Antibiotic sensitivity study of marketed preparations of Doxycycline HCl against different microorganisms

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In the name of ALLAH
The most Gracious And most Merciful

CERTIFICATE

This is to certify that, the thesis "Antibiotic sensitivity study of marketed preparations of Doxycycline HCL against different microorganisms" submitted to the Department of Pharmacy, East West University, Rampura, Dhaka for the partial fulfil of the requirements for the degree of Bachelor of Pharmacy was carried out by Zakia Sultana (ID: 2007-1-70-030).

……..
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Chairperson and Associate Professor
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CERTIFICATE

This is to certify that, the thesis "Antibiotic sensitivity study of marketed preparations of Doxycycline HCL against different microorganisms" submitted to the Department of Pharmacy, East West University, Rampura, Dhaka for the partial fulfil of the requirements for the degree of Bachelor of Pharmacy was carried out by Zakia Sultana (ID: 2007-1-70-030) 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 available of this connection are dully acknowledged.

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Abstract

Doxycycline is a semisynthetic broad-spectrum tetracycline antibiotic, active against a wide range of gram-positive and gram-negative organism. It is used to treat infection caused by susceptible bacteria. This study was employed to evaluate the microbial sensitivity pattern of two brands of doxycycline hydrochloride (Doxicap®, Impedox®) at different concentrations (200mg, 250mg, 300mg, 350mg and 400mg) against four microorganisms (*Staphylococcus aureas, Streptococcus pneumoniae, Escherichia coli, Haemophilus influenzae*). For *Staphylococcus aureas, Streptococcus pneumoniae, Escherichia coli* three concentrations were tested which were 300mg, 350mg and 400mg and for *Haemophilus influenzae* also three concentrations were tested which were 200mg, 250mg, 300mg. Kirby-Bauer disc diffusion method was used to investigate whether there is any significant difference with standard or not. Antimicrobial resistance is a very significant public health issue. The global presence of antimicrobial resistance is threatening the continue effectiveness of many current medications due to poor quality drug, counterfeit medications etc. The present study showed that, the amount of crude Doxycycline present in the two brands was effective in terms of purity, but whether the drug is therapeutically active or not can be determine by performing the quality control tests.

Key Words: Doxycycline hydrochloride, Tetracycline, Antimicrobial susceptibility testing, Kirby-Bauer method, Zone of inhibition, Antimicrobial resistance, counterfeit medication.

Chapter One:

Introduction

1.1 Overview

The vast majority of bacteria are harmless or beneficial; quite a few bacteria are pathogenic. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, meningitis, cellulites, skin infections etc, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*, and food borne illnesses, which can be caused by bacteria such as *Shigella, Campylobacter* and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis and leprosy. Antibiotics are substances produced by living organisms, such as *Penicillium* or *Bacillus that* kill or inhibit the growth of other organisms, primarily bacteria. Many antibiotics are chemically altered to reduce toxicity, increase solubility, or give them some other desirable characteristic that they lack in their natural form. Other substances have been developed from plants or dyes and are used like antibiotics. A better term for these substances is antimicrobials. Bacterial infections may be treated with antibiotics, which are classified as bacteriocidal if they kill bacteria, or bacteriostatic if they just prevent bacterial growth. There are many types of antibiotics and each class inhibits a process that is different in the pathogen from that found in the host. For example, the antibiotics doxycycline from the group of tetracycline inhibit the bacterial ribosome, but not the structurally-different eukaryotic ribosome, and so exhibit selective toxicity (Richard, 2007).

Doxycycline plays a vital role to protect from microorganisms. Because, this is an antimicrobial drug that acts against various types of bacteria. Doxycycline is a member of the tetracycline antibiotics group, and is commonly used to treat a variety of infections. Doxycycline is used to treat bacterial infections, including pneumonia and other respiratory tract infections, lyme disease, acne, infections of skin, genital, and urinary systems and anthrax (after inhalational exposure). It is also used to prevent malaria. It works by preventing the growth and spread of bacteria (Khachatourians, 1998). Doxycycline comes as a regular and a coated capsule, a tablet, a syrup, and a suspension (liquid), all to take by mouth. (Haque, 2005).

However, antibiotics are used both in treating human disease and animal disease. Uses may be contributing to the rapid development of antibiotic resistance in bacterial populations,

which is one of the major problem in the treatment of bacterial infections. Microbial Resistance to antibiotics is on the rise, in part because of inappropriate use of antibiotics in human medicine, poor quality medicine or substandard medicine. Antibiotic resistance may also occur due to over prescribing by doctors. So, people should be very careful in using antibiotics (Deshpande and Joshi, 2011).

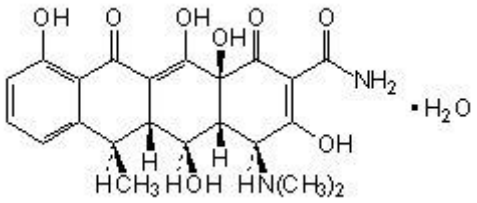
1.2 Doxycycline

Doxycycline belongs to the class of medicines known as tetracycline antibiotics. It works by killing bacteria or preventing their growth. It is a broad-spectrum antibiotic synthetically derived from oxytetracycline (Rang *et al,* 2003). The chemical designation of this light-yellow crystalline powder is alpha-6-deoxy-5-oxytetracycline. Doxycycline has a high degree of lipid solubility and a low affinity for calcium binding. Doxycycline works by interrupting the production of proteins by bacteria. It is effective against a wide variety of bacteria, such as *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, and many others. Doxycycline is used to treat bacterial infections in many different parts of the body. It is also used to treat pimples and abscesses (usually on the face) that are caused by rosacea, also known as acne rosacea or adult acne. Doxycycline delayed-release tablets are also used to prevent malaria and treat anthrax infection after possible exposure and other problems. However; this medicine will not work for colds, flu, or other virus infections (Schofield, 1974). It is available as doxycycline hydrochloride capsules for oral administration. Each capsule for oral administration contains 100 mg doxycycline hydrochloride, equivalent to 100 mg doxycycline (Korolkovas, 1988).

1.3 Chemistry

Doxycycline is a broad-spectrum antibiotic. The chemical designation of the light-yellow crystalline powder is 4 - (Dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - demonohydrate. Catalytic hydrogenolysis of oxytetracycline hydrochloride in water-dimethaylformamide (1:1) solution at pH 1.8 leads directly to doxycycline hydrochloride.

The structural formula is:



$C_{22}H_{24}N_2O_8 \bullet H_2O$, M.W. = 462.46

It is a yellow, crystalline powder, with a slight ethanolic odor and a bitter taste. It is soluble in water and in solutions of alkali hydroxides and carbonates, slightly soluble in alcohol and practically insoluble in chloroform and ether. Doxycycline has a high degree of lipid solubility and a low affinity for calcium binding. It is highly stable in normal human serum. Doxycycline will not degrade into an epianhydro form (Korolkovas, 1988).

1.4 Spectrum of activity

Doxycycline have a antimicrobial spectrum of activity against a wide range of gram-positive and gram-negative organisms.

1.4.1 Gram-Negative Bacteria

Neisseria gonorrhoeae

Calymmatobacterium granulomatis

Haemophilus ducreyi

Haemophilus influenzae

Yersinia pestis (formerly *Pasteurella pestis*)

Francisella tularensis (formerly *Pasteurella tularensis*)

Vibrio cholera (formerly *Vibrio comma*)

Bartonella bacilliformis

Brucella species

Escherichia coli

Klebsiella species

Enterobacter aerogenes

Shigella species

(Tripathi, 2008)

1.4.2 Gram-Positive Bacteria

Streptococcus pyogenes

Streptococcus pneumonia

Staphylococcus aureas

Enterococcus group (*Streptococcus faecalis* and *Streptococcus faecium*)

Alpha-hemolytic *streptococci* (*viridans* group)

(Tripathi, 2008)

1.4.3 Other Microorganisms:

Rickettsiae

Chlamydia psittaci

Chlamydia trachomatis

Mycoplasma pneumoniae

Ureaplasma urealyticum

Borrelia recurrentis

Treponema pallidum

Treponema pertenue

Clostridium species

Fusobacterium fusiforme

Actinomyces species

Bacillus anthracis

Propionibacterium acnes

Entamoeba species

Balantidium coli

Plasmodium falciparum

(Tripathi, 2008)

1.5 Therapeutic uses of doxycycline

Doxycycline is a prescription antibiotic licensed to treat a number of infections-

1.5.1 Rickettsial Infections

Doxycycline can be used to treat a number of different types of infections caused by *Rickettsiae*:

- Rocky Mounted spotted fevar
- Typhus including brills deseases
- Rickettsialpox
- Q. fevar

(Haque, 2005).

1.5.2 Acne vulgaris

For people with moderate to severe acne, doxycycline may be used. It is used to control acne by curbing the growth of bacteria and reducing inflammation (Tripathi, 2008).

1.5.3 Plague

Doxycycline is highly effective in both bubonic and pneumonic plague (Tripathi, 2008).

1.5.4 Chlamydial infection

Doxycycline can be used to treat a number of different types of Chlamydial infections:

- Lymphogranuloma venerum
- PID
- Psittacosis
- Inclusion conjunctivitis
- Trachoma
- Non specific urethritis

(Haque, 2005).

1.5.5 Gonnoria

Doxycycline is the drug of choice for the treatment of gonnoria caused by N. gonorrhoeae. It gives the most rapid, efficient, and predictable results of treating this disease (Schofield, 1974).

1.5.6 Mycoplasma infection

Doxycycline can be used to treat a Mycoplasma infections, such as Mycoplasma pneumonia (Haque,2005).

1.5.7 Cholera

Doxycycline have adjuvant value by reducing stool volume and limiting the duration of diarrhoea (Tripathi, 2008).

1.5.8 Borrelia infection

Doxycycline can be used to treat a Borrelia infections, such as lyme diseases and relapsing fever (Haque, 2005).

1.5.9 Doxycycline Uses in Children

Doxycycline should not be used in children under the age of 8. Permanent discoloration and altered development of teeth has been reported in children younger than 8 years old who have used doxycycline (Monson, 2009).

1.6 Dosage form

The recommended doxycycline dosage for most types of bacterial infections in adults is 100 mg at 12 hours intervals continued for at least 24 to 48 hours. For chronic or more serious infections, treatment can be carried out for a longer time (Korolkovas, 1988).

1.7 Mechanism of Action

Tetracycline antibiotics, including doxycycline, are bacteriostatic. They inhibit protein synthesis by reversibly binding to the 30S ribosomal subunit of susceptible organisms. As a

result, they prevent the binding of aminoacyl transfer RNA, thus inhibiting protein synthesis and bacterial cell growth (Tripathi, 2008).

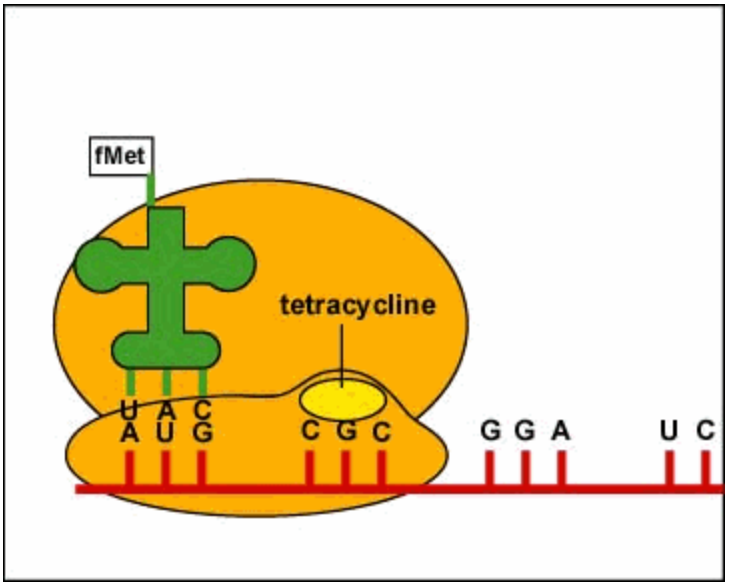


Figure 1.1: Inhibition of protein synthesis by Tetracycline

1.8 Pharmacokinetics

Doxycycline is second-generation tetracyclines. It is readily absorbed, distributed throughout the organism as a function of their lipophilicity and eliminated in both the urine and the faeces. Doxycycline is readily absorbed and is bound to plasma proteins in varying degree. They are concentrated by the liver in the bile, and excreted in the urine and feces at high concentrations and in a biologically active form. Doxycycline is virtually completely absorbed after oral administration (Saivin and Masterton, 1988).

1.8.1 Bioavailability

Doxycycline is almost completely absorbed from the gastrointestinal tract and reaches peak serum levels within 2 hours. When given by parenteral route, these levels are attains rapidly. Its half-life is approximately 18 hours (Korolkovas , 1988).

1.8.2 Biotransformation

It is 90% absorbed and 80 to 95% protein bound. It concentrate in the liver and is excreted primarily by external mechanisms, therefore, its half-life is approximately the same in normal patients ans in those with impaired renal function. The drug is eliminated in the feces, mainly as an inactive conjugate or perhaps as a chelate, which explains its relatively weak action on the intestinal microflora (Korolkovas, 1988).

1.9 Metabolism

Different people taking Doxycycline often have very different reactions. One person may tolerate Doxycycline well but not get the desired effect, another may have side effects or adverse reactions that make Doxycycline intolerable, and yet another may get the desired effect with no side effects. These variations in response and side effects are caused by many variables, but genetic variation and metabolism-based drug, herbal, and food interactions are among the most common. Doxycycline is metabolized by the body in much the same way as food, herbals, and environmental pollutants. It is broken down by liver and gut enzymes or other mechanisms so they can be absorbed and eliminated in the bile and urine (Cozza, 2003).

1.11 Adverse effects

The commonest adverse effect is gastrointestinal disturbance caused initially by direct irritation and later to modification of the gut flora. Phototoxicity has been seen which is dose related and produce vascular disturbance. High dose can decrease protein synthesis of host cell- an anti-anabolic effect, which could result in renal damage. Long term therapy can cause disturbances of bone marrow (Rang *et al*, 2003).

Renal toxicity is apparently dose related. Hypersensitivity reactions: urticaria, angioneurotic edema, anaphylaxis, anaphylactoid purpura, serum sickness, pericarditis, and exacerbation of systemic lupus erythematosus (Haque, 2005).

Increased sensitivity of the skin to UV light (photosensitivity), disturbances of the gut such as nausea, vomiting, diarrhoea, indigestion or abdominal pain, difficulty or pain when swallowing (dysphagia), loss of appetite, flushing, skin rashes (rare) can be seen sometimes. Mild increase in pressure within the skull (benign intracranial hypertension) which is rare. (Friedman, 2000).

1.12 Contraindication

Doxycycline is contraindicated during pregnancy, in breastfeeding women. It have been used at lower doses over many years for skin disorders. However, hepatic necrosis and serum sickness have been associated with prolonged use of a doxycycline (Rang *et al*, 2003). Although doxycycline and other antibiotics have been cited as a cause of oral contraceptive failure. Concurrent use of doxycycline with barbiturates, carbamazapine, or phenytoin may result in decreased doxycycline serum concentration due to induction of microsomal enzyme activity and resulting 50% reduction of the half-life of doxycycline (Marcia, 2003). When taken concomitantly with aluminium, calcium, iron and magnesium ions, or sodium bicarbonate, the gastrointestinal absorption of doxycycline is significantly decreases (Moffett *et al*, 1972). With coumarin type anticoagulents, it potentiates their effect. With drugs that increase its biotransformation, such as barbiturates, carbamazepine, or phenyltoin, its half-life may be decreased by almost 50%. With ferrous sulphate, there is interferences and even death, with oral contraceptives, it reduces their efficacy. With penicillin, potential antagonism or lack of enhanced therapeutic effect may occur (Korolkovas, 1988).

1.13 Antimicrobial resistance

Resistance is not a new phenomenon; it was recognized early as a scientific curiosity and then as a threat to effective treatment outcome. The global spread of antimicrobial resistance threatens the continued effectiveness of many medicines used today. Resistant and multidrug-resistant bacterial infections comprise a great problem. The increasing cost of health expenditures is a major worldwide problem. The overuse of antibiotics leads to the development of resistant bacteria, resulting in patients being exposed to such species during the course of their treatment (Mijovic *et al*, 2012).

1.13.1 Causes of antimicrobial resistance

One of the most important cause of antimicrobial resistance is the discontinuation of full course of antibiotic therapy and the lack of maintenance of schedule time for taking antibiotic. Resistance to first-line drugs in most of the pathogens ranged from minimal to almost complete resistance. Food-borne exposure is regarded as the most critical pathway for transfer of antimicrobial resistance from animals to humans. Resistant animal pathogens in some food products, especially meat, cause infections in humans that are difficult to treat. Initially, medical scientists overcame the problems of resistance by discovering and developing new classes of antimicrobial agents that were effective against resistant organisms. With the widespread use of antimicrobials, the prevalence of resistance to each new drug has increased (Deshpande and Joshi, 2011).

Many patients believe that new and expensive medications are more efficacious than older agents; this belief is shared by some prescribers and dispensers and often results in the unnecessary use of the newer agents. Self-medication with antimicrobials is often cited as a major factor contributing to drug resistance. Patients who fail to complete therapy have a higher likelihood of relapse, development of resistance and need for re-treatment; this applies especially to those patients requiring prolonged treatment. Transmission of antimicrobial-resistant strains from hospital personnel to patients or vice versa may also occur (Jarlier *et al*, 2012).

1.13.2 Mechanisms of antimicrobial resistance

Multiple drug resistance or Multidrug resistance is a condition enabling a disease-causing organism to resist distinct drugs or chemicals of a wide variety. Various microorganisms have survived for thousands of years by their being able to adapt to antimicrobial agents. They do so via spontaneous mutation or by DNA transfer. This process enables some bacteria to oppose the assault of certain antibiotics, rendering the antibiotics ineffective. A variety of transmissible genetic elements, that allow bacterial cells to survive and deal with antimicrobial agents are increasingly found together in multi-drug resistant organisms. Acquired resistance mechanisms involve mutations in genes targeted by the antibiotic and/ or

transfer of resistance determinants borne on plasmids, bacteriophages, transposons and other mobile genetic elements. The microorganisms employ several mechanisms in attaining multidrug resistance (Deshpande and Joshi, 2011).

1.13.3 Impact of antimicrobial resistance

Infections caused by resistant microbes often fail to respond to treatment, resulting in prolonged illness and greater risk of death. When treatment fails or response to treatment is slow, the patient remains infective for a longer time. This provides greater opportunities for the resistant strain to spread to other people. The enormous growth of global trade and travel results in greater spread of the resistant microbe in a short period of time. When infections become resistant to “first-line” drugs, treatment has to be switched to second- or third-line drugs, which are often more expensive drugs. Excess costs associated with resistant microorganisms may be due to: obligation to use more expensive antibiotics, longer hospital stay, higher mortality, delayed appropriate antibiotic therapy or a necessity to perform surgery. Infections caused by drug-resistant strains are associated with significant mortality and treatment is quite challenging (Arias, 2009).

1.13.4 Prevention and control of antimicrobial resistance

Antimicrobial resistance is a very significant public health issue. Although the prevalence of resistance to antimicrobials among various bacterial pathogens varies from place to place, it is truly a worldwide problem and deserves all of the attention that medical science can apply. In the future, preservation of existing drugs will be increasingly important. Optimal use of existing antimicrobial agents, using alternative treatment options (where possible), reducing the need for antimicrobials by increasing immunity, reducing the use of antimicrobials without providing an alternative form of treatment through education of health professionals and patients, antibiotic policies implementation of infection control measures (e.g., hand washing, screening and isolation) are the strategies aimed at prevention of emergence and spread of antibiotic resistance. Strategies such as early recognition of resistant microorganisms via more rapid diagnostic techniques, surveillance systems and screening of patients and staff, reduction of infectivity by use of antimicrobials and disinfectants will be

useful. Even improvement in the spacing of beds in hospitals is necessary. Many methods have been used to ensure adherence to antimicrobial therapy. These include the use of fixed dose combinations to minimize the number of tablets or capsules. It is also necessary not to use antibiotics for colds, coughs, bronchitis, sinus infections, and eye infections, which are mostly caused by viruses. Using the most cost-effective antibiotic with the least resistance-inducing capacity, combinations therapies and vaccination are of critical importance (Deshpande and Joshi, 2011).

Aim and Objective of the study

The aim and objective of this study was –

- To evaluate the microbial sensitivity pattern of two different brands of doxycycline by disc diffusion method at different concentrations (200µg, 250µg, 300µg, 350µg and 400µg).
- To investigate whether there is any significant difference between the standard and different brands of doxycycline.

Significance of the Study

An important task of the microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates with different antimicrobial agents. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. Manual methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods (Hudzicki, 2012). However, because of the widespread and sometimes inappropriate use of antibiotics, strains of bacteria have begun to emerge that are antibiotic-resistant. These new, stronger bacteria pose a significant threat to global world, general welfare and health and a challenge to researchers (Deshpande and Joshi, 2011). The spread of multiple antimicrobial-resistant pathogenic bacteria has been recognized by the World Organization for Animal Health, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem. The development of bacterial antimicrobial resistance is neither an unexpected nor a new phenomenon. It is, however, an increasingly troublesome situation due to the frequency with which new emerging resistance phenotypes are occurring among many bacterial pathogens and even commensally organisms (Lalitha, 2008).

One of the important cause of resistance is poor quality medicines. Poor quality medicine is medicine that does not meet official standards for strength, quality, purity, packaging, and/or labeling. They may be legally registered innovator or generic products, or they could be counterfeits, deliberately mislabeled for identity, strength, or source. Whether counterfeit or unintentionally substandard, poor quality drugs result in serious health implications including treatment failure, adverse effects, increased morbidity, mortality, development of drug resistance, and wasted resources. Recent reports indicate the availability of substandard and counterfeit drugs has reached a disturbing proportion in many low-income countries (Deshpande and Joshi, 2011). Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture (Todar, 2012).

Resistance has been observed to essentially all of the antimicrobial agents currently approved for use in human and veterinary clinical medicine. This, combined with the variety of

antimicrobial agents currently available, makes the selection of an appropriate agent an increasingly more challenging task. This situation has made clinicians more dependent on data from in vitro antimicrobial susceptibility testing, and highlights the importance of the diagnostic laboratory in clinical practice .There is a number of antimicrobial susceptibility testing (AST) methods available to determine bacterial susceptibility to antimicrobials. The selection of a method is based on many factors such as practicality, flexibility, automation, cost, reproducibility, accuracy, and individual preference (Lalitha, 2008).

Doxycycline is an antibiotic which inhibit the bacterial growth by stopping protein synthesis. They have been widely used for the past forty years as therapeutic agent in human and veterinary medicine but also as growth promotor in animal husbandry. Some of bacterial resistances to this antibiotic have nowadays limited their use. But in spite of this, doxycycline is effective against many causative microorganisms (Collard, 1999). Doxycycline is chosen for this study because it can treat a broad spectrum of bacterial infections that is already mentioned and it has a good safety profile that can treat the diseases in lower doses with a half-life of 18 hours.

Chapter two

Materials and Method

2.1 Apparatus and reagents

Table 2.1: List of Apparatus and reagents

Apparatus and reagents	
1. Petri dish	12. Distilled Water
2. Autoclave	13. Forceps
3. Laminar Air Flow	14. Bunsen Burner
4. Hot Air Oven	15. Inoculating Loop
5. Electronic Balance	16. Agar powder
6. Nutrient Agar(Media)	17. Ruler
7. Normal Saline(0.9% NaCl)	18. Disc (Prepared by filter paper)
8. Centrifuge Tube	19. Measuring cylinder
9. Cotton buds	20. Test tube
10. Incubator	21. 100ml Volumetric Flask
11.Micropipette	22. Beaker

2.2 Sample to be tested

Antimicrobial sensitivity pattern of doxycycline was performed for two different brands of antibiotics (Doxicap® and Impedox®) against four different types of microorganisms.

2.3 Preparation of standard

1. At first, 200mg, 250mg, 300mg, 350mg and 400mg powder of Doxycycline hydrochloride (given standard) was taken.

2. The measured amount of standard was dissolved in 10ml distilled water.

2.4 Preparation of sample

1. At first 3 capsules from given samples were weighed and recorded in the Record Book.

2. The amount of the Power of the capsules were calculated which was equivalent to 200mg, 250mg,300mg, 350mg and 400mg to the given standard.

3. The calculated amount of samples were dissolved in 10ml distilled water.

4. All the solutions were mixed by shaking carefully.

5. And finally filtered the solution by using filter paper.

2.5 Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter. At first small discs were prepared by using punching machine and sterilized in autoclave machine. Then 10µl sample was putted individually in these discs and air dried. The discs for standard and blank were prepared by following same procedure. Then these discs were placed in the agar plate by using a forcep.

2.6 Preparation of Agar plate

At first 28 gm agar powder was taken in 1000ml water which was mentioned in the label of agar bottle. It was autoclaved for 30-40 minutes. When the agar has been autoclaved, allowed it to cool until the bottle can be held with hands. Then the agar was carefully poured out into sterile petri dishes and labelled appropriately for each organism to be tested (Watase, 2012).

2.7 Organisms to be tested

Gram positive bacteria:

Staphylococcus aureus

Streptococcus pneumoniae

Gram negative bacteria:
Escherichia coli
Haemophilus influenzae

2.8 Procedure

2.8.1 Antimicrobial Susceptibility Test by Kirby-Bauer method

Firstly, obtained a plate culture of one of the organism which was to be tested. By using a sterile loop, emulsified a colony from the plate in the sterile saline solution and mix it thoroughly making sure that no solid material from the colony was visible. Then a sterile swab was dipped into the broth culture of the organism and the swab was used to streak a agar plate for a lawn of growth. This was accomplished by streaking the plate in one direction, then streaking at right angles to the first streaking, and finally streaking diagonally. The streaking was end by using the swab to streak the outside diameter of the agar. Then the plates were allowed to dry for about 5 minutes. Finally antibiotic disks were placed on the surface of the agar using sterilized forceps (Hudzicki, 2012).

The agar plates were then incubated in the incubator and a temperature range of 35°c ±2°c was maintained. Results were recorded in a record book after 18-24 hours of 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. When measuring zone diameter, it was round up to the next millimeter (Felmingham and Brown, 2001). Triplet test was done for each concentration of the each sample, and the average diameter was taken for recording the zone of inhibition.

Figure 2.1: Zone of Inhibition

Chapter Three:
Result

3.1 Antibiotic Sensitivity test Result

Blank was performed for each microorganism to ensure that whether the solvent has any effect on zone of inhibition or not. Blank test did not give any zone of inhibition for any microorganisms.

Table 3.1: Result of Zone of Inhibition for *Haemophilus influenza*

Concentration (µg/disc)	Standard (mm)	Average (mm)	Doxicap® (mm)	Average (mm)	Impedox® (mm)	Average (mm)
200	23	22.83	24	24.5	22.5	22.5
	23		24		23	
	22.5		25		22	
250	24	24.5	26	26.5	23	23.66
	24.5		26		24	
	25		27.5		24	
300	32	32.66	32	32.66	25	24.5
	32		33		24.5	
	33		32.5		24	

From the table 3.1, it was observed that, Doxicap® gave zone of inhibition almost close to the zone of inhibition of standard for *Haemophilus influenzae.* Impedox® also gave almost same zone of inhibition for this organism. But for the concentration 300µg/disc, Impedox® gave much less zone of inhibition then standard.

Table 3.2: Result of Zone of Inhibition for *Staphylococcus aureus*

Concentration (µg/disc)	Standard (mm)	Average (mm)	Doxicap® (mm)	Average (mm)	Impedox® (mm)	Average (mm)
300	17	17.33	18	17.33	19	18.66
	18		17		19	
	17		17		18	
350	17.5	18	18.5	17.5	19	19.33
	18		17		20	
	18.5		17		19	
400	19	18.66	18.5	19	19	19.5
	18		19		19.5	
	19		19.5		20	

From the table 3.2, it was seen that, Doxicap® and Impedox® gave zone of inhibition which is similar to the zone of inhibition of standard for *Staphylococcus aureus.*

Table 3.3: Result of Zone of Inhibition for *Escherichia coli*

Concentration (µg/disc)	Standard (mm)	Average (mm)	Doxicap® (mm)	Average (mm)	Impedox® (mm)	Average (mm)
300	14.5	15	17.5	17	17	16.33
	15		16.5		17	
	15.5		17		15	
350	19	18.66	18	17.66	19	18.66
	19		18		19	
	18		17		18	
400	24	24.33	19.5	19	19	19.66
	24		18.5		20	
	25		19		20	

From the table 3.3, Doxicap® and Impedox® showed almost similar sensitivity like the standard. But for the concentration 400µg/disc the zone of inhibition was deviated from the standard for *Escherichia coli.*

Table 3.4: Result of Zone of Inhibition for *Streptococcus pneumoniae*

Concentration (µg/disc)	Standard (mm)	Average (mm)	Doxicap® (mm)	Average (mm)	Impedox® (mm)	Average (mm)
300	25	25.33	22	22.33	18.5	18.5
	25		22		18	
	26		23		19	
350	28	27.66	26	25.66	21	20.66
	27		26		21	
	28		25		20	
400	30	29.66	27	26.5	22	22.16
	29		26		22	
	30		26.5		22.5	

From the table 3.4, it was find out that, Doxicap® and Impedox® gave zone of inhibition which is slighty deviated from the zone of inhibition of standard for *Streptococcus pneumoniae.* So it can be said that these two drugs are not so effective for inhibiting the growth of *Streptococcus pneumoniae.*

Chapter Four:
Discussion

4.1 Discussion

Antimicrobial resistance is one of the biggest challenges faced by public health at the beginning of the third millennium. The global presence of antimicrobial resistance is threatening to the continued effectiveness of many current medications. Many strains of bacteria has developed resistance to the antibiotics, which has reduced the effectiveness for treating some types of infection (Deshpande and Joshi, 2011).

In the past 60 years, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Wound infections, gonorrhea, tuberculosis, pneumonia, septicemia and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs. In 1998, in the United States, 80 million prescriptions of antibiotics for human use were filled. Nowadays, about 70 percent of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment (Todar, 2012).

One of the major cause of antimicrobial resistance is counterfeit medicines or substandard drug. Counterfeit drugs are deliberately and fraudulently mislabelled with respect to identity and/or source. Counterfeiting can apply to branded and generic products. Counterfeit drugs may include drugs with correct or incorrect ingredients, without active ingredients, with insufficient active ingredient, or with fake packaging. Substandard drugs are genuine drug products which do not meet the quality specifications set for them. Considering the vast scale of the global pharmaceutical industry and the incidence of potentially fatal diseases, any amount of poor-quality medicine is unacceptable because it increases drug resistance. The regular use of substandard or counterfeit medicines can lead to therapeutic failure or drug resistance (Newton *et al*, 2010).

Other factors for developing drug resistance are the unnecessary use of antibiotics. Self-medication with antimicrobials is often cited as a major factor contributing to drug resistance. The microorganisms employ several mechanisms in attaining multidrug resistance such as they lack of reliance on the glycoprotein cell wall; enzymatic deactivation of antibiotics, decreased cell wall permeability to antibiotics and altered target sites of antibiotic. Optimal

use of existing antimicrobial agents, using alternative treatment options, reducing the need for antimicrobials by increasing immunity, education of health professionals and patients, antibiotic policies and implementation of infection control measures are the strategies aimed at prevention of emergence and spread of antibiotic resistance. It is clear that bacteria will continue to develop resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information. Absence of appropriate legislation or its enforcement may result in the proliferation where untrained or poorly trained persons dispense antimicrobials, leading to overuse and inappropriate use. Using the appropriate drug at the appropriate dosage and for the appropriate duration is one important means of reducing the selective pressure that helps resistant organisms emerge (Deshpande and Joshi, 2011).

In this study, for *Staphylococcus aureas, Streptococcus pneumoniae, Escherichia coli,* three concentrations were tested which were 300mg, 350mg and 400mg. Because Doxicap® and Impedox® showed clear zone of inhibition at 300mg and higher concentrations. On the other hand *Haemophilus influenzae* showed clear zone of inhibition at 300mg and lower concentrations. So that 200mg, 250mg, 300mg were selected for *Haemophilus influenzae.* The data from this study revealed that all the different concentrations of both of the brands of doxycycline antibiotic exert effective actions against *Staphylococcus aureas, Escherichia coli, Haemophilus influenzae* and their inhibitory activity against these bacteria was almost similar with the standard. But for *Streptococcus pneumoniae,* both the drug was not effective in inhibiting the growth of this microorganism. In terms of sensitivity these drugs will not appropriate in treating infections caused by *Streptococcus pneumoniae.*

4.2 Conclusion

Developing countries are more susceptible to drug resistance than the developed countries due to lack of education, lack of monitoring of drug regulatory authorities, low standard of life, poor hygiene, irrational and substandard drug use. Thus we should be concerned about quality of drugs available in the market. The present study find out whether there is any significant difference between standard and two brands of doxycycline.. Because many strains of the gram-negative and gram-positive microorganisms have been shown to be resistant to doxycycline, susceptibility testing is recommended and other quality parameters should be check before the use of these medicines.

Chapter Five:
References

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