A study on pH profile and Sensitivity of Amoxicillin Dry Syrup/Suspension

A Thesis paper submitted to the Department of Pharmacy, East West University in conformity with the requirements for the Degree of Bachelor of Pharmacy.

Department of Pharmacy

East West University

December 2009

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Submitted By:

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ID# 2005-2-70-084

Department of Pharmacy

East West University

December 2009

This thesis paper is dedicated to my family

CERTIFICATE

This is to certify that, the thesis "A study on pH profile and Sensitivity of Amoxicillin Dry Syrup/Suspension" submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm) was carried out by Shagufta Shahzeen Siddiqui (ID# 2005-2-70-084) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

Sufia Islam

22.12.2009

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Abstract:

Antimicrobial agents are among the most commonly used and misused of all drugs. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, fueling an ever increasing need for new drugs. Antibiotics are antibacterial substances produced by various species of microorganisms (bacteria, fungi and actinomycetes) that suppress the growth of other microorganisms. The β-lactam antibiotics are useful and frequently prescribed antimicrobial agents that share a common structure and mechanism of action- inhibition of synthesis of the bacterial peptidoglycan cell wall.

To reduce the development of drug-resistant bacteria and maintain the effectiveness of amoxicillin and other antibacterial drugs, amoxicillin should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. Amoxicillin is similar to ampicillin in its bactericidal action against susceptible organisms during the stage of active multiplication. It acts through the inhibition of biosynthesis of cell wall mucopeptide.

Bacterial strains, even from same species, may vary widely in sensitivity to antibiotics. Information about the antimicrobial susceptibility of the infecting microorganism is important for appropriate drug selection. Several tests are available for determination of bacterial sensitivity to antimicrobial agents. The most commonly used are disk-diffusion tests, agar or broth dilution tests, and automated test system.

In our study, we measured pH of the reconstituted amoxicillin dry syrup/suspension to determine the stability profile of the product. The most stable pH range for amoxicillin according to British Pharmacopoeia is 4-7. The pH falls due to the formation of penicilloic acid which will further destroy the β-lactam ring and make the antibiotic lose its pharmacological action.

In Bangladesh, there are about 424 registered pharmaceutical companies who produce antibiotics. Among these 424 pharmaceutical companies, around 78 companies produce Amoxicillin. The most widely produced dosage form is capsule and suspension.

The sensitivity study was carried out against *E.coli* bacteria which are commonly responsible for many food borne diseases in Bangladesh. This study was carried out with 18 samples of different brands of amoxicillin dry syrup/suspension. Amoxicillin dry syrups/suspensions of 18 different brands were randomly selected from some big and small pharmaceutical companies. We have categorized the pharmaceutical companies (big and small) according to the market size taken from IMS data. The aim of the study was to evaluate in vitro sensitivity and pH of different brands of amoxicillin dry syrup and suspension of various national and multinational pharmaceutical companies in Bangladesh.

From our selected brands for experiment, we found that, all 18 brands (X_1-X_{18}) maintained the established pH criteria successfully. Among 18 brands, 17 brands showed established zone of inhibition that comply with the official monograph.

1. Introduction

1.1 Antimicrobial agents:

The use of the words "antibiosis" and "antibiotic substance" to designate antiliving processes in a very broad sense is found in the older biological literature as well as in many dictionaries. The scientific use of the word "antibiosis" dates from the concept first expressed, in 1889, by Vuillemin in the following terms:

"One creature destroying the life of another in order to sustain its own, one being in unrestricted opposition to the life of the other"

The active participant was named "antibiotic". This concept was also held by Marshal Ward, who applied the term "antibiosis" to an association of organisms whereby one injures ***(Waksman,1947).***

Antimicrobial agents are among the most commonly used and misused of all drugs. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, fueling an ever increasing need for new drugs. However, the pace of antimicrobial drug development has slowed dramatically, with only a handful of new agents, few of which are novel, being introduced into clinical practice each year. Reducing inappropriate use is thought to be the best way to control resistance. Although awareness of consequences of antibiotic misuse is increasing, over prescribing remains widespread, driven largely by patient demand, time pressure on clinicians, and diagnostic uncertainty. If the gains in the treatment of infectious disease are to be preserved, clinicians must be wiser and more selective in use of antimicrobial agents ***(Goodman and Gilman, 2006).***

In the strictest sense, antibiotics are antibacterial substances produced by various species of microorganisms (bacteria, fungi and actinomycetes) that suppress the growth of other microorganisms. Common use often extends the term *antibiotics* to include synthetic antimicrobial agents, such as *sulfonamides* and *quinolones*. Antibiotics differ markedly in physical, chemical, and pharmacological properties, in antimicrobial spectra, and in mechanisms of action ***(Goodman and Gilman, 2006)***.

1.1.a Classification:

General Classification of Antibiotics is as followed:

Antibiotics can be classified in many ways, such as:

A. According to the chemical Structure:

Antibiotic Class	Example
Sulfonamides and related drugs	Sulfadiazine and others, Sulfones- Dapsone (DDS), Paraaminosalicylic acid (PAS)
Diaminopyrimidines	Trimethoprim, Pyrimethamine
Quinolones	Nalixidic acid, Norfloxacin, Ciprofloxacin
β-lactam antibiotics	Penicillins, Cephalosporins, Monobactams, Carbapenems
Tetracyclines	Oxytetracycline, Doxycycline
Nitrobenzene Derivatives	Chloramphenicol
Aminoglycosides	Streptomycin, Gentamycin, Neomycin
Macrolide Antibiotics	Erythromycin, Roxithromycin, Azithromycin
Polypeptide Antibiotics	Polymyxin-B, Colistin, Bacitracin, Tyrothricin
Glycopeptides	Vancomycin, Teicoplanin
Oxazolidone	Linezolid
Nitrofuran derivatives	Nitrofurantoin, Furazolidone
Nitroimidazoles	Metronidazole, Tinidazole
Nicotinic acid derivatives	Isoniazid, Pyrazinamide, Ethionamide
Polyene antibiotics	Nystatin,Amphotericin-B, Hamycin
Azole derivatives	Miconazole, Clotrimazole, Ketoconazole, Fluconazole
Others	Rifampin, Lincomycin, Clindamycin, Spectinomycin, Sod. fusidate, Cycloserine, Viomycin, Ethambutol, Thiacetazone, Clofazimine, Griseofulvin

B. According to Mechanism of action:

Antibiotic Class	Example
Inhibit cell wall synthesis	Penicillins, Cephalosporins, Cyclosterine, Vancomycin, Bacitracin
Cause leakage from cell membranes	Polypeptides-Polymyxins, Colistin, Bacitracin, Polyenes-Amphotericin B, Nystatin, Hamycin
Inhibit Protein synthesis	Tetracyclines, Chloramphenicol, Erythromycin, Clindamycin, Linezolid
Cause misreading of m-RNA code and affect permeability	Aminoglycosides-Streptomycin, Gentamycin
Inhibit DNA gyrase	Fluroquinolones-Ciprofloxacin
Interfere with DNA function	Rifampin, Metronidazole
Interfere with DNA synthesis	Idoxuridine, Acyclovir, Zidovudine
Interfere with intermediary metabolism	Sulfonamides, Sulfones, PAS, Trimethoprim, Pyrimethamine, Ethambutol

C. According to the Type of organisms against which primarily active:

Antibiotic Class	Examples
Antibacterial	Penicillins, Aminoglycosides, Erythromycin
Antifungal	Griseofulvin, Amphotericin B, Ketoconazole
Antiviral	Idoxuridine, Acyclovir, Amantadine, Zidovudine
Antiprotozoal	Chloroquine, Pyrumethamine, Metronidazole, Diloxanide
Anthelmintic	Mebendazole, Pyrantel, Niclosamide, Diethyl carbamazine

D. Acoording to Spectrum of activity:

Narrow Spectrum	Broad Spectrum
Penicillin G	Tetracyclines
Streptomycin	Chloramphenicol
Erythromycin	

E. According to Type of Action:

Primarily Bacteriostatic	Primarily Bactericidal
Sulfonamides	Penicillins
Tetracyclines	Aminoglycosides
Chloramphenicol	Polypeptides
Erythromycin	Rifampin
Ethambutol	Cotrimoxazole
	Cephalosporins
	Vancomycin
	Nalidixic acid
	Ciprofloxacin

F. According to source Antibiotics are obtained from:

Fungi	Bacteria	Actinomycetes
Penicillin	Polymyxin B	Aminoglycosides
Cephalosporin	Colistin	Tetracyclines
Grisofulvin	Bacitracin	Chloramphenicol
	Tyrothricin	Macrolides
	Aztreonam	Polyenes

(*Tripathi, 2003*)

1.2 β-lactam Antibiotics:

The β-lactam antibiotics are useful and frequently prescribed antimicrobial agents that share a common structure and mechanism of action- inhibition of synthesis of the bacterial peptidoglycan cell wall. This class includes penicillin G and V, which are highly active against susceptible gram-positive cocci; penicillinase-resistant penicillins such as nafcillin, which are active against penicillinase-producung *Staphylococcus aureus;* ampicillin and other agents with an improved gram-negative spectrum, especially when combined with a β-lactamase inhibitor; and extended spectrum penicillin with activity against *Pseudomonas aeruginosa*, such as piperacillin.

Penicillin constitutes one of the most important groups of antibiotics. Although numerous other antimicrobial agents have been produced since the first penicillin became available, these still are used widely, and major antibiotics and new derivatives of basic penicillin nucleus still are being produced. Many of these have unique advantage of such that the members of this group of antibiotica are currently the drugs of choice for a large number of infectious disease ***(Goodman and Gilman, 2006)***.

1.2.a Mechanism of action of action of Penicillin:

The β-lactum antibiotics can kill susceptible bacteria. The cell walls of bacteria are essential for their normal growth and development. Peptidoglycan is a hetero polymeric component of the cell wall that provides rigid mechanical stability by virtue of its highly cross-linked latticework structure. In gram-positive microorganisms, the cell wall is 50-100 molecules thick but it is only 1 or 2 molecules thik in gram-negative bacteria.

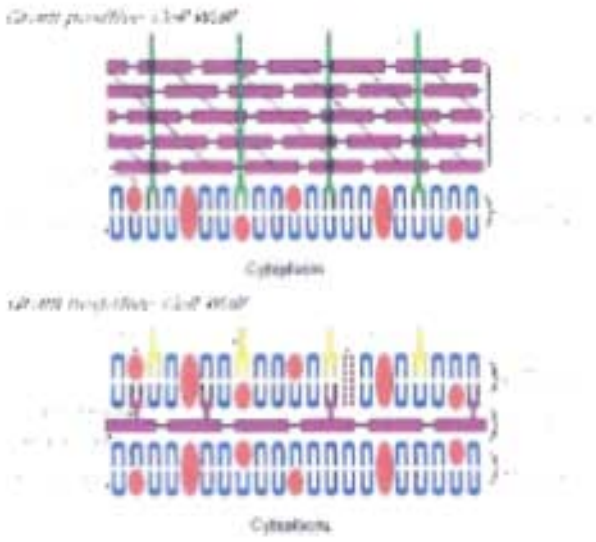

***Figure1.1*: Comparison of structure and composition of gram- positive and gram- negative cell walls.**

The peptidoglycan is composed of glycin chains, which are linear strands of two alternating amino sugars (N-acetylglucosamine and N-acetylmuramic acid) that are cross linked by peptide chains.

The biosynthesis of peptidoglycan involves about 30 bacterial enzymes and may be considered in three stages.

The first stage, precursor formation, takes place in the cytoplasm. The product, uridine diphosphate (UDP)-acetylmuramyl-pentapeptide, accumulates in cells when subsequent synthetic stages are inhibited. The last reaction in the systhesis of this compound is the addition of a dipeptide, D-alanyl-D-alanine. Synthesis of the dipeptide involves prior recemization of L-alanine and condensation catalyzed by D-alnyl-D alanine synthetase. D-cycloserine is a structural analog of D-alanine and acts as a compitative inhibitor of both the recemase and the synthatase.

During reactions of the second stage, UDP-acetylmuramyl-pentapeptide and UDP-acetylglucosamine are linked (with the release of uridine nucleotides) to form a long polymer.

The third and final stage involves completion of the cross-link. This is accomplished by a transpeptidation reaction that occurs outside the cell membrane. The transpeptidase itself is membrane-bound. The terminal glycine residue of the

pentaglycine bridge is linked to the fourth residue of the pentapeptide (D-alanine), releasing the fifth residue (also D-alanine). It is the last step in peptidoglycan synthesis that is inhibited by the β-lactam antibiotics and glycopeptide antibiotics such as *vancomycin* (by a different mechanism than the β-lactams). Stereomodels reveal that the conformation of penicillin is very similar to that of D-alanyl-D-alanine. The transpeptidase probably is acylated by penicillin; that is, penicilloyl enzyme apparently is formed, with cleavage of the –CO-N- bond of the β-lactam ring ***(Goodmann and Gilman, 2006)***.

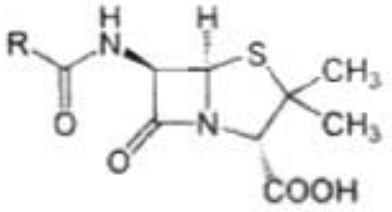

Figure 1.2: **Penicillin nucleus. β-lactam is the square at the centre**

Although inhibition of the transpeptidase just described is demonstrably important,there are additional, related targets for the actions of penicillins and cephalosporins; these are collectively termed as *penicillin-binding proteins* (PBPs). All bacteria have several such entities; for example, *S.aureus* has four PBPs, whereas *Escherichia coli* has at least seven. The PBPs vary in their affinities for different β-lactam antibiotics, although the interactions eventually become covalent. The higher-molecular-weight PBPs of *E.coli (*PBPs 1a and 1b) include the transpeptidases responsible for synthesis of the peptidoglycan. Other PBPs in *E.coli* include those that are necessary for septum formation at division. Inhibition of the transpeptidases causes spheroplast formation and rapid lysis. However, inhibition of the activities of other PBPs may cause delayed lysis (PBP2) or the production of long, filamentous forms of the bacterium (PBP 3). The lathelity of penicillin for bacteria appears to be

involve both lytic and nonlytic mechanisms. Penicillin's disruption of the balance between PBP-mediated peptidoglycan assembly and murein hydrolase activity results in autolysis. Nonlytic killing by penicillin may involve holing-like proteins in the bacterial membrane that collapse the membrane potential ***(Goodmann and Gilman , 2006)***.

1.3 Antibiotic market in Bangladesh:

Bangladesh has been producing antibiotic for a long period time. Now, there are around 300 registered pharmaceutical companies who produce antibiotics. Among these 300 pharmaceutical companies, around 78 companies are producing Amoxicillin. The most widely produced dosage form is capsule and suspension. Beside these dosage form; tablets, drop, injection are also available.

1.3.a Amoxicillin Market of Bngladesh:

Brand	Company	Dosage Form	Dose	Strength
AMOXYL	Aexim	Capsule	250 mg and 500 mg/capsule	-
Amoxyl	Aexim	Suspension		125mg/5ml
AMBEEXIN	Ambee	Suspension		125mg/5ml
AMBEEXIN	Ambee	Drop		125mg/1.25 ml
AMOCAP	Sonear	Capsule	250 mg and 500mg/capsule	
AMOCIL	Syntho	Capsule	250 mg and 500 mg/capsule	
AMOCIN	Pacific	Capsule	250mg and 500mg/capsule	

AMOCIN	Pacific	Suspension		125mg/5ml
AMOTID	Bio-pharma	Capsule	250mg and 500 mg/capsule	
AMOTID	Bio-pharma	Suspension		125/mg/5ml
AMOTID-F	Bio-pharma	Suspension		250mg/5ml
AMOTID	Biopharma	Drop		125mg/1.25ml
AMOX	Doctor's	Capsule	250 and 500 mg/capsule	
AMOX	Doctor's	Suspension		125mg/5ml
AMOX	Doctor's	Drop		125mg/1.25ml
AMOXI	Renata	Injection		500mg/vial
AMOXIC	Cosmo	Capsule	250 mg and 500mg/capsule	
AMOXIC	Cosmo	Suspension		125mg/5 ml
AMOXICAP	Renata	Capsule	250mg/capsule	
AMOXICON	Medicon	Capsule	250mg and 500mg/capsule	
AMOXICON	Medicon	Suspension		125mg/5ml
AMOXIL	Glaxosmithcline	Capsule	250mg and 500 mg/capsule	
AMOXIL	Glaxosmithcline	Suspension		125mg/5ml
AMOXIL Forte	Glaxosmithcline	Suspension		250mg/5ml
AMOXIMA	Modern	Capsule	250mg and 500mg/capsule	
AMOXIMA	Modern	Suspension		125mg/5ml
AMOXIMA	Modern	Drop		100mg/5ml
AMOXIPAN	Salton	Capsule	250mg and 500mg/capsule	
AMOXIPAN	Salton	Suspension		125mg/5ml

AMOXIZEN	Zenith	Capsule	250mg/capsule	
AMOXIZEN Ds Cap	Zenith	Capsule	500mg/capsule	
AMOXIZEN	Zenith	Suspension		125mg/5ml
AMOXON	Jayson	Capsule	250mg and 500mg/capsule	
AMOXON	Jayson	Suspension		125mg/5ml
AMOXON	Jayson	Drop		100mg/1ml
AMOXON	Jayson	Injection		250mg and 500mg/vial
ANTIF	Rangs Pharma	Capsule	250mg and 500mg	
ANTIF	Rangs Phar,a	Suspension		125mg/5ml
ANTIF DS	Rangs Pharma	Suspension		250mg/5ml
ANTIF	Rangs Pharma	Drop		100mg/1ml
APIMOX	Apollo	Capsule	250mg and 500mmg	
APIMOX	Apollo	Suspension		125mg/5ml
APIMOX	Apollo	Drop		100mg/1ml
APOXY	Apex	Capsule	250mg and 500mg/capsule	
APOXY	Apex	Suspension		125mg/5ml
APOXY DS	Apex	Suspension		250mg/5ml
ARISTOMOX	Aristopharma	Capsule	250 mg and 500mg/capsule	
ARISTOMOX	Aristopharma	Suspension		125mg/5ml
AVLOMOX	ACI	Capsule	250mg and 500mg/capsule	
AVLOMOX	ACI	Suspension		125mg/5ml
AVLOMOX DS	ACI	Suspension		250mg/5ml
AVLOMOX	ACI	Drop		100mg/ml
AVLOMOX	ACI	Injection		500mg/vial

BACTAMOX	Renata	Tablet	250mg and 500mg/tablet	
BACTAMOX	Renata	Suspension		125mg/5ml
BACTAMOX	Renata	Drop		100mg/ml
BACTAMOX	Renata	Injection		250mg and 500mg/vial
BENOXIL	Benham	Capsule	250mg and 500mg/capsule	
BENOXIL	Benham	Suspension		125mg/5ml
BENOXIL DS	Benham	Suspension		250mg/5ml
BENOXIL	Benham	Drop		100mg/ml
BOMOX	Bristol	Capsule	250mg and 500mg/capsule	
BPMOX	Bristol	Suspension		125mg/5ml
BPMOX	Bristol	Drop		100mg/ml
BROADMOX	Cosmic	Capsule	250mg/capsule	
BROADMOX DS	Cosmic	Capsule	500mg/capsule	
BROADMOX	Cosmic	Suspension		125mg/5ml
CEMOXIN	CPL	Capsule	250mg and 500mg/capsule	
CEMOXIN	CPL	Suspension		125mg/5ml
CLAMOX	Kumudini	Capsule	250mg and 500mg/capsule	
CLAMOX	Kumudini	Suspension		125mg/5ml
DEMOX	Desh Pharma	Capsule	250mg/capsule	
DEMOX-500	Desh Pharma	Capsule	500mg/capsule	
DEMOX	Desh Pharma	Suspension		125mg/5ml
DEMOX	Desh Pharma	Drop		100mg/ml
DEMOXIL	Drug International	Capsule	250mg and 500mg/capsule	
DEMOXIL	Drug	Suspension		124mg/5ml

	international			
DEMOXIL	Drug International	Injection		250mg,500mg and 1gm/vial
DOPEN	Hallmark	Capsule	250mg and 500mg/capsule	
DOPEN	Hallmark	Suspension		125mg/ml
ELIMOX	Elixir Pharma	Capsule	250 mg and 500mg/capsule	
ELIMOX	Elixir Pharma	Suspension		125mg/5ml
E-MOX	Edrue	Capsule	250mg and 500mg/capsule	
E-MOX	Edrue	Suspension		125mg/5ml
E-MOX	Edrue	Drop		100mg/ml
FIMOXYL	Sanofi Aventis	Capsule	250mg and 500mg/capsule	
FIMOXYL	Sanofi Aventis	Tablet	250 mg and 500mg/tablet	
FIMOXYL DS	Sanofi Aventis	Suspension		250mg/5ml
FIMOXYL	Sanofi Aventis	Suspension		125mg/5ml
FIMOXYL	Sanofi Aventis	Drop		100mg/ml
FIMOXYL	Sanofi Aventis	Injection		250mg and 500mg/vial
FLYMOX	White Horse	Capsule	500mg/capsule	
FLYMOX	White Horse	Suspension		125mg/5ml
G-AMOXYCILLIN	Gonoshastha	Capsule	250mg and 500mg/capsule	
G-AMOXYCILLIN	Gonoshastha	Suspension		125mg/5ml
G-AMOXYCILLIN	Gonoshastha	Injection		500mg/vial
GENAMOX	General	Capsule	250mg and 500mg/capsule	
GENAMOX	General	Suspension		125mg/5ml

GENAMOX	General	Drop		100mg/ml
HECTAMOX	Millat	Capsule	250mg and 500mg/capsule	
HECTAMOX	Millat	Suspension		125mg/5ml
HECTAMOX	Millat	Drop		100mg/ml
HICONCIL	Medimet	Capsule	250mg and 500mg/capsule	
HICONCIL	Medimet	Suspension		125mg/5ml
HI-MOX	Hudson	Capsule	250mg/capsule	
HI-MOX-DS	Hudson	Capsule	500mg/capsule	
HI-MOX	Hudson	Suspension		125mg/5ml
JAMOXIL	Jalalabad	Capsule	250mg and 500mg/capsule	
JAMOXIL	Jalalabad	Suspension		125mg/5ml
KAMOXY	Chemico	Capsule	250mg and 500mg/capsule	
KAMOXY	Chemico	Suspension		125mg/5ml
KAMOXY	Chemico	Drop		125mg/1.25ml
LOXYL-250	Aisatic	Capsule	250mg/capsule	
LOXYL-500	Asiatic	Capsule	500mg/capsule	
LOXYL.Susp	Asiatic	Suspension		125mg/5ml
MOCI	Belsen	Capsule	250mg and 500mg/capsule	
MOCI	Belsen	Suspension		125mg/5ml
MONAMOX	Amico	Capsule	250mg and 500mg/capsule	
MONAMOX	Amico	Suspension		125mg/5ml
MONAMOX DS	Amico	Suspension		250mg/5ml
MONAMOX	Amico	Drop		125mg/1.25ml
MOX 250	Proteety	Capsule	250mg/capsule	
MOX500	Proteety	Capsule	500mg/capsule	

MOX Susp	Proteety	Suspension		125mg/5ml
MOX Drop	Proteety	Drop		100mg/ml
MOXA	Decent	Capsule	250mg and 500mg/capsule	
MOXA	Decent	Suspension		125mg/5ml
MOXACIL	Square	Capsule	250mg and 500mg/capsule	
MOXACIL 875	Square	Tablet	875mg/tablet	
MOXACIL DT	Square	Tablet	250mg/tablet	
MOXACIL	Square	Suspension		125mg/5ml
MOXACIL Forte Susp	Square	Suspension		250mg/5ml
MOXACIL	Square	Drop		125mg/1.25ml
MOXACIL	Square	Injection		250mg and 500mg/vial
MOXAPEN	Nipa	Capsule	250mg and 500mg/capsule	
MOXAPEN	Nipa	Suspension		125mg/5ml
MOXAPEN	Nipa	Drop		100mg/ml
MOXATID	Marksman	Capsule	250mg and 500mg/capsule	
MOXATID	Marksman	Suspension		125mg/5ml
MOXATID	Marksman	Drop		100mg/ml
MOXICO	Supreme	Capsule	250mg and 500mg/capsule	
MOXICO	Supreme	Suspension		125mg/5ml
MOXICO DS	Supreme	Suspension		250mg/5ml
MOXILIN	Acme	Capsule	250 mg and 500mg/capsule	
MOXILIN	Acme	Suspension		125mg/5ml
MOXILIN DS	Acme	Suspension		250mg/5ml

MOXILIN	Acme	Drop		100mg/ml
MOXILIN	Acme	Injection		250mg and 500mg/vial
MOXIN	Opsonin	Capsule	250mg and 500mg/tablet	
MOXIN	Opsonin	Tablet	250mg/tablet	
MOXIN 875	Opsonin	Tablet	875mg/tablet	
MOXIN	Opsonin	Suspension		125mg/5ml
MOXIN PR	Opsonin	Suspension		250mg/5ml
MOXIN	Opsonin	Drop		125mg/1.25ml
MOXIN	Opsonin	Injection		250mg 500mg/vial
MOX-Plus	Hudson	Suspension		250mg/5ml
MUMOX	SAPL	Capsule	250 mg and 500mg/capsule	
MUMOX	SAPL	Suspension		125mg/5ml
MYMOXIL	Mystic	Capsule	250mg and 500mg/capsule	
MYMOXIL	Mystic	Suspension		125mg/5ml
NAVAMOX	Navana	Capsule	250mg and 500mg/capsule	
NAVAMOX	Navana	Suspension		125mg/5ml
ORIXYL	Orion	Capsule	250 mg and 500mg/capsule	
ORIXYL	Orion	Suspension		125mg/5ml
ORIXYL	Orion	Drop		125mg/1.25ml
PAMOXIL	Peoples	Capsule	250mg and 500mg/capsule	
PAMOXIL	Peoples	Suspension		125mg/5ml
PANOXYL	Globex	Capsule	500mg/capsule	
PEMOX	APC	Capsule	250mg/capsule	

PEMOX	APC	Suspension		125mg/5ml
PENMOX	Techno Drugs	Capsule	500mg/capsule	
PENMOX	Techno Drugs	Suspension		125mg/5ml
PENMOX	Techno Drugs	Injection		250mg and 500mg/vial
PHARMOXYL	Pharmadesh	Capsule	250mg and 500mg/capsule	
PHARMOXYL	Pharmadesh	Suspension		125mg/5ml
PHARMAXYL	Pharmadesh	Drop		100mg/ml
REMAMOX	Reman	Capsule	250mg and 500mg/capsule	
REMAMOX	Reman	Suspension		125mg/5ml
ROXYL	Rasa	Capsule	250mg/capsule	
ROXYL-DS	Rasa	Capsule	500mg/capsule	
ROXYL	Rasa	Suspension		125mg/5ml
ROXYL	Rasa	Drop		100mg/ml
REMOXIN	Rephco	Capsule	250mg and 500mg/capsule	
REMOXIN	Rephco	Suspension		125mg/5ml
REMOXIN DS	Rephco	Suspension		250mg/5ml
SAPOX	Alco Pharma	Capsule	250mg/capsule	
SAPOX DS Cap	Alco Pharma	Capsule	500mg/capsule	
SAPOX	Alco Pharma	Suspension		125mg/5ml
SAPOX DS Susp	Alco Pharma	Suspension		250mg/5ml
SAPOX	Alco Pharma	Drop		125mg/1.25ml
SEEMAXYL	Seema	Capsule	250mg and 500mg/capsule	
SEEMAXYL	Seema	Suspension		125mg/5ml
SERVIMOX	Sandoz/Novartis	Capsule	250mg and 500mg/capsule	
SERVIMOX	Sandoz/Novartis	Suspension		125mg/5ml

SIMOX	Silva	Capsule	250mg and 500mg/capsule	
SIMOX	Silva	Suspension		125mg/5ml
SIMOX DS	Silva	Suspension		250mg/5ml
SIMOX	Silva	Drop		100mg/ml
SINAMOX	Ibn Sina	Capsule	250mg and 500mg/capsule	
SINAMOX	Ibn Sina	Suspension		125mg/5ml
SINAMOX DS	Ibn Sina	Suspension		250mg/5ml
SINAMOX	Ibn Sina	Drop		100mg/ml
SK-MOX	SK+F	Capsule	250mg and 500mg/capsule	
SK-MOX	SK+F	Suspension		125mg/5ml
SK-MOX DS	SK+F	Suspension		250mg/5ml
SK-MOX	SK+F	Drop		125mg/1 25ml
SKYMOXIN	Skylab	Capsule	250mg and 500mg/capsule	
SKYMOXIN	Skylab	Suspension		125mg/5ml
SKYMOXIN	Skylab	Drop		125mg/1.25ml
TYCIL	Beximco	Capsule	250mg and 500mg/capsule	
TYCIL	Beximco	Suspension		125mg/5ml
TYCIL DS	Beximco	Suspension		250mg/5ml
TYCIL	Beximco	Drop		125mg/1.25ml
TYMOX	Somatec	Capsule	250mg and 500mg/capsule	
TYMOX	Somatec	Suspension		125mg/5ml
TYMOX	Somatec	Drop		125mg/1.25ml
ULTRAMOX	Globe	Caosule	250mg and 500mg/capsule	
ULTRAMOX	Globe	Suspension		125mg/5ml

UNIMOX	Gaco	Capsule	250mg and 500mg/capsule	
UNIMOX	Gaco	Suspension		125mg/5ml
UNIMOX	Gaco	Drop		125mg/1.25ml
UNIMOX	Gaco	Injection		500mg/vial
ZIMOXYL	Ziska	Capsule	250mg and 500mg/capsule	
ZIMOXYL	Ziska	Suspension		125mg/5ml

(QIMP-14, 2007)

1.4 Amoxicillin Suspension:

To reduce the development of drug-resistant bacteria and maintain the effectiveness of amoxicillin and other antibacterial drugs, amoxicillin should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

Formulations of amoxicillin for oral suspension, USP contain amoxicillin, a semisynthetic antibiotic, an analog of ampicillin, with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms. Chemically, it is (2S,5R,6R) - 6 - [(R) - (-) - 2 - amino - 2 - (p - hydroxyphenyl)acetamido] - 3,3 - dimethyl - 7 - oxo - 4 - thia - 1 - azabicyclo[3.2.0]heptane - 2 - carboxylic acid trihydrate. It may be represented structurally as:

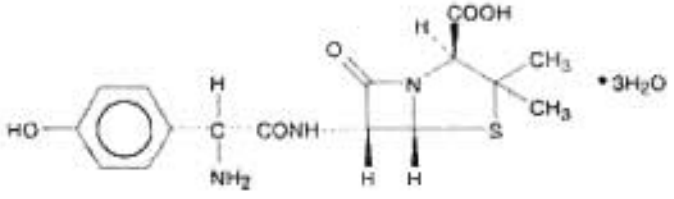

Figure 1.3: Amoxicillin

The amoxicillin molecular formula is C16H19N3O5S•3H2O, and the molecular weight is 419.45.

Amoxicillin for oral suspension is intended for oral administration.

Each 5 mL of reconstituted suspension contains amoxicillin trihydrate equivalent to 200 mg or 400 mg anhydrous amoxicillin. Each 5 mL of the 200 mg and 400 mg reconstituted suspension contains 0.16 mEq (3.61 mg) of sodium ***(Drugs.com, 2000-2009)***.

1.5 Microbiology of amoxicillin

Amoxicillin is similar to ampicillin in its bactericidal action against susceptible organisms during the stage of active multiplication. It acts through the inhibition of biosynthesis of cell wall mucopeptide. Amoxicillin has been shown to be active against most strains of the following microorganisms:

1. Aerobic Gram-Positive Microorganisms

a)*Enterococcus faecalis*:

Enterococcus faecalis – formerly classified as part of the Group D *Streptococcus* system – is a Gram-positivecommensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. It is among the main constituents of some probiotic food supplements.

Figure 1.4: Enterococcus faecalis

Acommensal organism like other species in the genus *Enterococcus*, *E. faecalis* can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity. Germ-free interleukin-10 knockout (IL-10

KO) mice developed inflammatory bowel disease (IBD) after they were colonized with a pure culture of *Enterococcus faecalis*. *E. faecalis* not only induced IBD (primarily in colon and rectum) but rectal dysplasia and adenocarcinoma was also found in the IL-10 KO mice. Conventional (complex-intestinal flora) IL-10 KO mice developed IBD within 10 to 15 weeks of age and showed more pathology in the cecum (typhlitis) than we observed with *E. faecalis*-induced IBD in gnotobiotic IL-10 KO mice. *E. faecalis* is a common intestinal microbe of man and animals that can trigger IBD, dysplasia, and carcinoma in a genetically susceptible murine host ***(Balish and Warner, 2002)***.

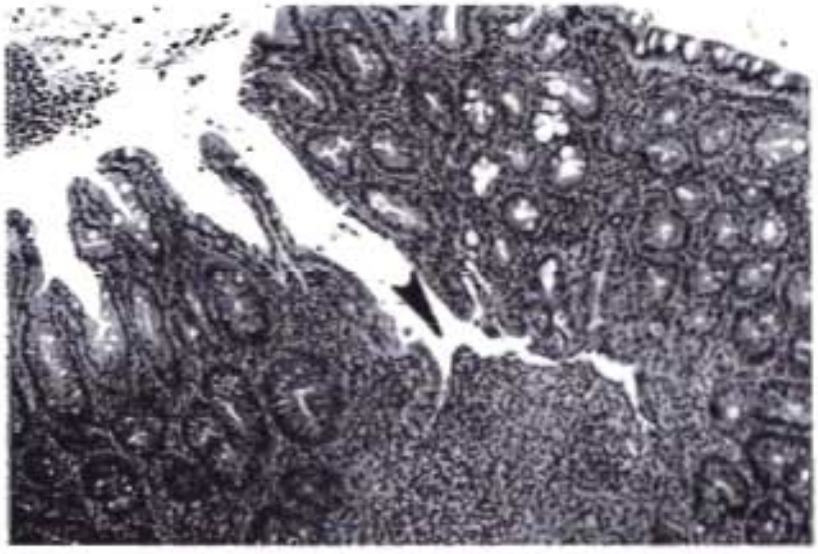

Figure 1.5: Fissure (**arrowhead**) in cecum of IL-$10^{-/-}$ mouse infected with *E. faecalis* for 20 weeks. H&E; original magnification, x100.

b)*Staphylococcus spp.* (β-lactamase–negative strains only):

Staphylococcus spp. are facultative anaerobes. Facultative anaerobes are capable of growth both aerobically and anaerobically. All species grow in the presence of bile salts and are catalase positive. Growth also occurs in a 6.5% NaCl solution. On Baird Parker Medium *Staphylococcus spp.* show as fermentative, except for *S. saprophyticus* which is oxidative.*Staphylococcus spp.* are resistant to Bacitracin (0.04 U resistance = <10mm zone of inhibition) and susceptible to Furazolidone (100μg resistance = <15mm zone of

inhibition). The staphylococci are important pathogenic bacteria responsible for a variety of diseases in humans and other animals. They are the most common cause of hospital acquired infection and antibiotic resistant strains (MRSA) have become endemic in hospitals in most countries causing major public health issues. In addition, the incidence of new strains that cause severe community-acquired infections in healthy people is increasing and MRSA strains are emerging in agricultural and domestic animals ***(Lindsay,2008)*** .

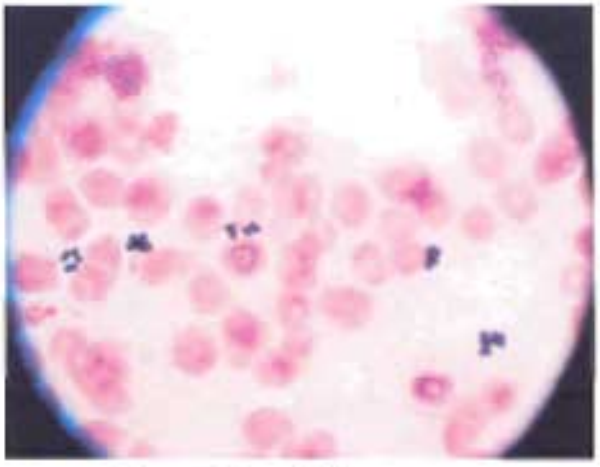

Figure1.6*: *Staphylococcus spp

c)*Streptococcus pneumoniae*:

Streptococcus pneumoniae, or pneumococcus, is Gram-positive, alpha-hemolytic, bile soluble diplococcus aerotolerant anaerobe and a member of the genus *Streptococcus*. *S pneumoniae* is the most common cause of bacterial meningitis in adults and children, and is one of the top two isolates found in ear infection, otitis media. Pneumococcal pneumonia is more common in the very young and the very old.

-Streptococcus spp. (α- and β-hemolytic strains only)

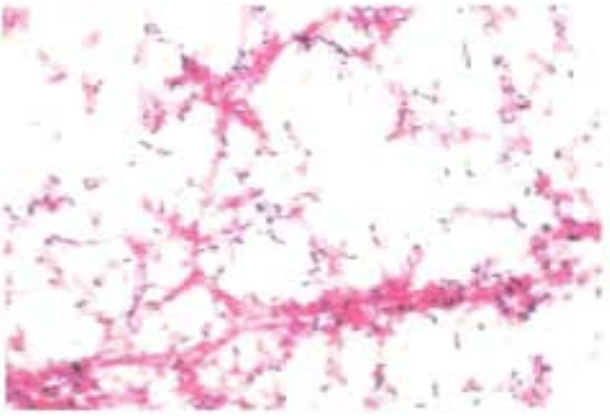

Figure1.7: Streptococcus

2. Aerobic Gram-Negative Microorganisms

-*Escherichia coli* (β-lactamase–negative strains only)
-*Haemophilus influenzae* (β-lactamase–negative strains only)
-*Neisseria gonorrhoeae* (β-lactamase–negative strains only)
-*Proteus mirabilis* (β-lactamase–negative strains only)
-*Helicobacter*
-*Helicobacter pylori* **(Drugs.com, 2000-2009)**

1.5.a Some Facts on *Escherichia coli:*

Escherichia coli is a bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E. coli* strains are harmless, but some, such as serotype O157:H7 can cause serious food poisoning in humans.

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. The bacteria can also be grown easily and its genetics are comparatively simple and easily-manipulated, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology. *E. coli* was

discovered by German pediatrician and bacteriologist Theodor Escherich in 1885, and is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria.

E. coli is Gram-negative, facultative anaerobic and non-sporulating. The cells are about 2 micrometers (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 μm^3. It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide.

Optimal growth of *E. coli* occurs at 37°C, but some laboratory strains can multiply at temperatures of up to 49°C. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide. Strains that possess flagella can swim and are motile, but other strains lack flagellum. *E. coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population.

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia. Recently it is thought that *E. coli* and certain other food borne illnesses can sometimes trigger serious health problems months or years after patients survived that initial bout.

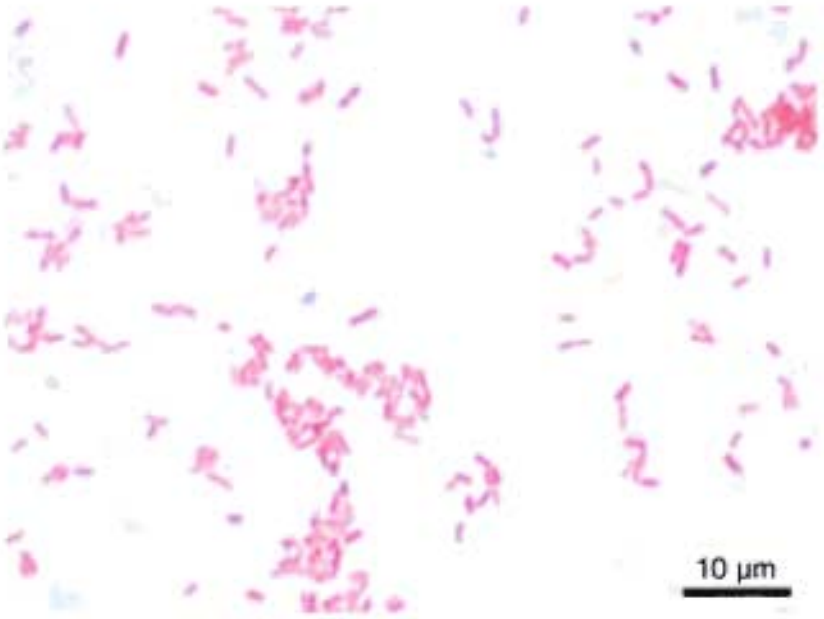

Figure 1.8: ***Escherichia coli***

In recent years, *Escherichia coli* (E. coli) 0157:H7 has developed into an emerging cause of foodborne illness. It has been identified as the leading cause of postdiarrheal hemolytic-uremic syndrome (HUS) and acute renal failure in infancy and childhood ***(Peacock, Jacob, Fallone,2001).***

E. coli O157:H7 is one of hundreds of strains of the gram-negative bacterium *Escherichia coli*. There are more than 170 serogroups of *E. coli*. Within each serogroup there are one or more serotypes. *E. coli* O157:H7 is the most common serotype and the most well-known enterohemorrhagic strain. The numbers assigned to the strain refer to the molecules on the bacteria surface that indicate the specific strain of *E. coli* ***(Peacock, Jacob, Fallone, 2001).***

1.6 Testing for microbial sensitivity and stability:

1.6.a Sensitivity Tests

Bacterial strains, even from same species, may vary widely in sensitivity to antibiotics. Information about the antimicrobial susceptibility of the infecting microorganism is important for appropriate drug selection. Several tests are available for determination of bacterial sensitivity to antimicrobial agent agents. The most commonly used are disk-diffusion tests, agar or broth dilution tests, and automated test system.

Disk diffusion technique provides only qualitative or semi-quantitative information on antimicrobial susceptibility. The test is performed by applying commercially available filter paper disks impregnated with a specific amount of the drug onto an agar surface, over which a culture of microorganism has been streaked. After 18-24 hours of incubation, the size of the clear zone of inhibition around the disk is measured. The diameter of the zone depends on the activity of the drugs against the test strain. Standardized values for zone size for each microbial species and each antibiotic permit classification of the clinical isolate as resistant, intermediate, or susceptible ***(Goodman and Gillman, 2006)***.

1.6.b Stability Tests

The stability of reconstituted amoxicillin trihydrate-potassium clavulanate oral suspension both in original containers and pre-packaged in commercially available oral syringes stored at various temperatures was determined. Amoxicillin trihydrate 125 mg/5 mL-potassium clavulanate 31.25 mg/5 mL and amoxicillin trihydrate 250 mg/5 mL-potassium clavulanate 62.5 mg/5 mL were reconstituted according to the manufacturer's instructions. The reconstituted suspensions in the original containers and in five brands of oral syringes were stored at 5 degrees C and 25 degrees C and -10 degrees C, 5 degrees C, and 25 degrees C, respectively, for 0, 2, 4, 7, and 14 days (Stiles, Olsen, Barton and Greenwood,1988).

Amoxicillin degrades rapidly under natural or acidic condition. Preformulation studies indicate that clavulanic acid undergoes acid-base catalyzed reactions depending on pH and the presence of buffer salts. Maximal stability of clavilanic acid is reported to be at pH 6.3. Although amoxicillin is subject to similar degradation pathways it appears to be more stable than clavunalic acid *(Vega, Manzo and Sola, Improving the stability of potassium clavulanate in admixture with amoxicillin).*

Our main reason behind this research is because Amoxicillin is the drug that affects the gram (-) organisms most effectively. For this we have conducted our in vitro experiments over *E.coli* bacterium which is gram (-) organism. The reason behind choosing *E.coli* bacterium particularly, because it is the most common gram (-) bacterium which is present over a wide range of bacterial infections/diseases which are prevalent in Bangladesh. So, we are investigating how effective the amoxicillin drugs are against *E.coli* bacterium.

In Bangladesh, many multinational and national pharmaceutical industries manufacture Amoxicillin dry syrup/suspension. So far from our knowledge, no study has been carried out to determine the pH profile of different brands of amoxicillin dry syrup/suspension manufactured in Bangladesh.

Therefore this study is carried out to determine the pH of some randomly selected amoxicillin dry syrup/suspension in order to find out the stability profile and sensitivity of these drugs.

Significance of the study:

As aforementioned we determined the pH profile and sensitivity of the reconstituted dry syrup/suspension of different brands of Amoxicillin. For this we have carried out the pH tests over them. The reason for carrying out the pH test is to confirm that if they remain reasonably consistent as per the guaranteed by the manufacturers and established by various drug policy regulation bodies. So, if the drugs deteriorate, one of the signs is a very erratic pH indication. A non consistent pH can badly affect the stability of the drug and if administered to a patient, its effect could be fatal.

The acidic pH leads to formation of penicilloic acid which degrades the drug and inhibits the drug to reach its therapeutic index and thus reduce efficacy. The pH profile is very much related to the sensitivity of the drug. If pH falls below the standard range, the sensitivity of the drug will also reduce gradually.

Sensitivity of the reconstituted amoxicillin dry syrup and suspension was also performed to determine the bactericidal action of amoxicillin against *E.coli*. If the reconstituted amoxicillin does not show the sensitivity against the organism, the appropriate action of the drug cannot be expected. Due to poor sensitivity of the syrup/suspension the bacteria might become resistant to the drug.

In this study we determined the stability (pH profile) and sensitivity of available amoxicillin dry syrup or suspension in Bangladesh. According to our knowledge, no study has been performed yet to determine the stability (pH profile) and sensitivity of reconstituted amoxicillin dry syrup/suspension from different pharmaceutical companies of Bangladesh. This study will ultimately help the health sector to evaluate the stability (pH profile) and sensitivity of amoxicillin dry syrup/suspension of different pharmaceutical companies of Bangladesh.

Objective of the study:

The objectives of the research are

1. To test the pH profile,
2. To determine the sensitivity of various brands of Amoxicillin

Chapter-2

Methodology

2.1 Methodology

A growth medium is a mixture of nutrients, moisture and other chemicals that bacteria need for growth. Media are used to grow bacterial colonies (millions of bacteria having arisen through the binary fission of a single progenitor).

Inoculation is the placement of something to where it will grow or reproduce. In this sensitivity test we used only MacConkeys agar because, our organism is *E.coli which* is gram (-)ve.Whenever bacterial colonies are growing on MacConkeys Agar, they are Gram-negative bacteria (since Gram+ do not grow on this type of medium). If the colonies are pink, they are Gram- lactose-fermenting bacteria. These pink colonies are typically coliform bacteria in the family Enterobacteriaceae, inlcuding the genera *Escherichia, Klebsiella, Enterobacter, Hafnia* and *Citrobacter*.

In our experiment, we used 18 different brands of amoxicillin dry syrup/suspension which was selected randomly from the market. According to brand names we labeled them in X_I to X_{IR}.

2.1.a Materials required

2.1.a.(i) Materials required for inoculation in media plates:-

- Petri dish
- Beaker
- Measuring cylinder
- Media preparation bottle
- Funnel
- Analytical balance
- Autoclave
- Hot air oven
- Laminar air flow chamber
- Micropipette
- Micropipette tips
- Test tubes
- Aluminum foil
- Nutrient agar
- MacConkey agar
- pH Meter
- Petri Dishes

2.2 Sterilization method

Sterilization was done using autoclave and/or hot air oven. Beakers, funnel, measuring cylinders, micropipette tips and test tubes, normal saline water and the culture media (the media that require autoclave) were sterilized using autoclave at 121 °C for 15 minutes. Petri dishes were sterilized in hot air oven at 200 °C for 90 minutes. All the items were wrapped in aluminum foil during sterilization This is to ensure that the glassware do not get contaminated after sterilization when they are transferred from the sterilizer to the laminar air flow chamber. After sterilization the items are kept in the laminar air flow chamber and all the activities are done here to prevent contamination.

2.3 Culture media used and methods of preparation:

2.3.a MacConkey agar

MacConkey Agar is used for the isolation and differentiation of Gram-negative enteric bacilli.

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey. The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the *coliform* group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and *coliforms*.

MacConkey Agar is recommended for the detection and isolation of Gram-negative organisms from clinical, dairy, food, water, pharmaceutical, and industrial sources.
Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting Gram-positive cocci and allowing Gram-negative organisms to grow. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Directions for Preparation

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and dispense into sterile petri dishes.

Expected results:

Microorganism	Response	Reactions	Bile ppt
Enterococcus faecalis	marked to complete inhibition	-	-
Escherichia coli	growth	pink colonies	+
Proteus mirabilis	growth with partial inhibition of swarming	colorless colonies	-
Salmonella typhimurium	growth	colorless colonies	-

Table 2.1:- Cultural response on MacConkey Agar at 37°C after incubation for 24 hours.

2.3.b Preparation of the Culture Media

- 2 ml of saline was taken in a test tube.
- The sample organism was taken with the tip of inoculation stick and mixed it with the saline.
- The saline containing the sample organism was shaken to mix with the saline evenly.

2.3.c Pour plate method:-

1. The media was prepared according to the instructions.
2. The media was poured in the petri dish up to 5mm.
3. The petri dish was swirled gently to mix with the media.
4. After mixing the media was allowed to solidify.
5. The sample was then stricken over the solid media.
6. The disks soaked with the drug and place it on the surface of the agar. With forceps gently tap the disk to ensure better contact with the agar.
7. After the media becomes solid the petri dish was incubated.

2.4 pH Test

For stability testing of the antibiotic, pH test was carried out. The most stable pH range for β-lactam antibiotic is 6-6.5. If pH falls, it will to construction of penicilloic acid which will further destroy the β-lactam ring and make the antibiotic lose its pharmacological action. There are several reasons why pH of the antibiotic falls, some of them are: increased temperature, hydrolysis etc.

2.4.a Method of Testing

- The pH meter was calibrated with pH solution having pH 7.
- Each drug was reconstructed.
- 10 ml of each suspension was taken into a beaker.
- The pH meter was submerged in the beaker making sure that the bulb fully sank under the suspension.

Chapter-3

Results

3. Results:

3.1 pH Data of 1st day of different brands of amoxicillin suspension (X_1 to X_6) after reconstitution:

Drugs	pH
X_1	5.2
X_2	5.4
X_3	5.3
X_4	5.9
X_5	5.6
X_6	5.6

Table 3.1: **pH Data of 1st day of different brands of amoxicillin suspension (X_1 to X_6) after reconstitution**

Brand X_1-X_6 showed good pH profile compared to the established standard range for amoxicillin, ranging between 4 and 7 which is ideal for the stability of antibiotic. At this pH, the drug do not hydrolyze to form penicilloic acid.

3.2 pH Data of 1st day of different brands of amoxicillin suspension after reconstitution (X_7 to X_{13}) in Bangladesh:

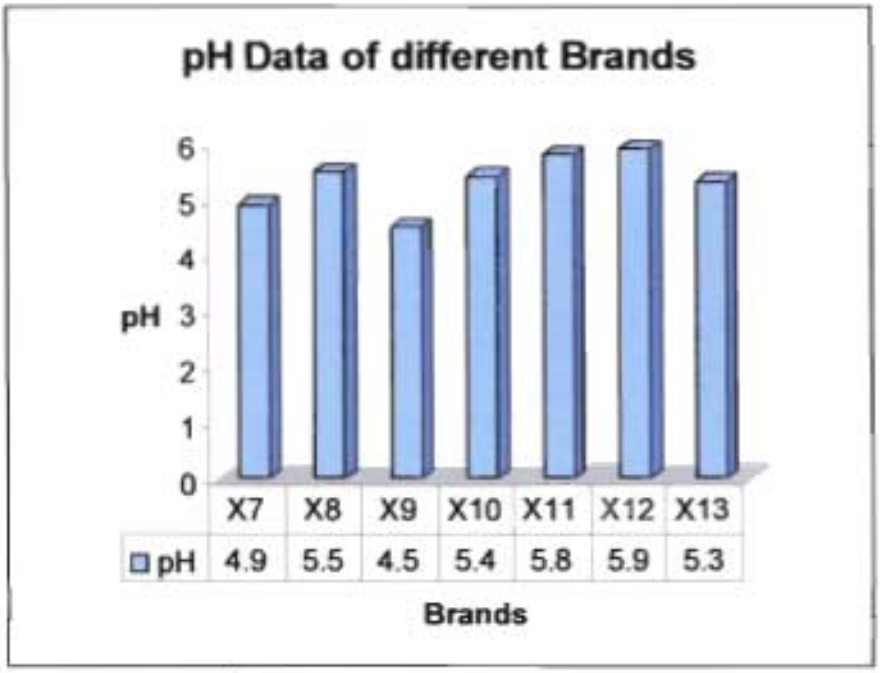

Figure 3.1: **pH Data of 1st day of different brands of amoxicillin suspension (X_7 to X_{13}) after reconstitution**

Brand X_7-X_{13} showed good pH profile compared to the established standard range for amoxicillin, ranging from 4-7 which is ideal for the stability of antibiotic.

3.3 pH Data of 1st day of different brands of amoxicillin suspension after reconstitution (X_{14} to X_{18}) in Bangladesh:

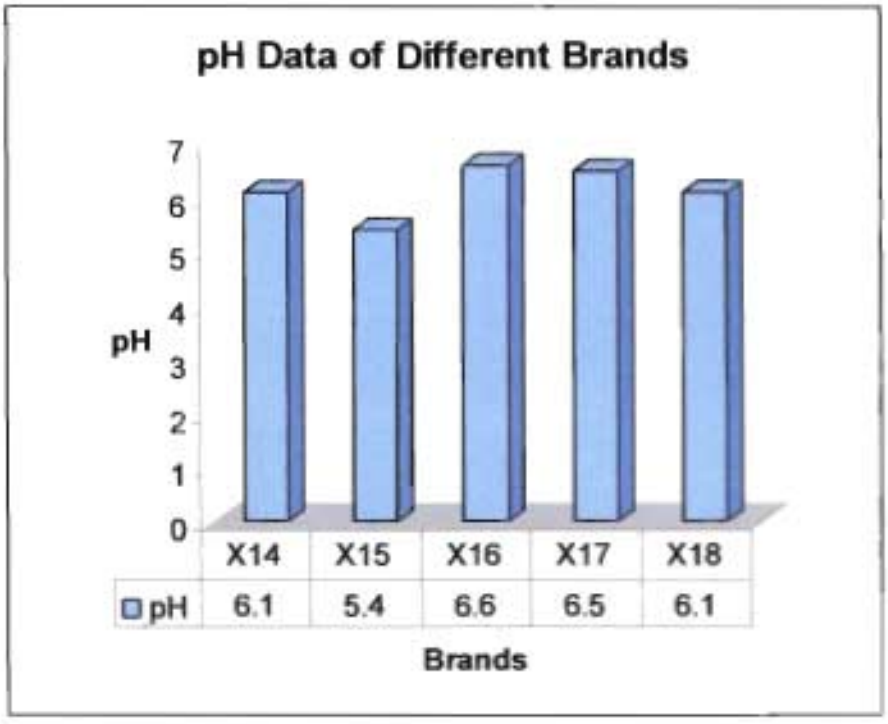

Figure 3.2: **pH Data of 1st day of different brands of amoxicillin suspension (X_{14} to X_{18}) after reconstitution**

Brand X_{14}-X_{18} showed a very good pH profile ranging from 4-7 which is ideal for the stability of antibiotic.

Zone of Inhibition of the Samples

3.4 Zone of inhibition of 1st day for X_1, X_2, X_3, X_4, X_5, X_6, X_{14} and X_{15}

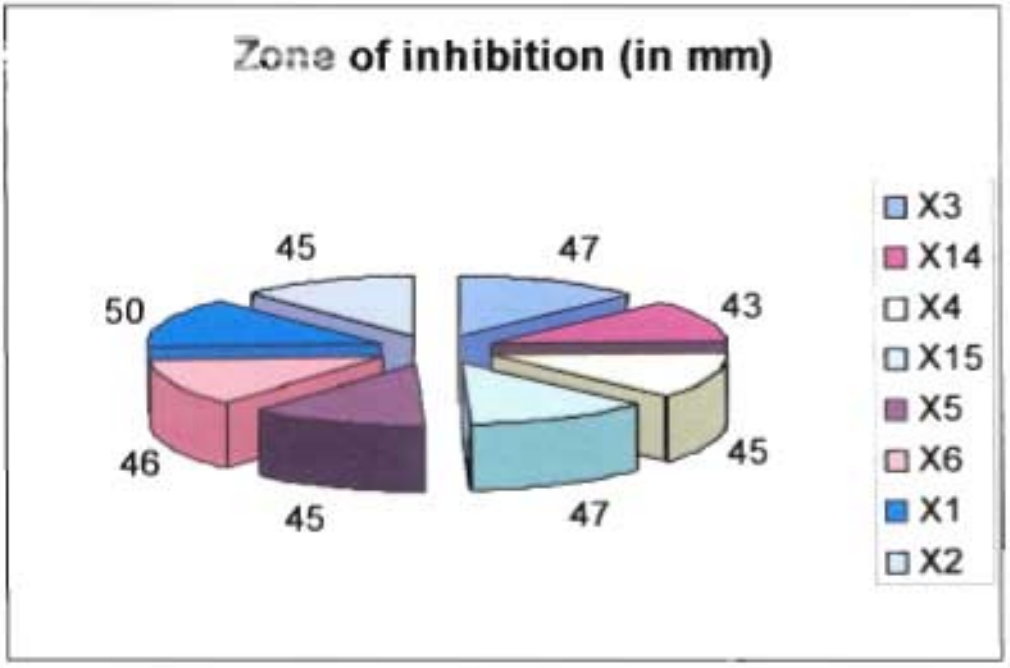

Fig 3.3: **Zone of inhibition of 1st day for X_1,X_2,X_3,X_4,X_5,X_6,X_{14} and X_{15}**

The 1st day data of above mentioned drugs showed good zone of inhibition which indicated the positive sensitivity of the drugs (X_1,X_2,X_3,X_4,X_5,X_6,X_{14} and X_{15})

3.5 Zone of inhibition of last day for X_1, X_2, X_3, X_4, X_5, X_6, X_{14} and X_{15}

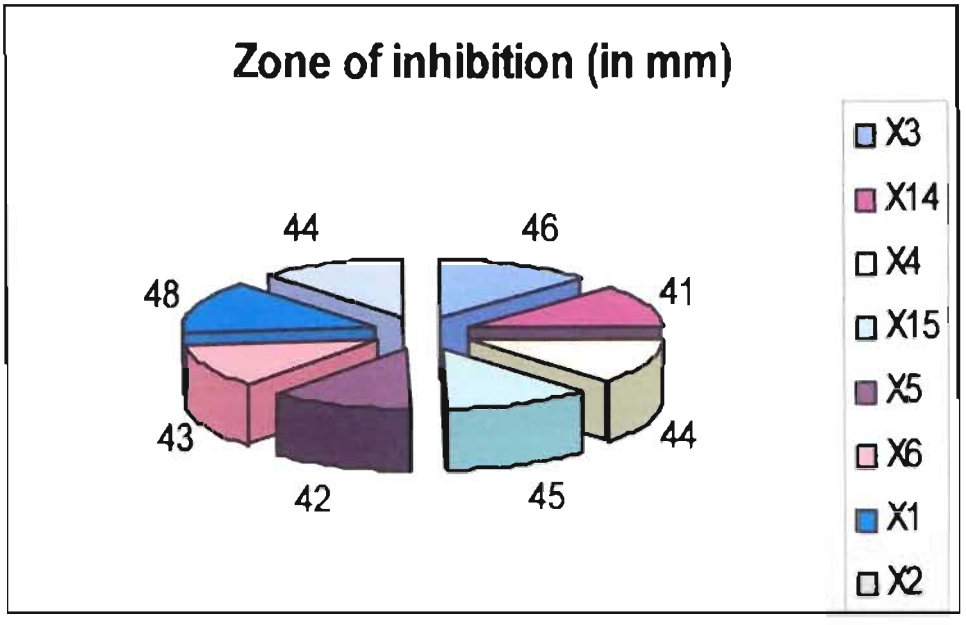

Fig 3.4: **Zone of inhibition of last day for X_1, X_2, X_3, X_4, X_5, X_6, X_{14} and X_{15}**

The last day data of above mentioned drugs showed reduced zone diameter as compared with the first day. However, they were within the established zone of inhibition of amoxicillin for *E.coli* which indicated the positive sensitivity of the drugs (X_1, X_2, X_3, X_4, X_5, X_6, X_{14} and X_{15})

3.6 Zone of inhibition of 1st day for X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{16}, X_{17}, X_{18}

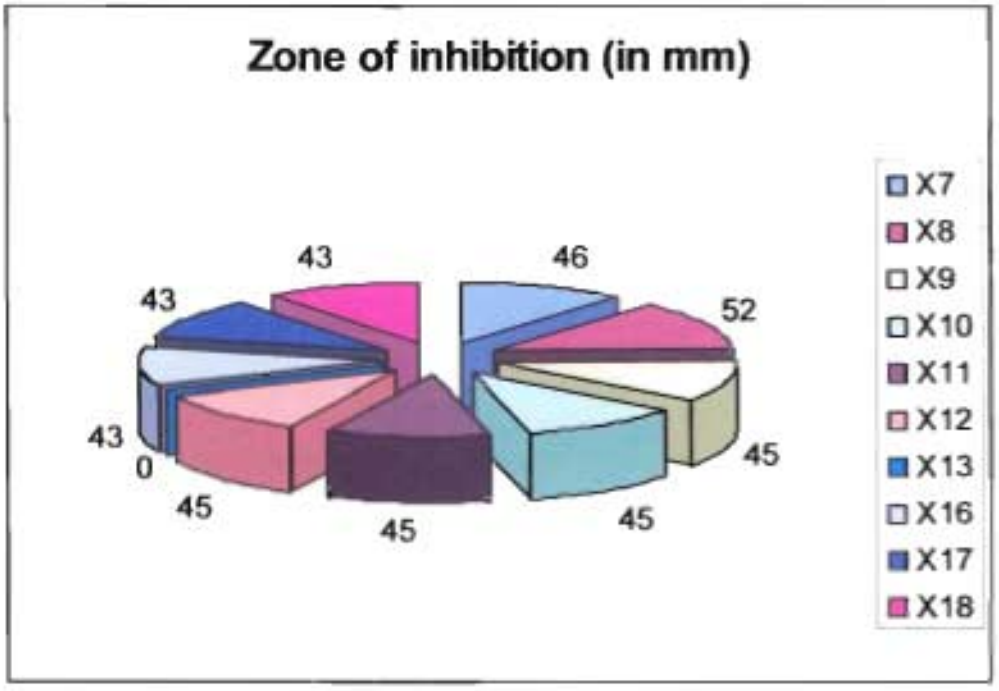

Fig 3.5: **Zone of inhibition of 1st day for X_7,X_8,X_9,X_{10},X_{11},X_{12},X_{13},X_{16}, X_{17}, X_{18}**

The 1st day data of above mentioned drugs showed good zone of inhibition which indicated the positive sensitivity of the sample drugs (X_7,X_8,X_9,X_{10},X_{11},X_{12},X_{13},X_{16}, X_{17}, X_{18})

3.7 Zone of inhibition of last day for X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{16}, X_{17}, and X_{18}

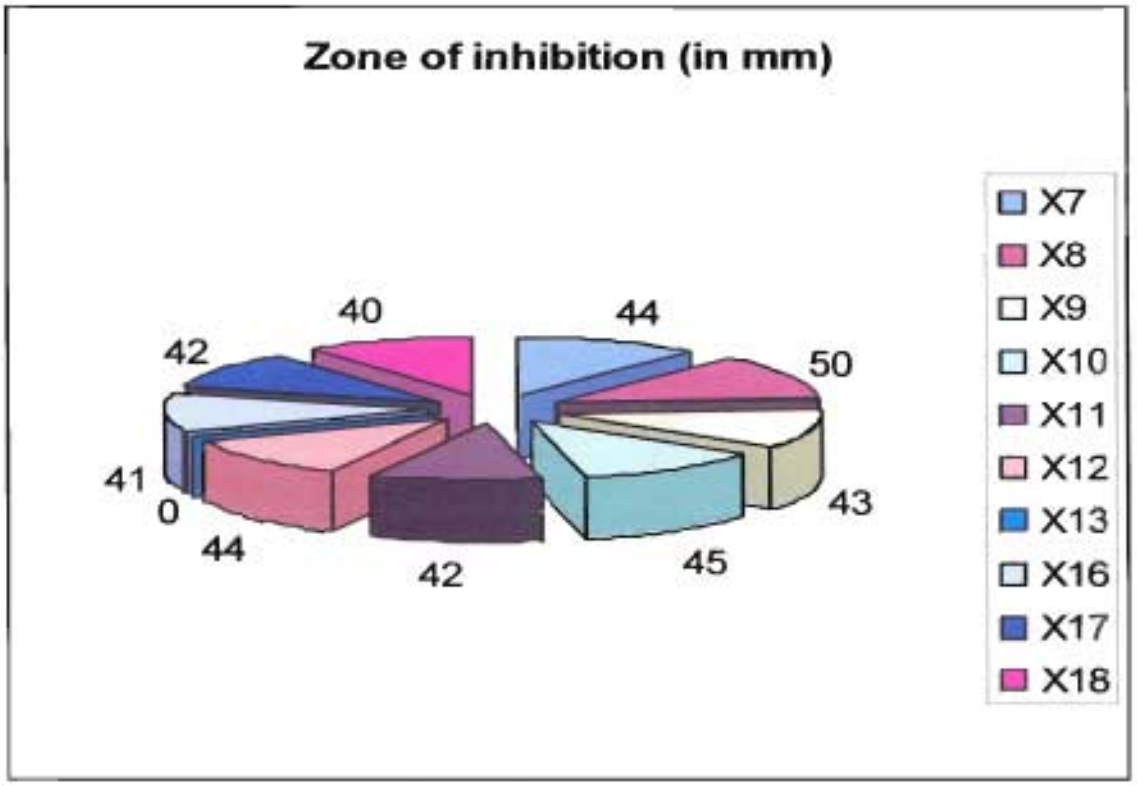

Fig 3.6: **Zone of inhibition of last day for X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{16}, X_{17}, X_{18}**

The last day data of above mentioned drugs showed reduced zone as compared to the zone shown on the first day. However, they were within the established zone of inhibition of amoxicillin for *E.coli* which indicated the positive sensitivity of the sample drugs (X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{16}, X_{17} and X_{18}).

Figure 3.7: **zone of inhibition of X_1 (50 nm)**

Figure 3.8.: **zone of inhibition of X_2 (45 nm)**

Figure 3.9: **zone of inhibition of X_3 (47 nm)**

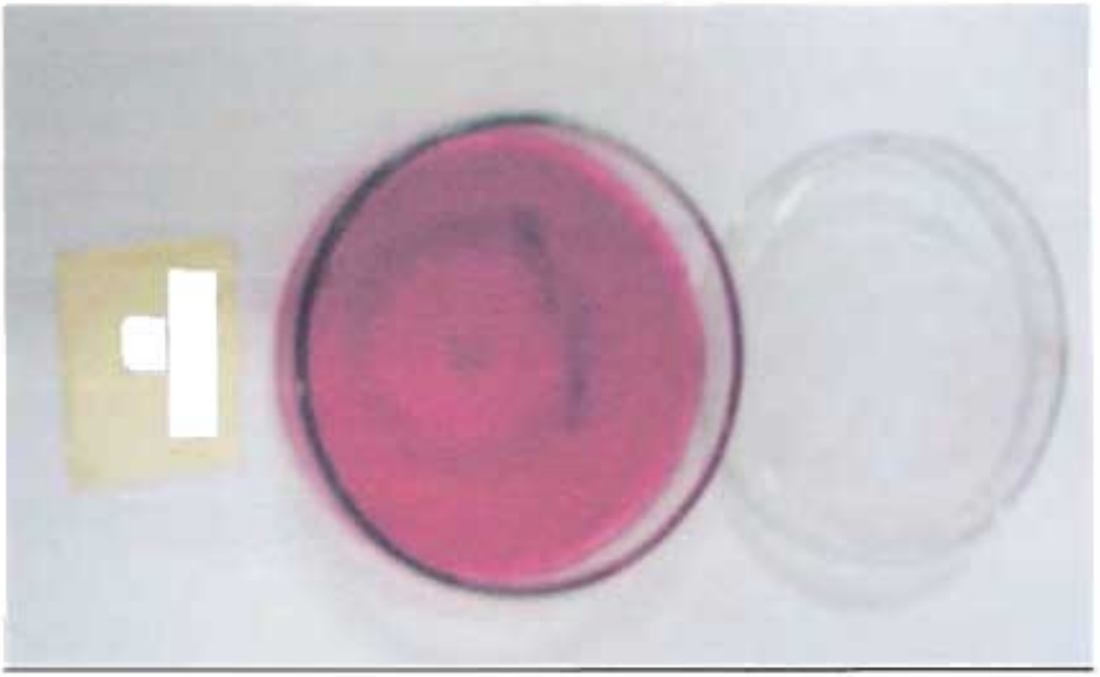

Figure 3.10: **zone of inhibition of X_4 (45)**

Figure 3.11: **zone of inhibition of X_5 (45 nm)**

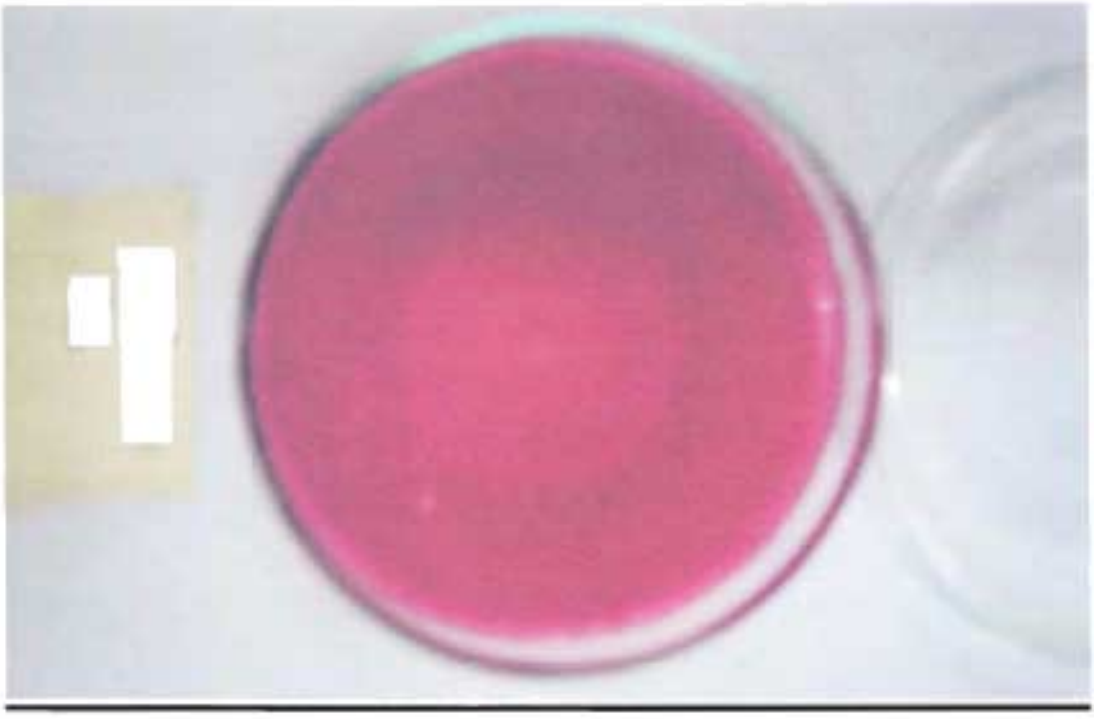

Figure 3.12: **zone of inhibition of X_6 (46 nm)**

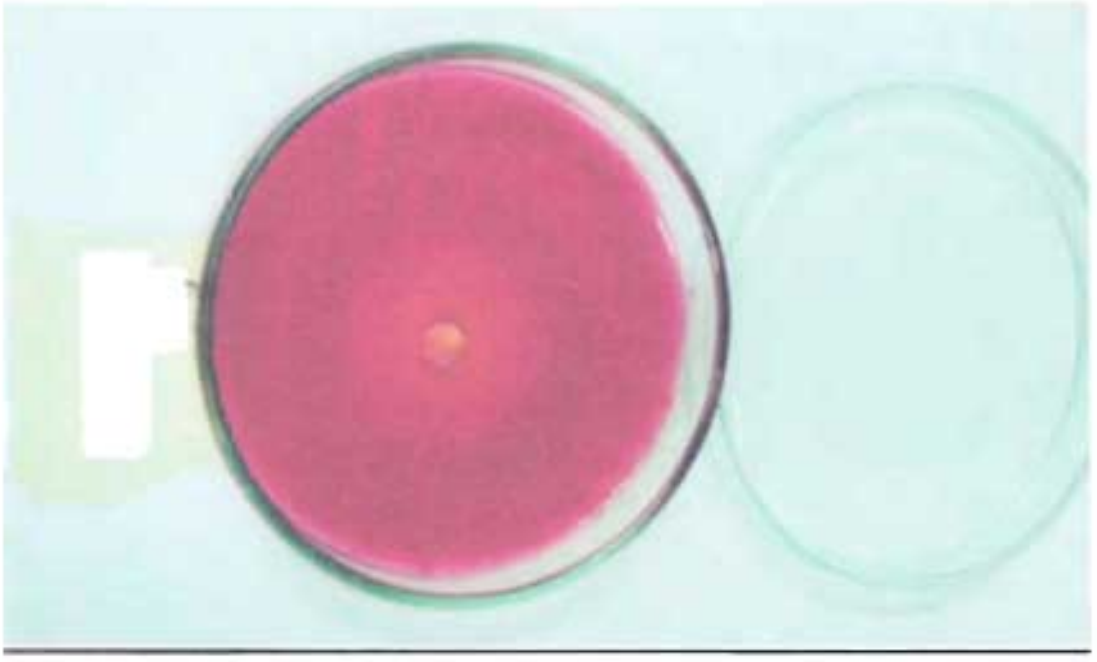

Figure 3.13 : **zone of inhibition of X_7 (46 nm)**

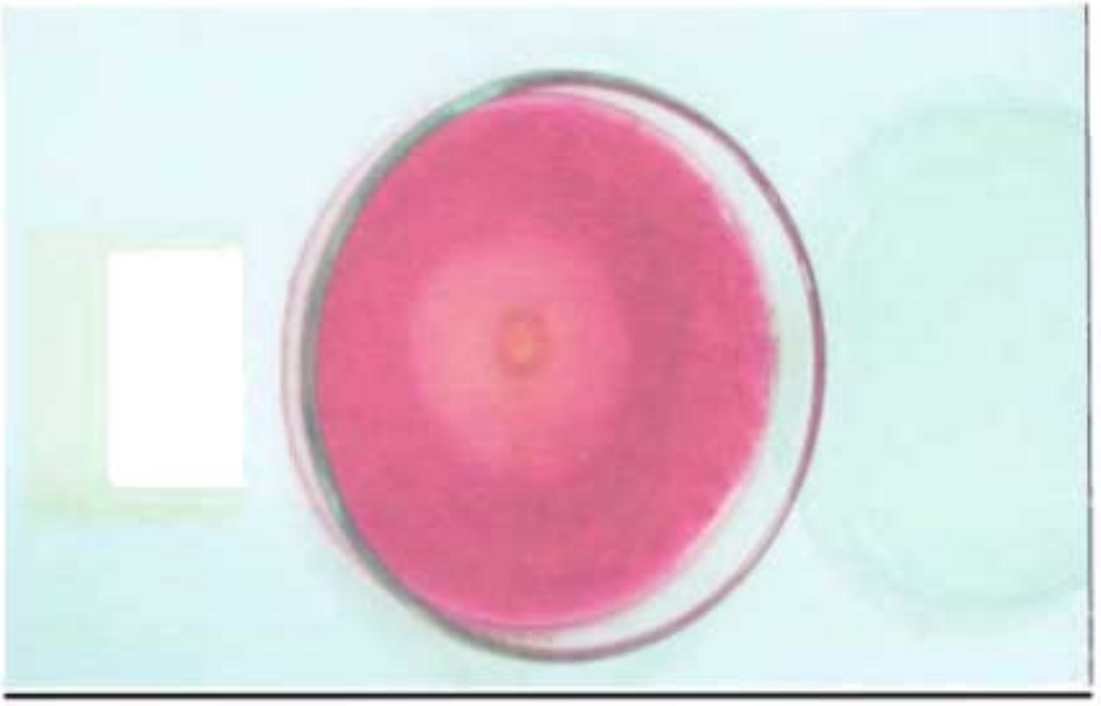

Figure 3.14: **zone of inhibition of Xs (52 nm)**

Figure 3.15: **zone of inhibition of X9 (45 nm)**

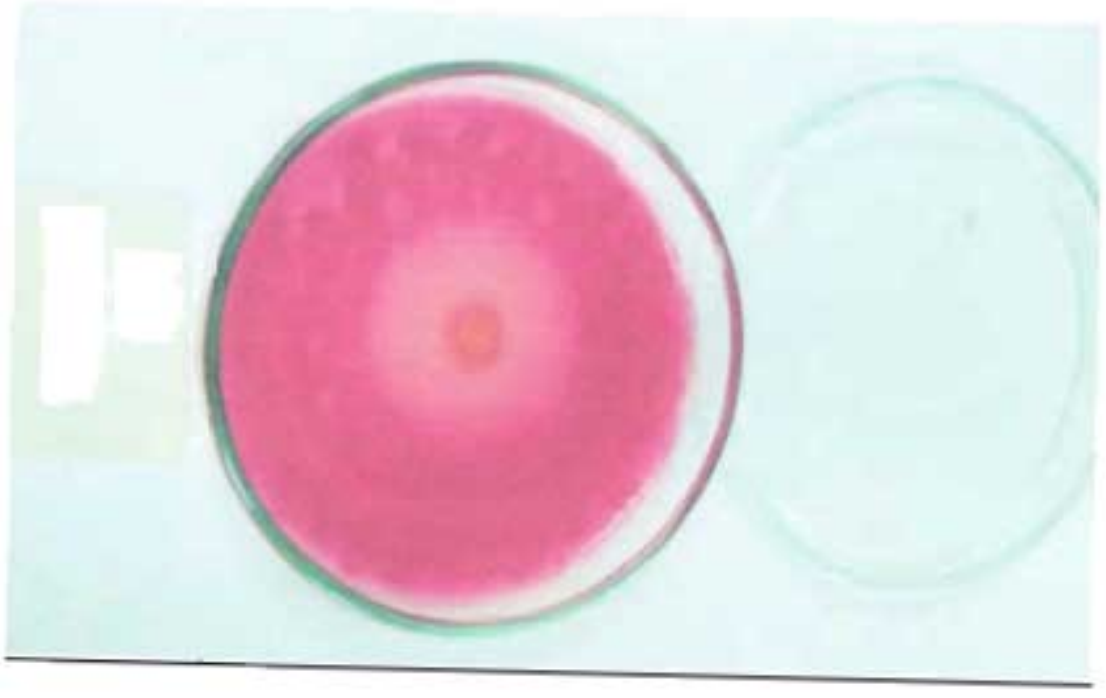

Figure 3.16: **zone of inhibition of X_{10} (45 nm)**

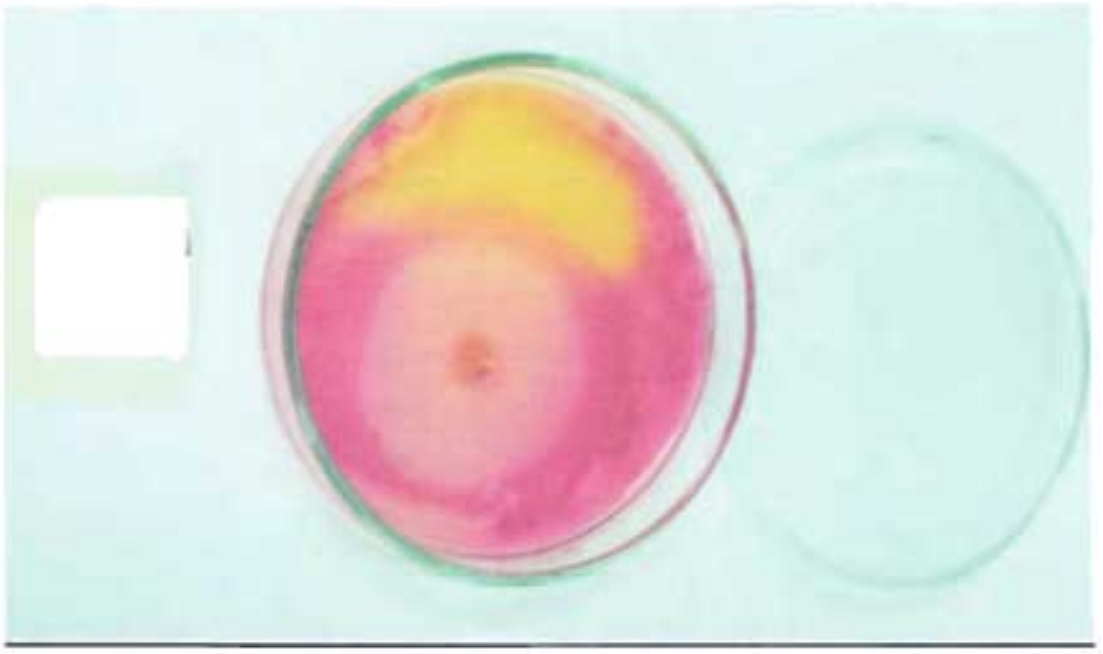

Figure 3.17: **zone of inhibition of X_{II} (45 nm)**

Figure 3.18: **zone of inhibition of X_{12} (45 nm)**

Figure 3.19: **zone of inhibition of X_{13} (0 nm)**

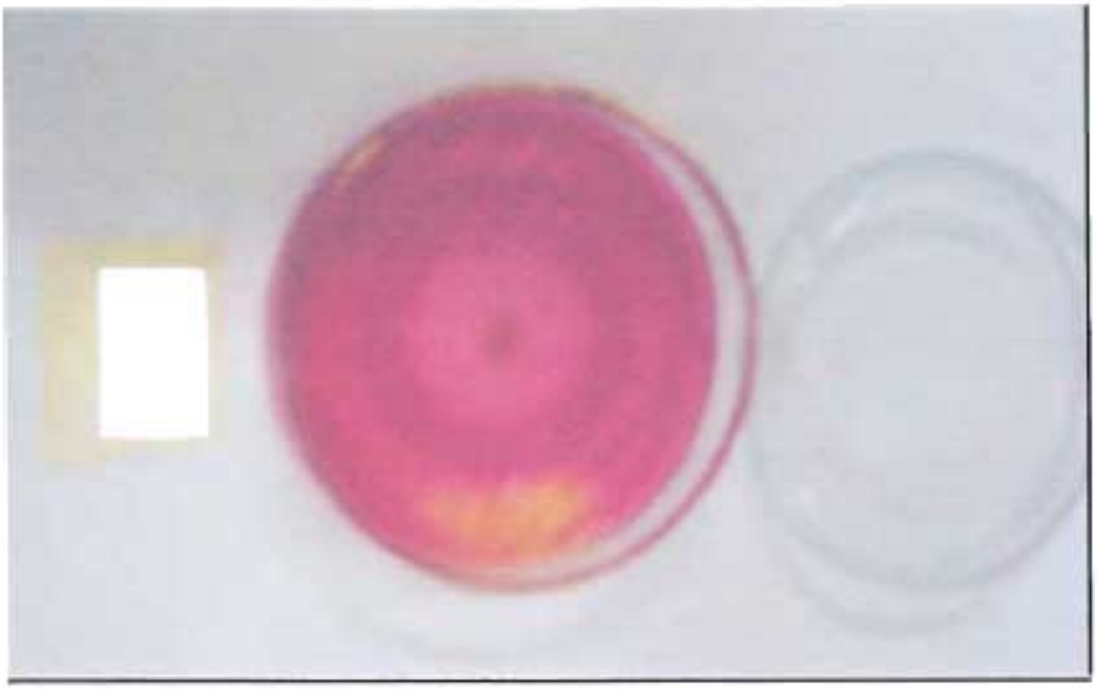

Figure 3.20: **zone of inhibition of X_{14} (43 nm)**

Figure 3.21: **zone of inhibition of X_{15} (47 nm)**

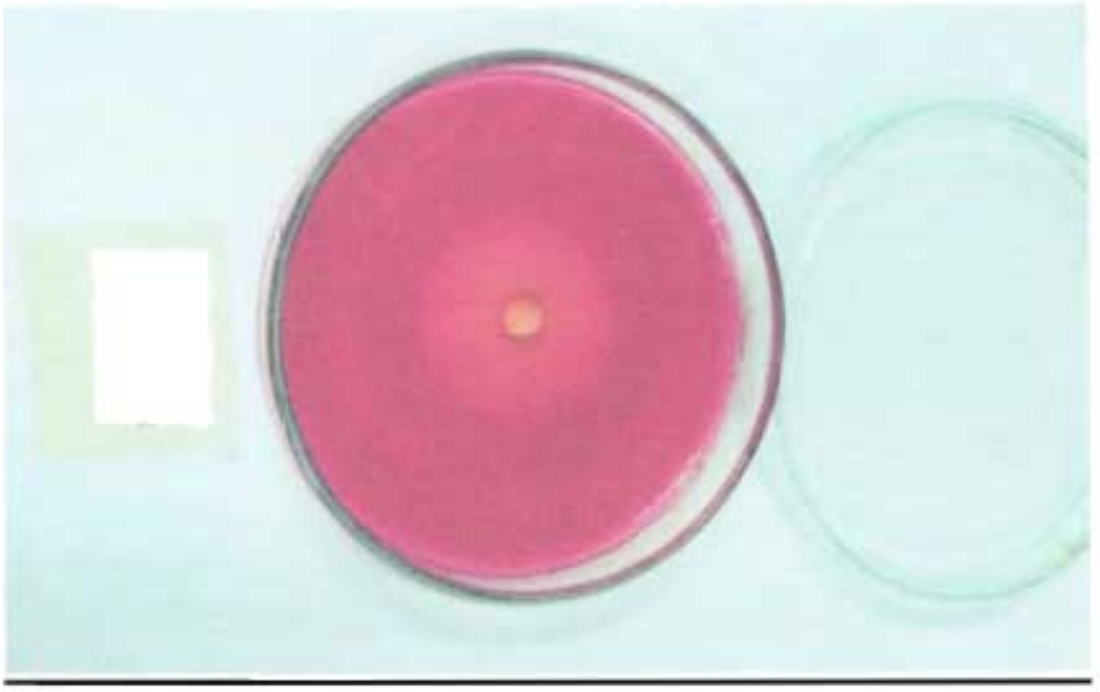

Figure 3.22: **zone of inhibition of X_{16} (43 nm)**

Figure 3.23: **zone of inhibition of X_{17} (43 nm)**

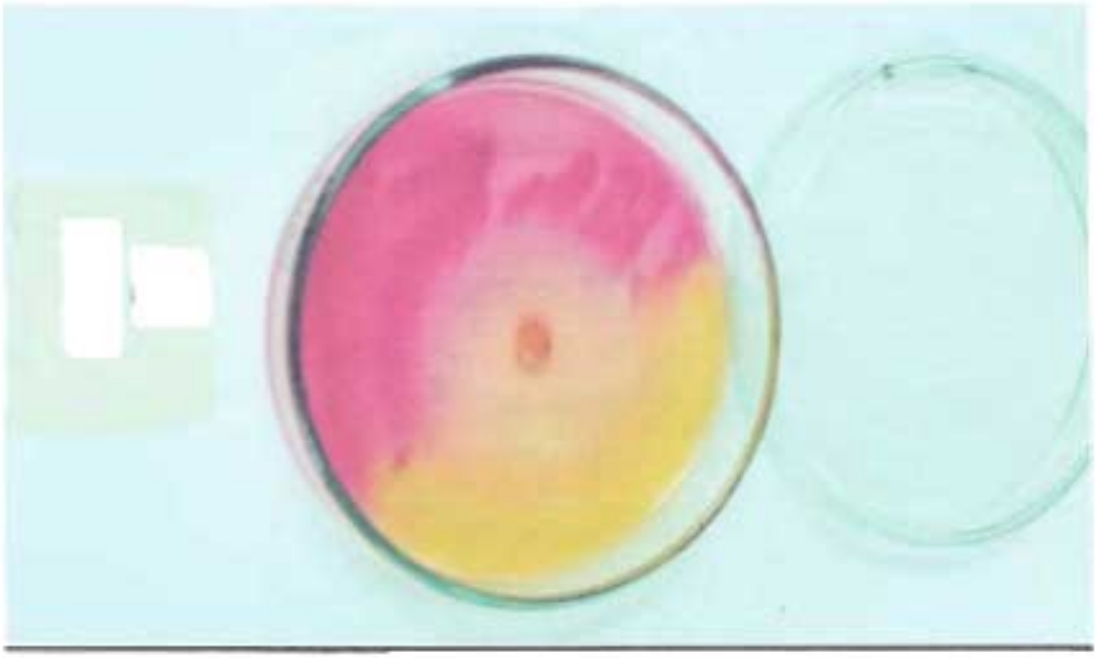

Figure 3.24: **zone of inhibition of X_{18} (43 nm)**

The zone of inhibition was fairly good in as per seen in result. It was higher than the usual zone (8-15mm standard) which was approximately 40 mm in average. 17 brands showed high zone of inhibition on sensitivity test, only one brand showed no zone. The high zone of inhibition might be due to high concentration of drug during disk diffusion.

Chapter-4

Discussion

Discussion:

Our research has been carried out on 18 randomly selected pharmaceutical companies. From the pH profile of our selected brands for experiment, we found that 13 brands (X_1-X_{13}) showed the established pH range which is between 4-7. Five brands (X_{14}-X_{18}) showed excellent pH criteria which ranges from 6-7 and considered the optimum pH for the stability of β-lactam antibiotics.

The optimum of pH of amoxicillin is between 4-7 (British Pharmacopoeia) If the pH falls and antibiotic becomes acidic, its β-lactam ring will be destroyed and penicilloic acid will form which will not give potent β-lactam ring activity. Consequent fall of pH leads to destruction of penicillin and thus it will lose it pharmacological action. Loss of activity can be significantly reduced by adding buffer to maintain the pH between 4-7 Hydrolysis of the product also lead to destruction of β-lactam ring of the antibiotic. The hydrolysis rate increases with rise of temperature. So, to, maintain the drugs' stability, we stored the drugs in low temperature (refrigerator) to retain its stability.

Our study was carried out to show, most drugs were within the stable pH range. From other studies we come to know that the heavily substituted beta-lactams are stable under physiological conditions including in the presence of enzymes of the digestive tract. The beta-lactams were unstable in base. At pH 11.3 and 37°C they were hydrolyzed with half-lives of 1.5-2 h ***(Knight, Green, et al, 1992)***.

The reasons for fluctuating pH profile (within the range) can be of various reasons. For example, it can be due to reconstitution error, manufacturing error, salt imbalance (used as buffer), storage condition, and impurity in ingredients, temperature and finally instrumental error

The zone of inhibition was fairly good in as per seen in result. It was higher than the usual zone which was approximately 40 mm in average. According to WHO, the zone of inhibition for *E.coli* in MacConkey agar is 8-15 mm. Seventeen brands of amoxicillin

showed high zone of inhibition on sensitivity test, only one brand showed no zone. The high zone of inhibition might be due to high concentration of drug in the dosage form.

In another study, it was shown that the sensitivities to penicillins and to a penicillin and β-lactamase inhibitor combination agent were determined for *Helicobacter pylori* strains that were sensitive, moderately resistant, or highly resistant to amoxicillin. All strains were resistant to nafcillin and oxacillin. Moderately resistant strains showed an intermediate zone of inhibition to ticarcillin, mezlocillin, piperacillin, and amoxicillin-clavulanic acid. High-level resistance was associated with the smallest zone size for all penicillins tested ***(Gore, Graham, et al, 1999)***.

The procedure followed throughout the experiment was intended to be a fair and neutral manner so that no condition can bias the result or the outcome. For this, firstly the MacConkey Agar was prepared. This agar was chosen because this agar is used especially for culturing gram (-)ve bacterium and as the target organism of this experiment is a gram (-)ve bacterium, the choice is naturally most desirable to make the result very reliable. Following the preparation of MacConkey Agar, live *E.coli* bacterium was cultured for 24 hours and after which, by Pour Plate Method, the cultured *E.coli* bacterium was transferred to another MacConkey Agar, which was provided all condition for a healthy culture of *E.coli*. Thus from this point onwards, the sensitivity of the amoxicillin drug samples were truly being tested. As for the pH test, a simple but accurate and reliable investigation was done by a pH meter, which provided accurate pH readings for all the randomly chosen samples of amoxicillin.

The overall result predicts that the brands which were used in this research act as good antibiotic considering the sensitivity test and they can be prescribed against *E.coli* for better treatment. The drugs varied in their pH profile within the established range. For this, storage condition and manufacturing process has to be performed with extra precaution. Only with proper stability and sensitivity action, amoxicillin dry syrup/suspension can become an important antibiotic drug against food borne disease and will be able to save many lives, especially children if doctors prescribe this drug based on satisfactory research result. Further studies are needed to determine the entire stability

and sensitivity of these drugs against different pathogenic strains in order to understand the quality of medicine available in Bangladesh.

Chapter-5

Conclusion

Conclusion:

From the research conducted, we can now fully appreciate the importance of the findings of the stability, sensitivity of the amoxicillin manufactured in Bangladesh. *E.coli* bacteria were the target organism of the experiment. If the drug amoxicillin is not effective or not up to the required standard in terms of its sensitivity and stability, it may leave the treatment uncompleted. It may also lead to the rise of strains of bacterium developing resistance against the antibiotic.

Based on the research conducted upon randomly selected amoxicillin manufactured in 18 companies in Bangladesh, all the brands showed the established pH range for amoxicillin to be stable.

It is important to note that out of the 18 selected brands as aforementioned 17 of them had shown impressive zone of inhibition in the bacterium culture. Only one of the brands, however, failed to show any zone of inhibition. On an average, the zone of inhibition was 40 mm.

The overall results from the experiment conducted indicate that the brands that have their amoxicillin tested can be safely prescribed to the patients suffering from *E.coli* infection. Although there are some shortcomings in some of the drugs, the remedies suggested are such that storage condition and manufacturing process has to be performed with extra precaution, which will hopefully enable the drugs to overcome their respective shortcomings.

Therefore, the antibiotic amoxicillin can be an effective fighter of food borne diseases caused by *E.coli* bacterium, especially in Bangladesh where food borne diseases by this bacterium is very common, only if the amoxicillin meet the required standard in terms of its potency, stability and sensitivity.

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