streptococcus pneumoniae*(Saikal Z., et al, 2003).

1.6 Adverse Effects

Most common side effects are nausea, vomiting, abdominalpain, photosensitivity, and vaginitis. Women prone to candida vaginitis should consider carrying a self-treatment course of an anti-fungal. Severe adverse effects are uncommon and includeesophagitis and esophageal ulcerations. Doxycycline can accumulate in calcium rich tissues such as bones and teeth during their formation, and may cause yellow and brown discoloration.The mechanism for this effect is believed to be deposition of the complex formed from chelation of doxycycline to calcium. Staining of the teeth is permanent and appears to be the result of enamel hypoplasia and formation of enamel hypoplasia in developing teeth inchildren. Photosensitivity reactions may occur due to accumulation of doxycycline in the skin.Doxycycline should not used in patients who have renal disease, especially renal insufficiency or renal failure(Tan R.K., et al, 2011).

1.7 Mechanism of Action

Doxycycline is a semi-synthetic structural isomer of the tetracyclinefamily that exhibits a bacteriostatic activity on many bacteria.Doxycycline is generally bacteriostatic against a wide variety of organisms, both gram-positive and gram-negative. In gram-negative bacteria, transportation of the doxycycline into the cell occurs either by passive diffusion or through an energy-dependent active transport system. The latter system is also believed to exist in gram-positive bacteria. Doxycycline is more lipophilic than the other tetracycline's, which allows it to pass easily through the lipid bilayer of bacteria. The main mechanism of tetracycline action is knownto be the inhibition of protein synthesis through binding to thebacterial ribosomal 30S subunit. Doxycycline works by inhibiting protein synthesis by reversibly binding to bacterial ribosomes (30s subunit), preventing the attachment of amino acyl tRNA and leading to the termination of translation. It is more selective for the bacterial 70s ribosome versus mammalian 80s ribosome. Doxycycline also has selective toxicity against bacteria because it has high efficiently transport into bacterial cells. They enter Gram negative bacteria through porins due to its hydrophilicity, and through their lipophilicity in Gram positive bacteria. The bacteria mistakes doxycycline for food thus passes through the cytoplasmic membrane by an energy requiring active transport. Doxycycline can also alter the cytoplasmic membrane and this in turn causes leakage of nucleotides and other compounds out of the cell. This does not directly kill the bacteria but instead inhibit it (La V., et al, 2007).

1.8 Pharmacokinetics

1.8.1 Absorption and Bioavailability

Doxycycline is said to be almost completely absorbed with a bioavailability of more than 80% with an average of 95% and absorption is not significantly affected by the presence of food in the stomach or duodenum. After intravenous infusion of the same dose peak plasma concentrations are higher, but become very similar to those after oral administration following equilibration into the tissues. The absolute bioavailability (BA) of doxycycline administered orally at a dose of 100–200mg is 90–100%. The average percent fraction doxycycline absorbed in humans (Fa %) was reported to be 95%.Doxycycline is rapidly absorbed; it can be detected in the blood within 15 min of administration.The absorption primarily occurs in the duodenum.A secondary peak of doxycycline in plasma is normally observed due to enterohepaticcycling. Linear pharmacokinetics of orally administered doxycycline has been demonstrated in the dose range 100– 600 mg. A peak plasma doxycycline concentration of about 2.6 mg/ml is reached within approximately 2–3.5 h after a dose of 200mg. Different salt forms demonstrated no significant influences on doxycycline absorption. Comparing between intravenous (IV) and oral (po) doses of doxycycline felt absorption was lower and in the range 73–77%. Its biological half-life varies

from about 12 to 24 hours. In addition, food has less effect on absorption with doxycycline (E.Jantrati.,et al,2009).

1.8.2 Distribution

Doxycycline is more lipid-soluble. It is widely distributed in body tissues and fluids. The volume of distribution of doxycycline at steady state varies between approximately 53 L and 134 L and the volume of central compartment is 22 L.These values can vary slightly according to the doxycycline salt. In the elderly, the volume of distribution is higher than in young patients. Doxycycline distributes effectively into the body tissues especially liver, kidney and digestive tract. The plasma protein binding of doxycycline is between 80% and 95% at usual therapeutic concentration,the peak concentration (Cmax, mg/L) varies with dose being 15.3 mg/L, 4 h after a dose of 500 mg orally. Doxycycline–metal ion complexes are unstable at acid pH; therefore. Serum concentrations being reduced by 20% by test meals compared with 50% for tetracycline (E.Jantrati.,et al,2009).

1.8.3 Metabolismand Excretion

Doxycycline is eliminated unchanged by both the renal and biliary routes. Tissue penetration depends on single time point estimations; however, penetration into sputum is 8–28% estimated over 16 h. Penetration into saliva is poor while biliary concentration exceeds serum by many fold. Penetration as assessed by a 3 h post-dose concentration measurement after a single peri-operative dose of doxycycline in orthopedics indicated levels below that of serum in bone, skin, fat, tendons and muscle. Concentrations are highest in the liver, kidney and digestive tract that are the excretory organs. Doxycycline is slowly eliminated via the kidneys to an extent of approximately 30–40% in patients with normal renal function. Renal clearance is 1.8–2.1 L/h. The rest of the dose is eliminated through the digestive tract and excreted in the feces;about 35–60% is excreted in urine and the remainder in faeces.Additionally, a decrease in the area under the plasma concentration–time curve (AUC) of doxycycline was observed during concomitant treatment with drugs that induce hepatic enzymes, for instance, alcohol (chronic use), rifampicin, carbamazepine, phenobarbital, phenytoin, owing to the increased metabolism of doxycycline (Tan R.K., et al, 2006).

1.9 Drug Interactions

Doxycycline interacts with many types of medications. Decreased absorption of doxycycline can occur if taken concurrently with medications with divalent or trivalent cations such as antacids, laxatives and oral iron preparations. It can increase the absorption of digoxin, which may lead to digoxin toxicity. The gastrointestinal side effects (nausea, vomiting, stomach upset) of

theophylline may be increased by doxycycline. The dosage of oral anticoagulants (blood thinners, such as warfarin) may need to be adjusted when this medication is started. Doxycycline may decrease the effectiveness of oral contraceptives (birth control pills) and pregnancy could result. Barbiturates, carbamazepine, phenytoin, and antacids can lower the levels of doxycycline in the blood, thus decreasing its effectiveness. Iron and antacid containing aluminum, calcium and magnesium can chelate doxycycline in the gastrointestinal tract and form an insoluble complex, which can decrease its absorption and therefore, its effectiveness (Kenneth N.et al, 2011).

1.10 Contraindications

Doxycycline is contraindicated during

- Pregnancy
- In breast feeding women
- In children less than 8 years of age
- GI disease
- Hepatic disease
- Renal disease
- Hypersensitivity
- Candidal infections of the vagina
- Sunlight (UV) exposure(Kenneth N.et al, 2011)

1.11 Dosage and administration

Doxycycline is commonly administered in tablet, capsule or oral suspension. It is also available for intravenous injection.

Adults: The usual dose of oral Doxycycline is 200 mg on the first day of treatment (administered 100 mg every 12 hours or 50 mg every 6 hours) followed by a maintenance dose of 100 mg/day. The maintenance dose may be administered as a single dose or as 50 mg every 12 hours. In the management of more severe infections (particularly chronic infections of the urinary tract), 100 mg every 12 hours is recommended. In severe cases a dosage of 200 mg doxycycline is maintained throughout the course of treatment. In the case of syphilis a daily dose of 300 mg may be given for 15 days.

Children: An initial dose of approximately 4 mg/mg/kg/day may be given, whereby the effects of drug on teeth and bones should be consideration (E.Jantrati. et al, 2009).

1.12 Resistance

1.12.1 Mechanism of Doxycycline Resistance

Doxycycline has been a widely used antibiotic because of its low toxicity and broad spectrum of activity. However, its clinical usefulness has been declining because of the appearance of an increasing number of doxycycline-resistant isolates of clinically important bacteria. Two types of resistance mechanisms predominate: doxycycline efflux and ribosomal protection. A third mechanism of resistance, doxycycline modification, but its clinical relevance is still unclear. In efflux genes found in gram-negative enteric bacteria, regulation is via a repressor that interacts with doxycycline. Gram-positive efflux genes appear to be regulated by an attenuation mechanism. Recently it was reported that at least one of the ribosome protection genes is regulated by attenuation. Doxycycline resistance genes are often found on transmissible elements. Efflux resistance genes are generally found on plasmids, whereas genes involved in ribosome protection have been found on both plasmids and self-transmissible chromosomal elements (conjugative transposons). One class of conjugative transposon, originally found in streptococci, can transfer itself from streptococci to a variety of recipients, including other gram-positive bacteria, gram-negative bacteria, and mycoplasmas. Another class of conjugative transposons has been found in the Bacteroides group. An unusual feature of the Bacteroides elements is that their transfer is enhanced by preexposure to tetracycline. Thus, tetracycline has the double effect of selecting for recipients that acquire a resistance gene and stimulating transfer of the gene(Speer B., et al, 1992).

1.12.2 Factors influencing of resistance development against Doxycycline

- Due to drug adulteration.
- Drug not contains sufficient active ingredients.
- Drugs are easily copied by another company, where standard formulation are not maintained.
- Inadequate access to medical services and antimicrobial drugs. This remains a key problem in developing countries, and is also likely to be important amongst the poorer sections of society in developed countries.

- Unnecessary use of antimicrobial drugs for the wrong infections or when no infection is present;
- Not taking a full course of treatment,
- Sharing medication with other people,
- Keeping part of the course for another occasion.
- Where antibiotics are available without prescription, patients may elect to buy the cheapest antibiotic without regard to its effectiveness (Davey P., et al,2002).

Aim of the study

The major objective of this study was to find out the effectiveness or efficacy of different brands of Doxycycline available in Bangladesh.

Significance of the study

The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections.Uses of antibiotic sensitivity testing help to guide the physician in choosing antibiotics.It helps the local pattern of antibiotic prescribing.Antibiotic sensitivity testing will control the use of Antibiotics in clinical practice.Testing will help the clinicians in the choice of drugs for the treatment of particular infections. Quality control and assurance is essential to ensure the quality of antibiotic susceptibility test by diffusion methods.Most standardized methods include capsule of acceptable zone size range for control strains and in addition to checking that control size diameters are within the published range. If control test indicate unacceptable performance, the source of the error should be investigated and may include problems with media, antibacterial disc, inoculums and plate reading.Education is an important factor for the quality assurance process. Knowledge of atypical result for different organism may provide possible erroneous result and an understanding of limitation and sources of error in disc diffusion method contribution significant to the recognition, resolution and avoidance of errors (King A. et al, 2001).

*Staphylococcus aureus*is a persistent nosocomial bacteria causing wide spectrum of infections in human. Its resistance to various groups of antibiotics is a worldwide public health concern. In view of this, a prospective study was undertaken to detect the microbial spectrum, hemolytic assay and antibiotic susceptibility of nosocomial bacteria (Jayavanth P.D et. al. 2011).

Haemophilusinfluenzae is a major community-acquired pathogen causing significant morbidity and mortality worldwide. Antibiotic resistance in this organism is more diverse and widespread than is commonly appreciated. Susceptibility testing of *H influenzae* is essential to ensure quality of the drug (TristramS.,et al, 2007).

E. coli is a major cause of human morbidity and mortality around the world. Each year *E. coli* causes more than two million deaths due to infant diarrhoea and extraintestinalinfections and is also responsible for approximately 150 million cases of uncomplicated cystitis. Since humans and food animals carry so many *E. coli* cells that may establish commensal or antagonistic interactions with their hosts it is mandatory to define the genetic and population determinants that drive commensal strains to adopt a pathogenic behavior (Touchon M., et al, 2009).

Streptococcus pneumoniae, one of the most common gram-positive pathogens to colonize the human upper respiratory tract, is responsible for many severe infections, including meningitis and bacteremia. Streptococcus pneumoniae is an important cause of bacteremia, community-

acquired bacterial pneumonia, and meningitis, especially among young children and older adults (Tomita Y., et al, 2011).

CHAPTER 2:
Materials and Methods

2.Kirby-Bauer Disc diffusion susceptibility test procedure

2.1 Sample Preparation

1. A 500mg Doxycycline capsule was weighed and recorded in the Record Book.

2. Then took required amount of powder of the capsule.

3. Added water with doxycycline powder to make 10ml solution.

4. Mixed the solution by shaking carefully where Doxycycline readily soluble with the Methanol.

5. Filtered the solution by using filter paper then ready to antimicrobial test.

2.2 Apparatus and reagents

Table 2.1: Apparatus and reagents

1. Petri dish	10.100ml Volumetric Flask
2. Autoclave	11. Beaker
3. Laminar Air Flow	12. Distilled Water
4. Hot Air Oven	13. Forceps
5.1000ml Bottle	14. Bunsen Burner
6. Nutrient Agar (Media)	15. Inoculating Loop
7. Normal Saline (0.9% NaCl)	16.Electronoc Balance
8. Cotton Buds	17.Calipter or Ruler
9. Micropipette	18. Disc (Prepared by filter paper)

2.3 Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in hot air oven.

The loop is used for delivering the antibiotics is made.

2.4 Preparation of Agar plate

1. Agar plate was allowed to come to room temperature.

2. If the surface of the agar has visible liquid present, the plate should be inverted, agar on its lid would allow the excess liquid to drain from the agar surface and to evaporate. The plates were placed in a laminar air flow.

3. Each agar plate was appropriately labeled for each organism to be tested.

2.5 Placement of the antibiotic and blank disc

Before the placement the antibiotic disc (Doxycycline) a parameter marker was used to marks the bottoms of the test plates with sections according to the number of antibiotic. The sections were numbered the sequentially.

Antibiotic Doxycycline 200µg, 250µg, 300µg, 350µg and 400µg discs were manually placed on the agar plate.

(a) The Muller-Hinton agar plate was placed over the disc template.

(b)One disc was removed from the cartridge using forceps that had been sterilized.

(c)The plate was lifted and the disc was placed over one of the positioning marks.

The disc was pressed with the forceps to ensure complete contact with agar surface. The lid of the plate was replaced between discs to minimize exposure to air borne contaminants.

2.6 Incubation of the plates

1. A temperature range of 35°c ±2°c was maintained.

2. Temperature above 35°c might not allow the detection of growth and zone of inhibition.

3. The plates were not incubated carbon dioxide as this would decrease the pH of the agar and result in error due to incorrect pH of the media.

4. Results were read after 24 hours of incubation.

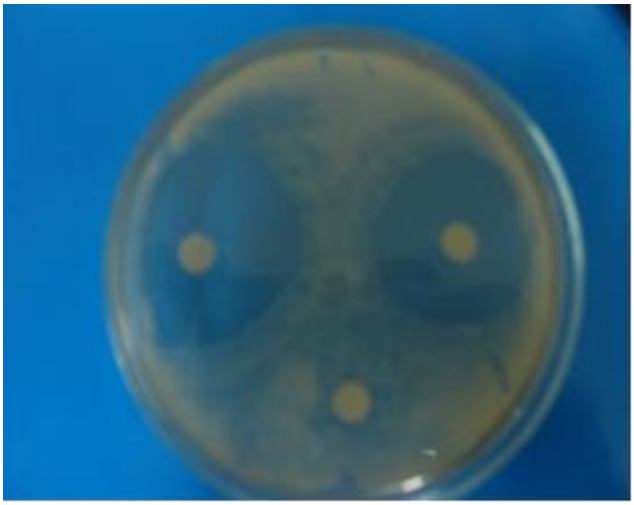

Fig.2.1 Zone of inhibition of sample and standard

2.7 Measuring zone sizes

1.Following incubation, the zone sizes were measured to the nearest millimeter using a ruler or calipter. The diameter of the disc was included in the measurement.

2. When measuring zone diameter, it was round up to the next millimeter.

3.The plate was held a few inches above a black, nonreflecting surface illuminated with reflected light.

4.The plate was viewed using a direct, vertical line of sight to avoid any parallax that might result in misreading.

5.The zone size was recorded on the recording sheet.

6.If the placement of the disc or the size of the zone did not allow to read the diameter of the zone, then it was measured from the center of the disc to a point on the circumference of the zone where a distinct edge was present(the radius) and the measurement was multiplied by 2 to determine the diameter.

2.8 Procedures

1. 800ml media (Agar) was prepared with Distilled Water. Where Nutrient Agar needed 22.4gm.

2. Media and other equipment's were sterilized in the Autoclave for 1 hour. Where temperature range is 60°C To 121°C and Pressure is 1 ATP.

3. Petri dish cleaned and kept in the Hot AirWoven to dry and sterilize.

4. Bacterial subculture was made before experiment at least 1 day.

5. There were four types of Bacteriasolution prepared with 1ml Normal Saline (0.9% NaCl) and microorganisms each.

6. Labeling was done before pouring the media into Petri dish.

7. Media was poured into Petridis equally and wait for being solid the media.

8. Spreaded the Bacterial solution on solid media with very careful.

9. Then placed the Antibiotic disc after drying the disc to each Petri dish very carefully.

10. The plates should be incubated soon after placing the disc.

11. The temperature range of 35°c ±2°c is normally required for incubation and the incubation time was 24 hours which were considered as standard for this test (Barth R., et al, 2009).

CHAPTER 3:
Results and Discussion

3. Antibiotic Sensitivity test Result

Antimicrobial sensitivity test was performed for two different antibiotics Doxacil® and Megadox® for standard Doxycycline solution using four types of microorganisms. The sensitivity was determined whether there is any difference in sensitivity between the referencesDoxycycline's

3.1 Comparison zone of inhibition of Doxycycline

*Table 3.1.1:*Result of Zone of inhibition of the microorganism *Escherichia coli*

Concentration	Standard(mm)	Doxacil®(mm)	Megadox® (mm)
300µg/Disk	15	15.66	19.33
350µg/Disk	18.66	19.33	20.33
400µg/Disk	24.33	19.50	20.50

From the table 3.1.1, it was observed that, the sample showed similar sensitivity like the standard against E.coli except Megadox® concentration of 300µg/Disk.

*Table 3.1.2:*Result of Zone of inhibition of the microorganism *Staphylococcus aureus*

Concentration	Standard(mm)	Doxacil® (mm)	Megadox® (mm)
300µg/Disk	17.33	14.33	18.83
350µg/Disk	18	16	19
400µg/Disk	18.66	19.16	19.66

From the table 3.1.2, it was observed that, the sample showed similar sensitivity like the standard against staphylococcus aureus.

*Table 3.1.3:*Result of Zone of inhibition of the microorganism *Sreptococcuspneumoniae*

Concentration	Standard(mm)	Doxacil® (mm)	Megadox® (mm)
300µg/Disk	25.33	26.33	26.33
350µg/Disk	27.66	26.66	26.83
400µg/Disk	29.66	28.16	28.83

From the table 3.1.3, it was observed that, the sample showed similar sensitivity like the standard against *Sreptococcuspneumonia.*

Table3.1 4: Result of Zone of inhibition of the microorganism *Haemophilusinfluenzae*

Concentration	Standard(mm)	Doxacil® (mm)	Megadox® (mm)
200µg/Disk	22.83	25.33	25.83
250µg/Disk	24.5	26.67	27
300µg/Disk	32.66	30.66	30.83

From the table 3.1.4, it was observed that, the sample showed similar sensitivity like the standard against *Haemophilusinfluenza.*

Discussion

Doxycycline is bacteriostatic against a wide variety of organisms, doxycycline resistance occurs due to drug adulteration and abuse of the related drugs. *E.coli* were subjected to antimicrobial sensitivity test againstdoxycycline (doxacil® and megadox®) and the observed sensitivity was recorded (Table 3.1). The variation found in the sensitivity pattern to these commonly used drugs in present study could be attributed to the prevailing usage and abuse of the drugs in the area under study. The lower sensitivity to the commonly used drugs indicates the dependence of the prescribers on these drugs, which is less commonly used. This further suggests the relation between antibiotic usage and the level of drug resistance encountered. The judicious use of antibiotic by the health professional and efforts to control procurement and use of antibiotics officially in the locality will probably help to limit the increasing rate of drug resistance in the pathogens. Rational drug policy should be in use before potent antibiotics are introduced to the country. Antibiotic administration should follow certain minimal requirements. In Bangladesh, empirical therapy is the rule rather than the exception and in this context of changing the dynamics of resistance to antibiotics, it is imperative for optimal patient care that constant evaluation of antibiotic sensitivity pattern of pathogens for commonly used antimicrobial agents in a particular environment is carried out. (Shahriar, et al, 2010)

*Staphylococcus aureus*was isolated from the collected samples. For hemolytic assay, *Staphylococcus aureus*culture was streaked on freshly prepared blood agar and incubated overnight. Antimicrobial susceptibility test was performed on *Staphylococcus aureus. Staphylococcus aureus*is responsible for wide range of infections in human namely, skin infections, septicemia, pneumonia, wound sepsis, osteomyelitis, post surgical infection and toxic shock syndrome. *Staphylococcus aureus*is normal micro flora that colonizes on skin and nose of healthy individual; it does not cause infection until favorable condition arises. *Staphylococcus aureus*initiatesinfection in human by production of toxin or through direct invasion and destruction of tissue. Due to the widespread use of antibiotics;*Staphylococcus aureus*has rapidly developed resistance to many antibiotics making treatment difficult (Jayavanth P.D et. al. 2011).

Reproducible susceptibility results for a wide range of agents against *H. influenzae* using a variety of media that support the growth of this fastidious species can therefore be obtained. When testing susceptibility of *H influenzae*, it is essential to ensure proper quality control of the freshness of the media in every run. *H. influenzae*, as is the case with most bacterial species, has a baseline, wild-type population with a defined, usually narrow, range of intrinsic activity of antimicrobial agents at the time of introduction of a new antimicrobial drug class. This defines the initial spectrum of activity of each antimicrobial agent when susceptibility breakpoints are established based on dosing regimens and sites of infection. Species can then be studied based on baseline activity and susceptibilities of strains with decreased activity, should they be present initially or should they develop. (TristramS.,et al,2007)

Doxycycline has a high degree of activity against many common respiratory pathogens and has been used in the outpatient management of lower respiratory tract infections, including pneumonia. Doxycycline is an effective and inexpensive therapy for the empirical treatment of hospitalized patients with mild to moderately severe community-acquired pneumonia.In patients hospitalized with the diagnosis of community-acquired pneumonia (CAP), antibiotics are usually chosen empirically to treat the most likely causative organisms until a microbiologic cause is established. However, a definitive cause is not usually established; and if there is a good clinical response, the course of therapy is completed with the initially chosen antibiotic. Combinations of antibiotics are often used and these regimens can be expensive. Considerations in choosing antibiotics should include the following: antibacterial spectrum, efficacy, adverse effects, ease of administration, and cost. (AilaniR.K.,et al.1999)

Conclusion

After performing all the quality control parameter tests for two different brands Doxacil® and Megadox® capsule manufactured by Square Pharmaceutical Co. Ltd and Beximco Pharmaceutical Co. Ltd, Some results showed significant difference with the reference standard for the antimicrobial sensitivity for the microorganisms. Bacteria have the ability to develop resistance following repeated or subclinical doses, so more advanced antibiotics and synthetic antibiotics are continually required to overcome them.

CHAPTER 4: Reference

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