In Vitro Antibacterial Susceptibility Test of Tridosil® and Azimex- 500®

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

Submitted By

Masudur Rahman
ID- 2007-1-70-004

DEPARTMENT OF PHARMACY
EAST WEST UNIVERSITY

In the name of ALLAH
The most Gracious
The most Merciful

CERTIFICATE

This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Masudur Rahman ID-2007-1-70-004.

Sufia Islam

.....

Sufia Islam, Ph.D.
Chairperson and Associate Professor
Department of Pharmacy
East West University

CERTIFICATE

This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Masudur Rahman ID-2007-1-70-039 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 fully acknowledged.

.....

Nishat Nasrin
Supervisor
Senior Lecturer
Department of Pharmacy
East West University

Acknowledgement

First, all praise and glory are to Allah for all the bounties granted to me and only with His guidance and help this achievement has become possible.

I would like to express my deepest gratitude to my supervisor and respected teacher, Nishat Nasrin, Senior Lecturer, Department of Pharmacy, East West University, for her constant supervision, intense support, enthusiastic encouragements and constructive criticism during the thesis work. Her timely advice and encouragement have made it possible for me to accomplish the task as per schedule.

I put forward my most sincere regards and profound gratitude to Dr. Sufia Islam, Associate Professor & Chairperson, Department of Pharmacy, East West University.

I would like to convey deepest love and obedience to my caring parents and elder brothers for their support and guiding me all through my life until today, which keeps me strong and honest to do the things I needed to do.

Cordial thanks to my friends who gave me support for my research work and for their extended cooperation for my study and thanks to all my well-wishers for their whole hearted inspiration throughout the period of the research work.

I would also like to express my deepest gratitude to Eskayef Bangladesh Limited.

List of Contents

LIST OF TABLES

LIST OF FIGURES

Abstract

Azithromycin is a semi-synthetic macrolide antibiotic chemically related to erythromycin and clarithromycin. It is used to treat certain infections caused by bacteria, such as bronchitis, pneumonia, sexually transmitted diseases (STD) and infections of the ears, lungs, skin, and throat. Quality of drugs must be controlled to get desired therapeutic effect. In a pharmaceutical sense, quality means checking and directing the degree or grade of excellence of processes and products. Antimicrobial susceptibility test is one of the quality control tests to estimate the quality and purity of antibiotics. It is performed by using disk diffusion test. The objective of the thesis is to measure the sensitivity and purity of the antibiotic by comparing with the standard. It is significant to determine the quality and purity of antibiotics because of rapid occurrence of antibiotic resistance due to use of substandard or poor quality drugs. The test was performed using Tridosil and Azimex-500 in three different concentrations such as 50 µg, 75 µg and 100 µg. Three different species of bacteria such as *E. coli, Staphylococcus aureus, Salmonella typhi* were inoculated on nutrient agar plates and different concentrations of sample were applied by disc diffusion method and incubated overnight. Inhibitory activity of the antibiotic was measured as zone of inhibition in mm. The zone of inhibition of the sample was compared with the zone of inhibition of the standard. Results show that both Tridosil and Azimex-500 contain sufficient amount of active ingredient which is required to achieve desired therapeutic activity. It indicates that the samples of different brands are manufactured according to GMP guidelines and have the quality which meets the standard specifications.

Chapter- 1
Introduction

1.1 Azithromycin

Azithromycin is a macrolide antibiotic. Its chemical name is 9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin (Richard *et al*, 2009).

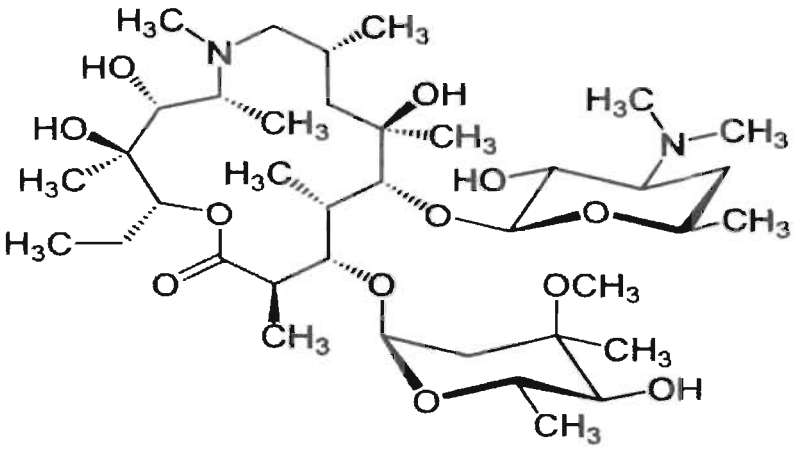

Figure1.1: Azithromycin

Azithromycin, a 15-atom lactone macrolide ring compound, is derived from erythromycin by addition of methylated nitrogen into the lactone ring. Its spectrum of activity and clinical uses are virtually identical to those of clarithromycin. Azithromycin is active against M avium complex and T gondii. Azithromycin is slightly less active than erythromycin and clarithromycin against staphylococci and streptococci and slightly more active against H influenzae. Azithromycin is highly active against Chlamydia (Tripathi *et al,* 2003).

1.2 Mechanism of Action

Macrolide antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to 50S ribosomal subunits of sensitive microorganisms at or very near the site that binds chloramphenicol. Azithromycin does not inhibit peptide bond formation *per se*, but rather inhibits the translocation step wherein a newly synthesized peptidyl tRNA molecule moves from the acceptor site on the ribosome to the peptidyl donor site (Goodman *et al,* 2008).

1.3 Azithromycin Resistance

Resistance to macrolides can result from:

(a) Drug efflux by an active pump mechanism

(b) Ribosomal protection by inducible or constitutive production of methylase enzymes that modify the ribosomal target and decrease drug binding

(c) Macrolide hydrolysis by esterases produced by Enterobacteriaceae

(d) Chromosomal mutations that alter a 50S ribosomal protein (found in B. subtilis, Campylobacter spp, mycobacteria, and gram-positive cocci) (Goodman *et al,* 2008).

1.4 Pharmacokinetics

1.4.1 Absorption

Azithromycin administered orally is absorbed rapidly and distributed widely throughout the body, except to the brain and CSF. Azithromycin should not be given with food. Azithromycin also can be given intravenously (Goodman *et al*, 2008).

1.4.2 Distribution

Azithromycin's unique pharmacokinetic properties include extensive tissue distribution and high drug concentrations within cells (including phagocytes), resulting in much greater concentrations of drugs in tissue or secretions compared to simultaneous serum concentrations. Protein binding is 50% at low plasma concentrations and less at higher concentrations (Goodman *et al,* 2008).

1.4.3 Elimination

Azithromycin undergoes some hepatic metabolism to inactive metabolites, but biliary excretion is the major route of elimination. Only 12% of drug is excreted unchanged in the urine. The t1/2, 40–68 hours, is prolonged because of extensive tissue sequestration and binding (Goodman *et al,* 2008).

1.5 Dosage

Azithromycin (tablet, oral suspension, and powder for intravenous injection) should be given 1 hour before or 2 hours after meals when administered orally. For outpatient therapy of

Community-acquired pneumonia, pharyngitis, or skin and skin-structure infections, a loading dose of 500 mg is given on the first day, followed by 250 mg/day for 4 additional days. Treatment or prophylaxis of M. avium intracellulare infection in AIDS patients requires 500 mg daily in combination with other agents for treatment, or 1200 mg once weekly for primary prevention. Azithromycin is useful in treatment of sexually transmitted diseases, especially during pregnancy when tetracyclines are contraindicated. Uncomplicated nongonococcal urethritis presumed to be due to C. trachomatis is treatedwith a single 1-g dose of azithromycin, which also is effective for chancroid. Azithromycin (1 g/weekfor 3 weeks) is an alternative drug for granuloma inguinale or lymphogranuloma venereum (Goodman *et al*, 2008).

In children, the recommended dose of azithromycin oral suspension for acute otitis media and pneumonia is 10 mg/kg on the first day (maximum 500 mg) and 5 mg/kg (maximum 250 mg/day) on days 2–5. The dose for tonsillitis or pharyngitis is 12 mg/kg/day, up to 500 mg total, for 5 days (Goodman *et al,* 2008).

1.6 Indications

1.6.1 Bronchitis

It is inflammation of the mucous membranes of the bronchi, the airways that carry airflow from the trachea into the lungs. Bronchitis can be divided into two categories, acute and chronic, each of which has unique etiologies, pathologies, and therapies (Cohen *et al*, 2004).

Acute bronchitis is characterized by the development of a cough, with or without the production of sputum, mucus that is expectorated (coughed up) from the respiratory tract. Acute bronchitis often occurs during the course of an acute viral illness such as the common cold or influenza. Viruses cause about 90% of cases of acute bronchitis, whereas bacteria account for fewer than 10% (Cohen *et al*, 2004).

Chronic bronchitis, a type of chronic obstructive pulmonary disease, is characterized by the presence of a productive cough that lasts for three months or more per year for at least two years. Chronic bronchitis most often develops due to recurrent injury to the airways caused by inhaled irritants. Cigarette smoking is the most common cause, followed by air pollution and occupational exposure to irritants (Cohen *et al*, 2004).

1.6.2 Pneumonia

Pneumonia is an inflammatory condition of the lung which especially affecting the microscopic air sacs (alveoli) associated with fever, chest symptoms and a lack of air space (consolidation) on a chest X-ray (McLuckie *et al*, 2009); (Leach *et al,* 2009).Typical symptoms include cough, chest pain, fever, and difficulty breathing (Ashby *et al,* 2007).

Pneumonia is a common illness affecting approximately 450 million people a year and occurring in all parts of the world (Ruuskanen *et al* 2011). It is a major cause of death among all age groups resulting in 4 million deaths (7% of the world's yearly total) (Lutfiyya *et al,* 2006). Rates are greatest in children less than five, and adults older than 75 years of age (Ruuskanen *et al,* 2001). It occurs about five times more frequently in the developing world versus the developed world (Ruuskanen *et al,* 2001).

1.6.3 Streptococcal pharyngitis

It is also called streptococcal tonsillitis, or streptococcal sore throat (known colloquially as strep throat) is a type of pharyngitis caused by a group A streptococcal infection. It affects the pharynx including the tonsils and possibly the larynx. Common symptoms include fever, sore throat, and enlarged lymph nodes. It is the cause of 37% of sore throats among children (Shaikh *et al,* 2010).

Pharyngitis, the broader category into which Streptococcal pharyngitis falls, is diagnosed in 11 million people annually in the United States (Choby *et al*, 2009). Although most cases are viral, group A beta-hemolytic streptococcus is the cause in 15–30% of the pharyngitis cases in children and 5–20% in adults. Cases usually occur in late winter and early spring (Choby *et al*, 2009).

1.6.4 Typhoid Fever

Typhoid fever, also known as Typhoid, is a common worldwide bacterial disease, transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella enterica*, *Salmonella typhi*. The bacteria then perforate through the intestinal wall and are phagocytosed by macrophages. The organism is a Gram-

negative short bacillus that is motile due to its peritrichous flagella. The bacterium grows best at 37°C / 98.6°F – human body temperature.

This fever received various names, such as gastric fever, abdominal typhus, infantile remittant fever, slow fever, nervous fever, pythogenic fever, etc. The name of "typhoid" comes from the neuropsychiatric symptoms common to typhoid and typhus (Giannella *et al*, 1996).

1.6.5 Gonorrhea

It is also known as the clap which is a common sexually transmitted infection caused by the bacterium *Neisseria gonorrhoeae*. The usual symptoms in men are burning with urination and penile discharge. Women, on the other hand, are asymptomatic half the time or have vaginal discharge and pelvic pain. If gonorrhea is left untreated, it may spread locally causing epididymitis or pelvic inflammatory disease or throughout the body, affecting joints and heart valves (Max *et al*, 1898).

Gonorrhea is a common infectious disease. According to a study there are 196 per 100,000 males 20 to 24 years old and 133 per 100,000 females 16 to 19 years old were diagnosed in 2005 (Moran *et al*, 2007). The CDC estimates that more than 700,000 people get new gonorrheal infections each year. Only about half of these infections are reported. In 2004, 330132 cases of gonorrhea were reported to the CDC. After the implementation of a national gonorrhea control program in the mid-1970s, the national gonorrhea rate declined from 1975 to 1997. After a small increase in 1998, the gonorrhea rate has decreased slightly since 1999. In 2004, the rate of reported gonorrheal infections was 113.5 per 100,000 persons. It is the second most common sexually transmitted disease after chlamydia (Max *et al*, 1898).

1.6.6 Chlamydia:

Chlamydia infection is a common sexually transmitted infection (STI) in humans caused by the bacterium *Chlamydia trachomatis*. The term Chlamydia infection can also refer to infection caused by any species belonging to the bacterial family *Chlamydiaceae*. *C. trachomatis* is found only in humans. Chlamydia is a major infectious cause of human genital and eye disease. Chlamydia infection is one of the most common sexually transmitted

infections worldwide; it is estimated that about 1 million individuals are infected with Chlamydia.

Chlamydia causes more than 250,000 cases of epididymitis each year. Women infected with chlamydia are up to five times more likely to become infected with HIV, if exposed (STD *et al* 2007).

1.7 Side Effects

Table 1.1: Side Effects of Azithromycin

Mild gastric upset	Abdominal pain
Headache	Dizziness
diarrhoea,	nausea,
shortness of breath,	rash or candida infection

(Centre for disease control *et al*, 2009)

1.8 Precautions

1.8.1 General

Because azithromycin is principally eliminated via the liver, caution should be exercised when azithromycin is administered to patients with impaired hepatic function.

Prolonged cardiac repolarization and QT interval, imparting a risk of developing cardiac arrhythmia and torsades de pointes, have been seen in treatment with other macrolides. A similar effect with azithromycin cannot be completely ruled out in patients at increased risk for prolonged cardiac repolarization. (Murray *et al*, 1997)

1.8.2 Pregnancy

Pregnancy Category B: No evidence of impaired fertility or harm to the fetus due to azithromycin was found. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human

response, azithromycin should be used during pregnancy only if clearly needed. (Shaikh *et al*, 2010)

1.8.3 Nursing Mothers

It is not known whether azithromycin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when azithromycin is administered to a nursing woman.

1.8.4 Geriatric Use

Pharmacokinetic parameters in older volunteers (65–85 years old) were similar to those in younger volunteers (18–40 years old) for the 5-day therapeutic regimen. Dosage adjustment does not appear to be necessary for older patients with normal renal and hepatic function (Murray *et al*, 1997)

1.9 Drug Interaction

Theophylline is strongly associated with erythromycin interaction; clarithromycin may also interact with this drug. Azithromycin however, do not appear to have any effect on theophylline pharmacokinetics. The other therapeutic agents considered are cyclosporin, the antiepileptics, carbamazepine and phenytoin, terfenadine, warfarin, oral contraceptives, agents used in the management of gastritis and peptic ulcer and zidovudine. With the exception of interaction with antacids, there is no evidence that azithromycin, unlike most other macrolides, interacts with any of these agents to produce clinically significant adverse effects. The explanation for this variation appears to be azithromycin's inability to induce and bind to the cytochrome P450 IIIA enzyme system (Foulds *et al*, 1991).

In a single-dose crossover study, ten volunteers took either azithromycin alone or immediately following a dose of an aluminium/magnesium combination antacid. Although the mean maximum serum concentrations of azithromycin were significantly reduced by concurrent antacid administration, the extent of total azithromycin absorption was unaffected. This finding probably has little clinical implication as azithromycin's activity is not directly dependent on serum concentration. In the same study, when oral cimetidine was given 2 hour

before azithromycin, there were no changes in the serum concentrations of the antibiotic (Foulds *et al*, 1991).

1.10 Drug Resistance

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive. Resistant organisms (they include bacteria, viruses and some parasites) are able to withstand attack by antimicrobial medicines, such as antibiotics, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist and may spread to others. AMR is a consequence of the use, particularly the misuse, of antimicrobial medicines and develops when a microorganism mutates or acquires a resistance gene (WHO). Major causes of antimicrobial resistance are-

- Inadequate national commitment to a comprehensive and coordinated response, ill-defined accountability and insufficient engagement of communities
- Weak or absent surveillance and monitoring systems
- Inadequate systems to ensure quality and uninterrupted supply of medicines
- Inappropriate and irrational use of medicines, including in animal husbandry
- Poor infection prevention and control practices

1.10 Mechanism of Antibiotic Resistance:

The many mechanisms that bacteria exhibit to protect themselves from antibiotics can be classified into four basic types. These are-

1.10.1 Drug Inactivation or Modification

The resistant bacteria retain the same sensitive target as antibiotic sensitive strains, but the antibiotic is prevented from reaching it. This happens, for example, with β lactamases—the β lactamase enzymatically cleaves the four membered β lactam ring, rendering the antibiotic inactive. Over 200 types of β lactamase have been described. Most β lactamases act to some degree against both penicillins and cephalosporins; others are more specific—namely, cephalosporinases (for example, AmpC enzyme found in *Enterobacter* spp) or penicillinases

(for example, *Staphylococcus aureus* penicillinase). β Lactamases are widespread among many bacterial species (both Gram positive and Gram negative) and exhibit varying degrees of inhibition by β lactamase inhibitors, such as clavulanic acid (Livermore *et al,* 1995).

1.10.2 Reduced Drug Accumulation

Some antibiotic resistant bacteria protect the target of antibiotic action by preventing the antibiotic from entering the cell or pumping it out faster than it can flow in (rather like a bilge pump in a boat). β Lactam antibiotics in Gram negative bacteria gain access to the cell that depends on the antibiotic, through a water filled hollow membrane protein known as a porin. In the case of imipenem resistant *Pseudomonas aeruginosa*, lack of the specific D2 porin confers resistance, as imipenem cannot penetrate the cell. This mechanism is also seen with low level resistance to fluoroquinolones and aminoglycosides. Increased efflux via an energy-requiring transport pump is a well recognised mechanism for resistance to tetracyclines and is encoded by a wide range of related genes that have become distributed in the enterobacteriaceae (Chopra *et al*, 1992).

1.10.3 Alteration of Primary Site

Alterations in the primary site of action may mean that the antibiotic penetrates the cell and reaches the target site but is unable to inhibit the activity of the target because of structural changes in the molecule. Enterococci are regarded as being inherently resistant to cephalosporins because the enzymes responsible for cell wall synthesis (production of the polymer peptidoglycan)—known as penicillin binding proteins—have a low affinity for them and therefore are not inhibited. Most strains of *Streptococcus pneumoniae* are highly susceptible to both penicillins and cephalosporins but can acquire DNA from other bacteria, which changes the enzyme so that they develop a low affinity for penicillins and hence become resistant to inhibition by penicillins. The altered enzyme still synthesises peptidoglycan but it now has a different structure. Mutants of *Streptococcus pyogenes* that are resistant to penicillin and express altered penicillin binding proteins can be selected in the laboratory, but they have not been seen in patients, possibly because the cell wall can no longer bind the anti-phagocytic M protein (Tomasz *et al*, 1995); (Garcia-Bustos *et al*, 1990).

1.10.4 Alteration of target site

The final mechanism by which bacteria may protect themselves from antibiotics is the production of an alternative target (usually an enzyme) that is resistant to inhibition by the antibiotic while continuing to produce the original sensitive target. This allows bacteria to survive in the face of selection: the alternative enzyme "bypasses" the effect of the antibiotic. The best known example of this mechanism is probably the alternative penicillin binding protein (PBP2a), which is produced in addition to the "normal" penicillin binding proteins by methicillin resistant *Staphylococcus aureus* (MRSA). The protein is encoded by the mecA gene, and because PBP2a is not inhibited by antibiotics such as flucloxacillin the cell continues to synthesise peptidoglycan and hence has a structurally sound cell wall. The appearance in 1987 of vancomycin resistant enterococci has aroused much interest because the genes involved can be transferred to *S aureus*, and this can thus theoretically result in a vancomycin resistant MRSA. The mechanism also represents a variant of the alternative target mechanism of resistance. In enterococci sensitive to vancomycin the normal target of vancomycin is a cell wall precursor that contains a pentapeptide that has a d-alanine-d-alanine terminus, to which the vancomycin binds, preventing further cell wall synthesis. If an enterococcus acquires the vanA gene cluster, however, it can now make an alternative cell wall precursor ending in d-alanine-d-lactate, to which vancomycin does not bind (Michel *et al,* 1997); (Leclercq *et al*, 1997).

In general development of drug resistance occurs gradually or in several discrete steps. When organisms or cells are exposed to suboptimal and thus sublethal levels of a drug they tend to respond to the stress situation by adaptation involving one or more of the above mentioned mechanisms. In addition to resistance to a single drug, multiple drug resistant is becoming wide spread. The exposure to sub-optimal drug levels through self medication in the management of fever in developing countries is probably one of the most important reasons of multiple drug resistant.

To reduce the risks of development of drug resistance, chemoprophylaxis and treatment using more than one different drug is absolutely essential. New drugs are not given anymore alone, but only in combination with another one.

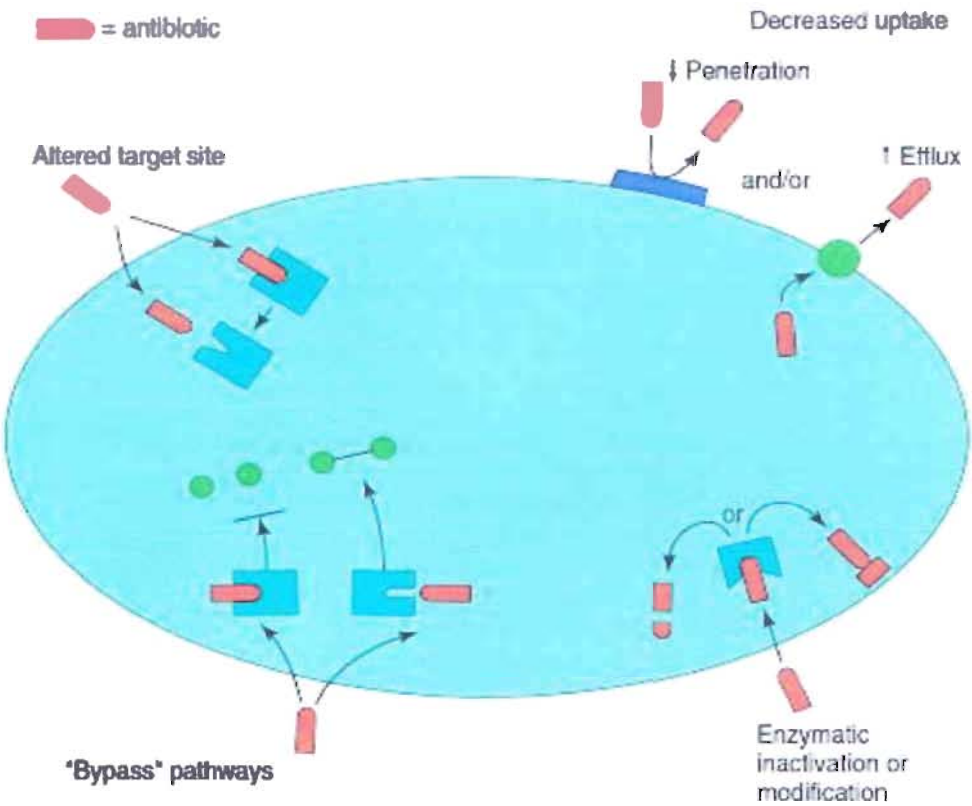

Figure 1.2 Major Antibacterial Resistance Mechanisms

1.12 Quality Assurance and Quality Control

ISO defines quality as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs."

In case of quality of pharmaceutical products, the suitability of drugs for their intended use is determined by:

1. Their efficiency weighed against safety, according to label claim, or as promoted or publicized

2. Their conformity to specifications regarding identity, purity and other characteristics.

The quality of a pharmaceutical product is ensured by quality assurance system of a pharmaceutical industry (WHO, 2011).

Quality assurance is defined as the overall program that ensures that the final results reported by the laboratory are correct.

The quality assurance of pharmaceutical products is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangements made to ensure that pharmaceutical products are of the quality required for their intended use.

The quality control is the core part of quality assurance. Quality control refers to the measures that must be included during each assay run to verify that the test is working properly. The aim of quality control is simply to ensure that the results generated by the test are correct. However, quality assurance is concerned with much more: that the right test is carried out on the right specimen, and that the right result and right interpretation is delivered to the right person at the right time.

Quality control of a pharmaceutical products is a concept that covers all measures taken, including the setting of specifications, sampling, testing and analytical clearance, to ensure that the raw materials, intermediates, packaging materials and finished pharmaceutical products conform with established specifications for identity, strength, purity and other characteristics.

Quality of antibiotics should maintain throughout the manufacturing process, from the bulk materials to packaging. Quality control of antibiotics is essential. Lack of quality control of antibiotics may lead to bacterial resistance to those antibiotics. Standard pharmacopoeias recommend various quality control tests for antibiotic tablets. The general quality control tests that are applied for all types of tablets to determine quality involve-

1.12.1 Tablet Hardness Test

The test measures crushing strength property defined as the compressional force applied diametrically to a tablet which just fractures it. Among a large number of measuring devices, the most favored ones are Monsanto tester, Pfizer tester, and Strong cobb hardness tester. All are manually used. So, strain rate depends on the operator. Heberlein Schleuniger, Erweka, Casburt hardness testers are motor driven.

1.12.2 Friability Test

The tablet may well be subjected to a tumbling motion. For example, Coating, packaging, transport, which are not severe enough to break the tablet, but may abrade the small particle from tablet surface. To examine this, tablets are subjected to a uniform tumbling motion for specified time and weight loss is measured. Roche Friabilator is most frequently used for this purpose.

1.12.3 Tablet Diameter

Tablet diameter is also an important test. Pfizer tester is used for checking the diameter of the tablets; screw gauge and caliper are also used. Tablet thickness can be measured by micrometer or by other device. Tablet thickness should be controlled within a ± 5% variation of standard value.

1.12.4 Tablet thickness

Tablet thickness is an important quality control test for tablet packaging. Very thick tablet affects packaging either in blister or plastic container. Tablet thickness is determined by the diameter of the tablet. Pfizer tester is used for checking tablet thickness.

1.12.5 Weight Variation

In this test tablets are weighed individually. The individual tablet weight is compared to the average. The tablet pass the U.S.P. test if no more that 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.

1.12.6 Content Uniformity Test

In content uniformity test tablets are assayed individually and 90 % of test batch must not contain less than 85% or not more than 115% of the labeled drug content.

1.12.7 Disintegration Test

The breakage of tablet into smaller fragments is called disintegration of tablet. To test for disintegration time the service temperature is 37 ± 20° C. Disintegration time for uncoated tablet is 5-30 minutes. For coated tablet, it is 1-2 hours.

1.12.8 Dissolution test

The release of drug from the tablet into solution per unit time under standardize condition is called dissolution test.

In dissolution test a single tablet is placed in a small wire mesh basket attached to the bottom of the shaft connected to a variable speed motor. The flask is cylindrical with a hemispherical bottom. The flask is maintained at 37±0.50C by a constant temperature bath. The motor is adjusted to turn at the specified speed and sample of the fluid are withdrawn at intervals to determine the amount of drug in solutions.

1.12.9 Antimicrobial Susceptibility Test

The potency (or activity) of antibiotics may be demonstrated by their inhibitory effect on microorganisms. Under current USP and EP standards, two methods are generally employed i.e. the "plate assay" or the "turbidimetric assay". The potency of the antibiotic is estimated by comparing the inhibition of growth obtained from known concentrations of the selected antibiotic against sensitive microorganism(s) to the inhibition of growth obtained from a reference standard. This test is generally performed on raw materials and finished product to ensure that the antibiotic potency specifications are met.

Purity of a drug means the actual amount of active ingredient present in the drug along with its other excipients. Purity level of antibiotics also can be determined by disk diffusion method. The zone produced by the antibiotic is compared with the standard. It helps to estimate the level of purity present in the antibiotic. If the zone produced by the antibiotic is similar to the zone produced by the standard then it indicates that the antibiotic contain active ingredient equal to the standard. Thus this experiment assures that that antibiotic is a quality product which is manufactured according to GMP guidelines and its quality meet USP/ BP specifications (James *et al*, 2011).

It is essential to determine the purity of antibiotics because various pathogenic bacteria become resistance to different class of antibiotics. The uses of antibacterial agents tend to increase every year which increase the risk of various resistance problems. Effectiveness of an antimicrobial agent depends on the concentration of the active ingredient. If concentration

of active ingredient varies from the required concentration then bacterial resistance, toxicity or other problems can arise. Thus purity of antibiotics must be determined. The purity of Azithromycin can be determined by disc diffusion method by following Standard Pharmacopeia's recommendation on antimicrobial susceptibility test (Donald *et al*, 2006).

The disk diffusion susceptibility method is simple and practical and has been well-standardized (Jorgensen *et al*, 2007).The test is performed by applying a bacterial inoculum of approximately 1–2×108CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface (Nijs *et al*, 2003).

Plates are incubated for 16–24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI *et al*, 2009) or those included in the US Food and Drug Administration (FDA)-approved product inserts for the disks. The results of the disk diffusion test are "qualitative," in that a category of susceptibility (ie, susceptible, intermediate, or resistant) is derived from the test rather than an MIC. However, some commercially-available zone reader systems claim to calculate an approximate MIC with some organisms and antibiotics by comparing zone sizes with standard curves of that species and drug stored in an algorithm (Korgenski *et al*, 1998); (Nijs *et al*, 2003).

The advantages of the disk method are the test simplicity that does not require any special equipment, the provision of categorical results easily interpreted by all clinicians, and flexibility in selection of disks for testing. It is the least costly of all susceptibility methods (CLSI *et al*, 2009).

Aim of the Study

The main goals of the study are-

- To measure the susceptibility of various micro-organism against azithromycin.
- To compare the purity of drugs available in the market with the standard.

Significance of the Study

An important task of the microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. 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. Each method has strengths and weaknesses, including organisms that may be accurately tested by the method. Some methods provide quantitative results (eg, minimum inhibitory concentration), and all provide qualitative assessments using the categories susceptible, intermediate, or resistant. In general, current testing methods provide accurate detection of common antimicrobial resistance mechanisms. However, newer or emerging mechanisms of resistance require constant vigilance regarding the ability of each test method to accurately detect resistance (James *et al*, 2011).

Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth. The bacteria targeted adapt by natural selection to become 'resistant' and continue to multiply despite the presence of the antibiotic. Controlling the deadliest infectious diseases in the world such as diarrheal diseases, respiratory tract infections, sexually transmitted infections. meningitis, pneumonia, and hospital acquired infections, is more difficult today because of the emergence of antimicrobial drug resistance. Resistance has emerged for most bacterial infections, which causes a significant proportion of the burden of disease in developing countries (Ramanan *et al*, 2006).

In 1990 it was estimated that 78% of world's population lived in developing countries and of 39.5 million deaths in the developing world, 9.2 million were estimated to have been caused by infectious and parasitic disease. Infections of the lower respiratory tract were the third most common cause of death worldwide (Murray *et al*, 1997). Ninety eight per cent of deaths in children occur in the developing world, mostly as a result of infections (C A Hart *et al*, 1998).

Bacterial resistance to different antibiotics is more severe in developing countries. Inappropriate, excessive use of antibiotics, insufficient control on drug prescribing, inadequate compliance with treatment regimens, prescribing inappropriate doses and

irrational use of antibiotic provides favorable conditions for resistant microorganisms to emerge and spread. For example, when patients do not take the full course of a prescribed antimicrobial or when poor quality antimicrobials are used, resistant microorganisms can emerge and spread (Ramanan *et al*, 2006).

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 (US Pharmacopoeia).

Azithromycin is chosen for the study because it can treat a broad spectrum of bacterial infections and it is the first line choice of drug for lower respiratory tract infections. It is relatively new macrolide antibiotic that has a longer half-life (approximately 60 hours) and better pharmacokinetic properties compared to the macrolide erythromycin. It is widely available and used in developing countries such as Bangladesh. It has an attractive safety profile and could be an option for use in pregnancy (Nosten *et al,* 2006). Several trials have used azithromycin to treat sexually-transmitted diseases such as, gonnorhoea, chlamydia trachomatis and genital infections during pregnancy with no reports of adverse neonatal outcomes (Adair *et al,* 1998).

Azithromycin is indicated mostly for the conditions when micro-organisms show resistance to penicillin, ciprofloxacin or quinolones; for example in case of gonnorhoea. It is also indicated when patient shows allergic reaction to penicillin and its derivatives. Thus effectiveness and purity of azithromycin must be maintained otherwise drug resistance may occur.

It is a study in which effectiveness and purity of different brands of azithromycin available in Bangladesh can be evaluated which can be helpful to estimate the quality of antibiotics available in Bangladesh.

Chapter- 2
Materials and Method

2.1 Sample

• Tridosil- 500 mg azithromycin tablet of Incepta.

• Azimex- 500- 500 mg azithromycin tablet of Drug International.

2.2 Materials

Names and sources of materials required for sensitivity testing are described in table no 2.1

Table 2.1: Name & sources of materials required for sensitivity test.

Materials	Sources
Micro pipette	Eppendrof, Germany
Nutrient agar	Himedia laboratories, India
Agar powder	BDH laboratory, England
Laminar air flow	EQU/03-EHC, ESCO, USA
Autoclave	Hydroclave MC8, Barnsted International
Incubator	BK 4266
Hot air oven	YCO-N01, Gemmy industrial crop, Taiwan
Electronic balance	ELB 3000, Shimadzu, Japan.

2.3 Sample Preparation

• A 500mg Azithromycin Tablet was weighed and recorded in the Record Book.

• The tablet was crushed gently by mortar and pestle then 50mg, 75mg and 100mg equivalent tablet powder were weighed and each of them kept in different tubes.

• Methanol was added with Azithromycin powder kept in each tube to make 10ml solution.

• The solutions were mixed by shaking carefully where Azithromycin readily soluble with the Methanol.

• The solutions were filtered by using filter papers. The filtrate solutions were used for antimicrobial test.

2.4 Standard Preparation

•50mg, 75mg and 100mg of standard Azithromycin powder was weighed and kept in three different tubes.

•Then methanol was added to each tube to make 10ml of solution.

•The solutions were mixed by shaking the tubes carefully where standards of Azithromycin powder readily soluble with the methanol.

2.5 Media preparation

•11.2 mg of nutrient agar and 3mg of agar powder were weighed and mixed with 400ml of distilled water.

•Then the solution was mixed vigorously to create a homogenous mixture.

•The mixture was kept into an autoclave for a certain period of time under specific conditions of sterilization.

2.6 Method

• Nutrient agar plates with appropriate turbidity were prepared by pour plate method.

• A sterile cotton bud swapped in the bacterial suspension and the cotton bud was streaked in at least three directions over the surface of the nutrient agar for obtaining uniform growth.

• Then the plates are allowed to dry at least for five minutes.

• Disks containing the test antibiotic were placed on the surface of the agar, using autoclave-sterile forceps to dispense each antibiotic disk one at a time.

• The disks was placed on the surface of the agar such a way that the distribution of the disks should apart from each other and not close to the edges of the plate.

• Then the plates were incubated within 15 minutes after applying the disks.

• The temperature range of $35^0 \pm 2^0$C is normally required for incubation and the incubation time was 24 which were considered as standard for this test.

Chapter- 3
Result

3.1 Comparison zone of inhibition of Tridosil

3.1.1 Result of zone of inhibition for 50ug/disc

Table 3.1.1: result of zone of inhibition for 50ug/disc

SL NO.	Name of the microorganisms	Zone of Inhibition (mm)		
		Blank	Standard	Sample
01.	*E.coli*	0	20	20
02.	*Staphylococcus aureus*	0	21	21
03.	*Salmonella typhi*	0	21	20

From the table 3.1.1, it is observed that, the sample showed similar sensitivity like the standard against *E.coli*, *Staphylococcus aureus* and *Salmonella typhi*.

3.1.2: Result of zone of inhibition for 75ug/disc

Table 3.1.2: result of zone of inhibition for 75ug/disc

SL NO.	Name of the microorganisms	Zone of Inhibition (mm)		
		Blank	Standard	Sample
01.	*E. coli*	0	23	22
02.	*Staphylococcus aureus*	0	25	24
03.	*Salmonella typhi*	0	23	23

From the table 3.1.2, it is observed that, the sample showed similar sensitivity like the standard against *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*.

3.1.3: Result of zone of inhibition for 100 ug/disc

Table 3.1.3: result of zone of inhibition for 100ug/disc

SL NO.	Name of the microorganisms	Zone of Inhibition (mm)		
		Blank	Standard	Sample
01.	*E. coli*	0	28	27
02.	*Staphylococcus aureus*	0	29	28
03.	*Salmonella typhi*	0	30	30

From the table 3.1.3, it is observed that, the sample showed similar sensitivity like the standard against *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*.

3.2 Comparison zone of inhibition of Azimex-500

3.2.1 Result of zone of inhibition for 50ug/disc

Table 3.2.1: result of zone of inhibition for 50ug/disc

SL NO.	Name of the microorganisms	Zone of Inhibition (mm)		
		Blank	Standard	Sample
01.	*E. coli*	0	20	18
02.	*Staphylococcus aureus*	0	22	21
03.	*Salmonella typhi*	0	21	19

From the table 3.2.1, it is observed that, the sample showed almost similar sensitivity like the standard against *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*.

3.2.2: Result of zone of inhibition for 75ug/disc

Table 3.2.2: result of zone of inhibition for 75ug/disc

SL NO.	Name of the microorganisms	Zone of Inhibition (mm)		
		Blank	Standard	Sample
01.	*E.coli*	0	24	22
02.	*Staphylococcus aureus*	0	25	23
03.	*Salmonella typhi*	0	24	22

From the table 3.2.2, it is observed that, the sample showed almost similar sensitivity like the standard against *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*.

3.3.3 Result of zone of inhibition for 100ug/disc

Table 3.3.3: result of zone of inhibition for 100ug/disc

SL NO.	Name of the microorganisms	Zone of Inhibition (mm)		
		Blank	Standard	Sample
01.	*E.coli*	0	29	26
02.	*Staphylococcus aureus*	0	28	27
03.	*Salmonella typhi*	0	30	28

From the table 3.3.3, it is observed that, the sample showed almost similar sensitivity like the standard against *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*.

Chapter -4
Discussion and Conclusion

Discussion

Quality of pharmaceutical product is very important because drugs must be marketed as safe and therapeutically active formulations whose performance is consistent and predictable. The evaluation of various quality parameters of the pharmaceutical products can ensure their quality as well as bioavailability and impart optimum therapeutic activity.

The quality of pharmaceuticals is a global concern and the lack of reliable drug quality assurance systems in many developing countries often contributes to the devastation of diseases, particularly those that have built up resistance to traditional first-line medicines.

Drug resistance does not only affect the individual but the whole community. Along with irrational prescription and inappropriate use of antibiotics, another major cause of antibiotic resistance in developing country is lack of active ingredient or poor quality of antibiotics. If the drug is not pure then it will not able to reach the plasma level required for therapeutic activity. Thus the microorganisms become tolerated at the low concentration of antibiotic.

Pharmaceutical market of Bangladesh is booming day by day which gives rise to a number of pharmaceutical industries which are involved in manufacturing various antibiotics. We have chosen two antibiotics under different brand names for the study and performed the antibacterial susceptibility test to determine the quality and purity of antibiotics available in Bangladesh.

This study data revealed that all the different concentrations of both of the antibiotics exert effective actions against various strains of pathological micro-organisms and their inhibitory activity against different bacteria is almost similar with the standard. All the blank test results were negative which indicated that solvent does not affect the activity of the antibiotics or the standard.

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 thesis results show that Tridosil and Azimex- 500 contain the sufficient amount of active ingredient required for antimicrobial activity and *in vivo* use of these agents can be as effective as the standard which indicates that these are quality products. .

There are many analytical methods by which purity as well as various other parameters of a quality drug can be determined more precisely, efficiently and rapidly. We can further study to determine the potency of the drugs by various tests such as Microtest. Microtest is a quantitative testing to measure microgram quantities of antibiotics in biological products, drug products or animal tissue.

References

Adair CD, Gunter M, Stovall TG, McElroy G, Veille JC, Ernest JM (1998). Chlamydia in pregnancy: a randomized trial of azithromycin and erythromycin. Obstetrics and Gynecology. pg.165-179

Ashby, Bonnie; Turkington, Carol (2007). "The encyclopedia of infectious diseases" ed.3

C A Hart and S Kariuki (1998).'Antimicrobial resistance in developing countries'. pg. 647–650.

Centre for disease control (Sep, 2009).Department of health, Northern Territory Government.

Choby BA (March 2009)."Diagnosis and treatment of streptococcal pharyngitis". Am Fam Physician 79 (5): 383–90.

Chopra I, Hawkey PM, Hinton M (1992). Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother. pg. 247–277

Cohen, Jonathan and William Powderly (2004). "Bronchitis, Bronchiectasis, and Cystic Fibrosis" Infectious Diseases. ed. 2nd

Donald C.Sockett DVM, PhD and Ann Valley BS (2006). "Antimicrobial Susceptibility Testing". Wisconsin Veterinary Diagnostic Laboratory.

Foulds, G., Hilligoss, D. M., Henry, E. B. & Gerber, N. (1991). "The effects of an antacid or cimetidine on the serum concentrations of azithromycin". Journal of Clinical Pharmacology. pg.31, 164-7.

Garcia-Bustos J, Tomasz A (1990). A biological price of antibiotic resistance: major changes in the peptidoglycan structure of penicillin-resistant pneumococci. Proc Natl Acad Sci USA.;87: 5415–5419.

Giannella RA (1996). "Salmonella". Baron's Medical Microbiology,. Univ of Texas Medical Branch. ed. 4th

Goodman & Gilman (2008). "Antibacterial Activity; Mechanism of Action; Absorption, Distribution And Elimination; Dosage". Manual of Pharmacology & Therapeutics. United States of America: The McGraw-Hill Companies, Inc. pg. 769-773

James H. Jorgensen1, Mary Jane Ferraro, (2011). "Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices"

Jorgensen JH, Turnidge JD (2007). Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA,

editors. Manual of clinical microbiolog. 9th ed. Washington, DC: American Society for Microbiology; 2007. pg. 1152-72.

Leach, Richard E. (2009). "Acute and Critical Care Medicine at a Glance" ed.2

Leclercq R, Courvalin P(1997). Resistance to glycopeptides in enterococci. Clin Infect Dis. pg. 545–556.

Livermor DM (1995). "β-lactamases in laboratory and clinical resistance". Clin Microbiol Rev. pg. 557–584

Lutfiyya MN; Henley E, Chang LF, Reyburn SW (February 2006)."Diagnosis and treatment of community-acquired pneumonia" Am Fam Physician 73 (3): 442–50.

Max Bender (1898). "Ueber neuere Antigonorrhoica (insbes. Argonin und Protargol)". pg. 43

McLuckie, A. (2009). Respiratory disease and its management. New York: Springer. pg. 51.

Michel M, Gutmann L (1997). Methicillin-resistant Staphylcoccus aureus and vancomycin-resistant enterococci: therapeutic realities and possibilities. pg. 1901–1906.

Moran JS (2007). "Gonorrhoea". Clin Evid. pg. 123-134

Murray CJL, Lopez AD (1997). Mortality by cause for eight regions of the world: global burden of disease study. Lancet. pg. 349

Nosten F, McGready R, d'Alessandro U, Bonell A, Verhoeff F, Menendez C (2006). Antimalarial drugs in pregnancy: a review. Current Drug Safety. pg.1-15.

Ramanan Laxminarayan (2006)."Drug Resistance." In Disease Control Priorities in Developing Countries, ed. 2nd

Rang HP, Dale MM, Ritter JM, Moore PK (2003). "Pharmacology". Churchill Livingstone Elsevier.

Richard A. Harvey, Pamela C. Champe (2009). "Lippincott's Illustrated Reviews: Pharmacology". Lippincott Williams & wilkins.

Ruuskanen, O; Lahti, E, Jennings, LC, Murdoch, DR (2011)."Viral pneumonia". Lancet 377 (9773): 1264–75.

Shaikh N, Leonard E, Martin JM (2010). "Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis". Pediatrics 126 (3): e557–64.

S. Karlsmose (2010). "Laboratory Protocol: Susceptibility testing of Enterobacteriaceae using disk diffusion".

Tomasz A, Munoz R(1995). "β-lactam antibiotic resistance in Gram positive bacteria pathogens of the upper respiratory tract: a brief overview of mechanisms". Microbial Drug Resistance. pg.103–109.

Tripathi KD(2003) "Azithromycin". Essentials of Medical Pharmacology. New Delhi. India: Jaypee Brothers Medical Publishers (P) Ltd. pg. 689