In Vitro Antimicrobial Susceptibility Test of Doxin®&Doxysina® (OPSONIN & IBNSINA) Brands of Bangladesh

Submitted By

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ID: 2007-3-70-060

Submission Date: 11.07.12

Department of Pharmacy

EAST WEST UNIVERSITY

In Vitro Antimicrobial Susceptibility Test of Doxin®&Doxysina® (OPSONIN & IBNSINA) Brands of Bangladesh

A thesis report submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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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, Aftabnagar, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Quazi Tanzeem-Ul-Haque (ID-2007-3-70-060)

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CERTIFICATE

This is to certify that the thesis in vitro antimicrobial susceptibility test of Doxin® and Doxysina® of Bangladesh submitted to the Department of Pharmacy, East West University, Aftabnagar, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Quazi Tanzeem-Ul-Haque (ID-2007-3-70-060) 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, laboratory facilities availed of this connection is dully acknowledged.

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THIS RESEARCH PAPER IS DEDICATED TO MY PARENTS, MY ELDER SISTER, MY RESPECTABLE SUPERVISORS, ALL MY FRIENDS & WELL WISHERS

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Abstract:

Objective: The study is carried out to evaluate the antimicrobial susceptibility test of two different brands of Doxycycline available in the market of Bangladesh. Doxin® 100mg and Doxysina® 100mg were selected for the study that were manufactured by Opsonin Pharmaceutical Co. Ltd. and Ibnsina Pharmaceutical Co. Ltd respectively. This study also helped us to investigate whether these brands were manufactured according to the standard specification or not by the companies. **Method**: Two different brands of antibiotic were collected from the local market of Bangladesh. 200µg, 250µg, 300µg, 350µg, 400µg doses were selected for both of the brands and antimicrobial test was carried out according to the Kirby-Bauer disc diffusion method to determine the zone of inhibition against four different microorganisms (*S. pneumoniae, Staphylococcus aureus, H. influenzae and E. coli*). **Results:** From the experiment it was observed that all the capsules of doxycycline of two brands in different concentrations showed significant zone of inhibition and that were approximately similar to that of the standard. **Conclusion**: The studied two brands of doxycycline were capable to inhibit the growth of susceptible microorganisms. Further investigation need to be carried out to determine the quality parameters of the two brands of doxycycline.

CHAPTER ONE:

DOXYCYCLINE

CHAPTER TWO:

MATERIALS & METHODS

CHAPTER THREE:

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1.1 Introduction

Doxycycline (alpha-6-deoxytetracycline) is a second-generation tetracycline with increased oral bioavailability and tissue penetration as a result of its improved lipophilicity compared with earlier tetracyclines. It is a semisynthetic derivative of oxytetracyclin and became available in 1967. It is available as both doxycycline monohydrate and doxycycline hyclate. Tetracyclines were once a very popular antibiotic, but have not been used as much lately due to the resistance problems that have developed. Newer broad-spectrum antibiotics have replaced tetracyclines in many cases. They are still popular in certain cases though. Some examples are low dose oral and topical acne therapy, upper respiratory tract infections, sexually transmitted diseases, Rocky Mountain spotted fever and many others. Among the tetracyclines, doxycycline is probably the most popular. Some reasons for this are that it is rapidly absorbed, causes less gastro-intestinal disturbance and has a half-life that allows it to be dosed once daily. While the use of tetracyclines is on the decline, doxycycline will likely be used for years (Damon P Eisen, 2012).

1.2 Chemistry of Doxycycline

The chemical designation of the light-yellow crystalline powder is alpha-6-deoxy-5 oxytetracycline. The molecular formula of doxycycline is $C_{22}H_{24}N_2O_8.HCl$, ½ $[C_2H_5OH.H_2O]$; the molecular weight is 512.9 and the chemical structure is shown in Figure 1.1.

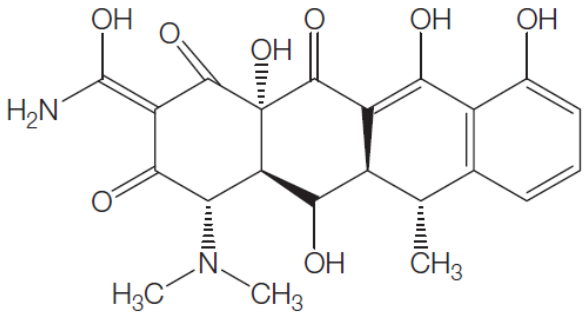

Fig 1.1: Doxycycline. (Damon P Eisen, 2012)

The basic structure of doxycycline contains a napthacene ring structure with a number of different groups attached at various sites. Doxycycline has significant improvements to the original tetracycline, including a replacement of the 6-hydroxyl group with a hydrogen, allowing

for better stability in both acidic and basic conditions, as well a better absorption profile and a longer half-life (Medicinal Chemistry Project, 2012)

1.3 Mechanism of Action

Bacteria need to synthesize proteins in order to ensure their reproduction. This biological activity requires the capture of nutrients from the surrounding environment at the expense of the host. Among those nutrients are the amino acids, which are incorporated in the bacteria's ribosomes, the cell organites where protein synthesis takes place. Doxycycline is generally bacteriostatic against a wide variety of organisms, both gram-positive and gram-negative. In gram-negative bacteria, transportation of the doxycycline into the cell occurs either by passive diffusion or through an energy-dependent active transport system. The latter system is also believed to exist in gram-positive bacteria. Doxycycline is more lipophilic than the other tetracyclines, which allows it to pass easily through the lipid bilayer of bacteria. Doxycycline penetrates the bacterial cell and interferes with the protein biosynthesis, stopping the process of bacteria reproduction. Bacteria cannot reproduce and die or are killed by the defense mechanisms (white cells) of the host. Doxycycline can also alter the cytoplasmic membrane and this in turn causes leakage of nucleotides and other compounds out of the cell. This does not directly kill the bacteria but instead inhibit it (Damon P Eisen, 2012).

1.4 Spectrum of Activity

The tetracyclines are primarily bacteriostatic and are thought to exert their antimicrobial effect by the inhibition of protein synthesis. The tetracyclines, including doxycycline, have a similar antimicrobial spectrum of activity against a wide range of gram-positive and gram-negative organisms. Cross-resistance of these organisms to tetracyclines is common.

Gram-Negative Bacteria

- *Neisseria gonorrhoeae*
- *Calymmatobacterium granulomatis*
- *Haemophilus ducreyi*

- *Haemophilus influenzae*
- *Yersinia* pestis (formerly Pasteurella pestis)
- *Francisella tularensis* (formerly *Pasteurella tularensis*)
- *Vibrio cholera* (formerly *Vibrio comma*)
- *Bartonella bacilliformis*
- *Brucella* species

Because many strains of the following groups of gram-negative microorganisms have been shown to be resistant to tetracyclines, culture and susceptibility testing are recommended:

- *Escherichia coli*
- *Klebsiella* species
- *Enterobacter aerogenes*
- *Shigella* species
- *Acinetobacter* species (formerly Mima species and Herellea species)
- *Bacteroides* species (Medicinal Chemistry Project, 2012)

Gram-Positive Bacteria

Because many strains of the following groups of gram-positive microorganisms have been shown to be resistant to tetracycline, culture and susceptibility testing are recommended. Up to 44 percent of strains of *Streptococcus pyogenes* and 74 percent of *Streptococcus faecalis* have been found to be resistant to tetracycline drugs. Therefore, tetracycline should not be used for streptococcal disease unless the organism has been demonstrated to be susceptible.

- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*
- Enterococcus group (*Streptococcus faecalis* and *Streptococcus faecium*)
- Alpha-hemolytic streptococci (*viridans* group)

Other Microorganisms:

- *Rickettsiae*
- *Chlamydia psittaci*
- *Chlamydia trachomatis*
- *Mycoplasma pneumoniae*
- *Ureaplasma urealyticum*
- *Borrelia recurrentis*
- *Treponema pallidum*
- *Treponema pertenue*
- *Clostridium* species
- *Fusobacterium fusiforme*
- Actinomyces species
- *Bacillus anthracis*
- *Propionibacterium acnes*
- *Entamoeba species*
- *Balantidium coli*
- *Plasmodium falciparum*

Doxycycline has been found to be active against the asexual erythrocytic forms of *Plasmodium falciparum* but not against the gametocytes of *P. falciparum* (Medicinal Chemistry Project, 2012).

Table 1.1: Doxycycline susceptibility data emphasizing organisms for which this antibiotic is an important therapeutic choice

	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Range (mg/l)
Gram-positive bacteria			
Staphylococcus aureus	0.25	4	0.03 to >16
Streptococcus pyogenes	0.12	8	
Streptococcus pneumoniae	0.25	8	0.06-8
Enterococcus fecalis	16	32	12-64
Bacillus anthracis	0.63	0.63	0.031–0.125
Listeria monocytogenes	0.25	0.25	0.125–1
Nocardia asteroides	2	32	<0.125-32
Nocardia farcinica	8	16	2-16

Gram-negative bacteria			
Escherichia coli	0.5	32	0.5-64
Klebsiella spp.	1	64	1-64
Haemophilus influenzae	0.5	1	
Burkholderia pseudomallei	1	1.5	0.125-4
Burkholderia mallei	0.5	2	0.125-4
Stenotrophomonas maltophilia	1	2	0.5-4
Leptosira interrogans		1.56	0.1-12.5
Yersinia pestis	0.5	1	0.125-2
Neisseria meningitidis	0.5	1	0.12-2
Brucella melitensis	0.032	0.064	0.0156–0.094
Mycoplasma pneumoniae	0.12	0.25	0.06-0.25
Legionella pneumophila	1	2	0.5-2
Chlamydia pneumoniae	0.12	0.25	0.06-0.50
Chlamydia trachomatis	0.125	0.25	0.125-0.25
Chlamydia psittaci	0.1		0.05-0.2
Rickettsiae			0.06–0.125
Coxiella burnetti			2-4
Bartonella spp.	0.03	0.12	<0.016-0.12
Plasmodium falciparum	1.2–5.4		
Mycobacterium marinum	2	6	0.5-12

(Damon P Eisen, 2012)

1.5 Therapeutic Uses

To reduce the development of drug-resistant bacteria and maintain effectiveness of Doxycycline capsules USP and other antibacterial drugs, Doxycycline capsules USP should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.

Doxycycline capsules USP is indicated for the treatment of the following infections:

- Rocky mountain spotted fever, typhus fever and the typhus group, Q fever, rickettsial pox, and tick fevers caused by *Rickettsiae*.
- Respiratory tract infections caused by *Mycoplasma pneumoniae*.
- Lymphogranuloma venereum caused by *Chlamydia trachomatis*.
- Psittacosis (ornithosis) caused by *Chlamydia psittaci*.
- Trachoma caused by *Chlamydia trachomatis*, although the infectious agent is not always eliminated as judged by immunofluorescence.
- Inclusion conjunctivitis caused by *Chlamydia trachomatis*.
- Uncomplicated urethral, endocervical or rectal infections in adults caused by *Chlamydia trachomatis*.
- Nongonococcal urethritis caused by *Ureaplasma urealyticum*.
- Relapsing fever due to *Borrelia recurrentis*.

Doxycycline is also indicated for the treatment of infections caused by the following gram-negative microorganisms:

- Chancroid caused by *Haemophilus ducreyi*.
- Plague due to *Yersinia pestis* (formerly *Pasteurella pestis*).
- Tularemia due to *Francisella tularensis* (formerly *Pasteurella tularensis*).
- Cholera caused by *Vibrio cholerae* (formerly *Vibrio comma*).
- Campylobacter fetus infections caused by *Campylobacter fetus* (formerly *Vibrio fetus*).
- Brucellosis due to *Brucella* species (in conjunction with streptomycin).
- Bartonellosis due to *Bartonella bacilliformis*.
- Granuloma inguinale caused by *Calymmatobacterium granulomatis*.
- Because many strains of the following groups of microorganisms have been shown to be resistant to Doxycycline, culture and susceptibility testing are recommended.

Doxycycline capsules USP is indicated for treatment of infections caused by the following gram-negative microorganisms, when bacteriologic testing indicates appropriate susceptibility to the drug:

- *Escherichia coli*
- *Enterobacter aerogenes* (formerly *Aerobacter aerogenes*)
- *Shigella* species
- *Acinetobacter* species (formerly *Mima* species and *Herellea* species)
- Respiratory tract infections caused by *Haemophilus influenzae*.
- Respiratory tract and urinary tract infections caused by Klebsiella species.
- Doxycycline capsules USP is indicated for treatment of infections caused by the following gram-positive microorganisms when bacteriologic testing indicates appropriate susceptibility to the drug:
- Upper respiratory infections caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*).
- Skin and skin structure infections caused by *Staphylococcus aureus*.
- Anthrax due to *Bacillus anthracis*, including inhalational anthrax (post-exposure): to reduce the incidence or progression of disease following exposure to aerosolized *Bacillus anthracis*.
- Doxycycline capsules USP is not the drug of choice in the treatment of any type of staphylococcal infections.
- When penicillin is contraindicated, Doxycycline is an alternative drug in the treatment of the following infections:
  i) Uncomplicated gonorrhea caused by *Neisseria gonorrhoeae*.
  ii) Syphilis caused by *Treponema pallidum*.
  iii) Yaws caused by *Treponema pertenue*.
  iv) Listeriosis due to *Listeria monocytogenes*.
  v) Vincent's infection caused by *Fusobacterium fusiforme*.
  vi) Actinomycosis caused by *Actinomyces israelii*.
  vii) Infections caused by *Clostridium* species.
  viii) In acute intestinal amebiasis, Doxycycline may be a useful adjunct to amebicides.
  ix) In severe acne, Doxycycline may be useful adjunctive therapy.

(Doxycycline, 2012)

Table 1.2: Major clinical uses and outcome data for doxycycline

Indication	Outcomes
Randomized controlled trials	
Non-gonococcal urthethritis	Doxycycline for 1 week equivalent to one dose azithromycin, cure C. trachomatis 97% versus 98%
Pelvic inflammatory disease	Doxycycline plus one shot i.m. cefoxitin equivalent to inpatient doxy plus cefoxitin
Brucellosis	Doxycycline –gentamicin equivalent to Doxycycline –streptomycin
Lyme disease	Prevention; 200 mg Doxycycline effective prevention after *Ixodes* tick bite
Leptospirosis	Doxycycline equivalent to penicillin G and cefotaxime for severe leptospirosis
Q-fever endocarditis	Doxycycline –hydroxychloroquine superior to doxy–ofloxacin with reduced relapse
Malaria prophylaxis	Protective efficacy 93–100% for *P. falciparum* Equivalent protective efficacy to mefloquine Protective efficacy 99–100% for *P. vivax*
Rickettsia	Mediterranean spotted fever (MSF); 2 days Doxycycline equivalent to 2 days ciprofloxacin Mild scrub typhus; 1 week Doxycycline equivalent to one dose azithromycin
Onchocerciasis (Wolbachia)	6 weeks of Doxycycline 100 mg interrupts O.

	volvulus embryogenesis for 18 months
Melioidosis	Doxycycline combined with chloramphenicol and cotrimoxazole used for 20 weeks continuation treatment
Plague	Doxycycline equivalent to gentamicin (and streptomcyin historical controls)
Bartonella quintana	Doxycycline –gentamicin reduces the risk of relapsed bacteremia compared with placebo

(Damon P Eisen, 2012)

1.6 Side Effects

Medicines and their possible side effects can affect individual people in different ways. The following are some of the side effects that are known to be associated with this medicine. Just because a side effect is stated here does not mean that all people using this medicine will experience that or any side effect.

- Disturbances of the gut, such as nausea, vomiting, diarrhoea, indigestion or abdominal pain.
- Difficulty or pain when swallowing (dysphagia).
- Inflammation of the food pipe (oesophagitis).
- Loss of appetite.
- Flushing.
- Sensation of ringing or other noise in the ears (tinnitus).
- Increased sensitivity of the skin to UV light
- Aching muscles or joints.
- Disturbances in the normal numbers of blood cells in the blood (rare). Consult your doctor if you experience unusual bruising or bleeding, tiredness, sore throat, fever or

other signs of infection while taking this medicine, as these could be symptoms of problems with your blood cells.

- Liver problems, including jaundice, hepatitis or liver failure (rare).
- Inflammation of the pancreas (pancreatitis - rare).
- Skin rashes (rare).
- Mild increase in pressure within the skull (benign intracranial hypertension). This is rare. However, you should stop taking this medicine and consult your doctor if you experience a severe persistent headache or changes in your vision while taking this medicine, e.g. blurred or double vision or loss of vision, as these could be symptoms of this condition.
- Rarely, severe allergic reactions such as swelling of the lips, throat and tongue (angioedema), severe skin rashes or anaphylactic shock. (Doxycycline, 2012)

1.7 Adverse Effects

Mechanism of action due to oral Doxycycline's virtually complete absorption, side effects to the lower bowel, particularly diarrhea, has been infrequent. The following adverse reactions have been observed in patients receiving Doxycyclines.

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, glossitis, dysphagia, enterocolitis, and inflammatory lesions (with monilial overgrowth) in the anogenital region. Hepatotoxicity has been reported. These reactions have been caused by both the oral and parenteral administration of tetracyclines.

Skin: Maculopapular and erythematous rashes, Stevens-Johnson syndrome, toxic epidermal necrolysis, and erythema multiforme have been reported. Exfoliative dermatitis has been reported but is uncommon.

Renal Toxicity: Rise in BUN has been reported and is apparently dose related.

Hypersensitivity Reactions: Urticaria, angioneurotic edema, anaphylaxis, anaphylactoid purpura, serum sickness, pericarditis, and exacerbation of systemic lupus erythematosus.

Blood: Hemolytic anemia, thrombocytopenia, neutropenia, and eosinophilia have been reported with tetracyclines.

Other: Bulging fontanels in infants and intracranial hypertension in adults.

When given over prolonged periods, doxicyclines have been reported to produce brown-black microscopic discoloration of the thyroid gland. No abnormalities of thyroid function are known to occur. (Doxycycline, 2012)

1.8 Pharmacokinetics & Pharmacodynamics

1.8.1Bioavailability

Doxycycline is almost completely absorbed in the duodenum after oral administration, and it has a prolonged serum half-life (12–16 hours). Eighty-two percent to 93% of doxycycline is protein bound (Agwuh and MacGowan, 2006). Co-administration with food has minimal impact on doxycycline levels – these being reduced by only 20%. All tetracyclines form complexes with metal ions in food, but doxycycline complexes are unstable in the acid contents of the stomach, so that the drug enters the duodenum in a free state, where it is absorbed. However, metal complexes formed in the alkaline contents of the small bowel, into which doxycycline diffuses as part of its mode of excretion, are stable and are not absorbed. The net effect is that the total absorption of doxycycline is partially impaired by the presence of multivalent cations, such as ferrous sulphate (Neuvonen et al., 1970), and by subsalicylate bismuth given simultaneously or 2 hours before doxycycline (Ericsson *et al.*, 1982). Iron reduces the AUC0–24 by 10% (Newton *et al.*, 2005).

The bioavailability of doxycycline is not reduced by proton pump inhibitors or H2 blockers but serum levels are lowered and bioavailability reduced by 85% if the drug is taken together with aluminum magnesium hydroxide (Maalox) (Deppermann *et al.*, 1989). Aluminum hydroxide taken orally also lowers the serum levels after I.V. doxycycline administration. This interaction may be due in part to an interference of aluminum ions with the enteric reabsorption of doxycycline (Nguyen *et al.*, 1989). Co-administration of doxycycline and cytochrome P450 3A4

inducers, including phenytoin, carbamazepine and rifampicin, reduce serum doxycycline levels presumably because of increased hepatic metabolism of the drug (Colmenero *et al.*, 1994)

1.8.2 Drug distribution

Table 1.3: Summary of doxycycline pharmacokinetics after a 200-mg dose

C_{max} (lg/ml)	t_{max} (h)	$t_{1/2}$ (h)	AUC (µg/ml/h)	Comments
5.27±1.5	2.7±0.8	132.7±0.85	90±16	
9.3	-	14	112	I.V. dose
3.17 (1.63–7.72)	2 (1.5–4)	10.5 (6.9–17.9)	32 (18.7–79.7)	Acute phase P. falciparum
4.44 (1.52–8.64)	3 (1.5–8)	11.6 (8.5–17.2)	48.6 (18.3–69.8)	Convalescence P. falciparum

(Damon P Eisen, 2012)

The key pharmacokinetic features of doxycycline are summarized in Table 1.3. After oral administration, the peak serum level is usually attained 2–3 hours later. After a 200 mg oral dose of doxycycline, peak serum levels of 5.0–5.4 mg/ml at 3–4 hours were found in fasted subjects, with subsequent levels of 2.9–4.0 and 1.3–2.2 mg/ml after 8 and 24 hours, respectively (Welling *et al.* 1977). There are few data on dose linearity with regards to doxycycline. When a single oral dose of 500 mg doxycycline was administered after food, a mean peak serum level of 15.29 mg/ml was obtained at 4 hours, and this fell to levels of 6.60, 3.42, 1.24, and 1.0 mg/ml after 24, 48, 72, and 96 hours, respectively (Adadevoh *et al.*, 1976). After a 200 mg i.v. infusion of doxycycline, a peak serum level of 5–10 mg/ml is usually attained (Alestig, 1973), which falls slowly and levels, ranging between 1–2 mg/ml persisting for 24 hours (Klastersky *et al.*, 1972). After a 100 mg single oral dose the AUC is 37–40 mg h/l (Malmborg, 1984), and after a 200 mg single dose, 90716 mg h/l (Welling *et al.*, 1977).

Doxycycline is more highly lipid soluble than earlier tetracyclines, and thus it has better tissue penetration. In dogs, lipophilicity of the tetracyclines has been correlated with many of their transport characteristics; it facilitates their transport across lipid-rich cell membranes and, therefore, doxycycline penetrates more readily than tetracycline into the brain, eyes and intestinal

epithelium. Doxycycline also penetrates more readily into bacterial cells (Nikaido and Thanassi, 1993). Interstitial fluid concentrations of doxycycline are 54% of serum evels, as shown by a blister fluid model study in healthy volunteers (Schreiner and Digranes, 1985). Doxycycline is concentrated in the bile with levels 10–25 times that in serum (Alestig, 1973). Doxycycline concentrations in thoracic duct lymph and peritoneal fluid are maintained at about 75% of simultaneous serum levels (Andersson *et al.*, 1976), and those in colonic tissue and particularly ileal tissue are equivalent to or exceed serum levels. Prostatic concentrations are up to 60% of serum levels (Eliasson and Malmborg, 1976; Oosterlinck *et al.*, 1976). Pleural fluid penetration was determined after 200 mg I.V. doxycycline was given to patients with pleurisy, with levels up to 25% of serum detected at 2 hours (Lode, 1979). Salivary concentration of doxycycline is poor, and, after an oral dose of 600 mg doxycycline, peak salivary concentrations occurred at 8 hours and were only 8% of the mean serum level at the time (Marlin and Cheng, 1979). After oral doses of 100 mg daily, mean salivary levels were 0.1–0.5 mg/ml (Heimdahl and Nord, 1983), and such concentrations were unaffected by parotitis (Eneroth *et al.*, 1978). Penetration into sputum is poor, having been shown to be 8– 28% over multiple time-points (Marlin *et al.*, 1981). Low concentrations of doxycycline are achieved in bone (Dornbusch, 1976), skin, subcutaneous fat, and tendon tissue, but levels in muscle are higher (Gnarpe *et al.*, 1976).

Therapeutic concentrations of doxycycline may occur in the aqueous humor, but cerebrospinal fluid (CSF) concentrations do not exceed 1 mg/ml in subjects with noninflamed meninges (Andersson and Alestig, 1976). Effective concentrations of doxycycline may be achieved in the CSF, as the range of CSF penetration in patients with central nervous system (CNS) disease is broad. In patients with Lyme disease treated with doxycycline 200 mg orally 12-hourly, CSF penetration 2–3 hours after a dose was 15% with a concentration of 1.1 mg/ml. With a doxycycline dose of 100 mg 12-hourly, the CSF concentration at that time was only 0.6 mg/ml (Dotevall and Hagberg, 1989). Yim *et al.* (1985) detected a mean CSF doxycycline level of 1.3 mg/ml (range 0.8–2.0) in five patients with latent or neurosyphilis receiving 200 mg twice daily for 7 days.

Doxycycline penetrates well into breast milk (Chow and Jewesson, 1985), with levels of up to 40% of plasma (British Columbia’s Children’s and Women’s Pharmacy, 2003), as it is less

bound to calcium than other tetracyclines, there is the potential for toxicity in the breast-fed infant.

1.8.3 Clinically important pharmacokinetic and pharmacodynamic features

The pharmacodynamics of doxycycline is poorly studied, but the available data indicate that there is concentration-dependent killing of *S. aureus, S. pneumoniae*, and *Escherichia coli* (Cunha *et al.*, 2000). Doxycycline has a postantibiotic effect for these same bacteria (Cunha *et al.*, 2000). There are no AUC/MIC static effect targets published currently to aid in determining clinical breakpoints. Breakpoints, if available, are therefore largely determined by epidemiologic MIC distributions and clinical experience (Sader *et al.*, 2007)

1.8.4 Excretion

Renal excretion of doxycycline occurs solely by glomerular filtration. Urinary excretion accounts for 30–65% of an orally administered dose of doxycycline (Steigbigel *et al.*, 1968; Alestig, 1973; Alestig 1974; Mahon *et al.*, 1976). In renal impairment this is reduced, but increased fecal excretion prevents accumulation of the drug High concentrations of doxycycline, up to 14 mg/ml, are attained in bile, but this route of elimination normally accounts for only a small percentage of an administered dose (Mahon *et al.*, 1970; Alestig, 1974). A large proportion of doxycycline excreted in bile is reabsorbed from the intestine.

With doxycycline, that part of an administered dose which is not excreted in the urine is excreted in the feces. Doxycycline diffuses from blood across the small bowel wall into the lumen, where cationic chelation occurs, preventing absorption (Whelton *et al.*, 1974). The contents of the small bowel, being constantly added to from the stomach and other secretions, easily cope with the binding of successive amounts of doxycycline. Biliary excretion contributes only a small amount to the fecal excretion of doxycycline. In the presence of renal impairment, increased amounts of doxycycline are excreted in the feces, thereby preventing accumulation of the drug in the serum (Alestig, 1974; Whelton *et al.*, 1974; Mahon *et al.*, 1976). For instance, Whelton *et al.* (1974) found that 77% of an orally administered dose given to end-stage renal failure patients was excreted in the feces.

Doxycycline is not substantially metabolized or inactivated by enzymatic means *in vivo*, with no human biometabolites identified. However, concomitant administration of barbiturates, phenytoin or carbamazepine and rifampicin (Colmenero *et al.*, 1994) shorten the serum half-life of doxycycline, suggesting that these drugs increase the metabolism of doxycycline potentially through liver metabolism and it is also possible that these drugs interfere with the protein binding of doxycycline, thereby encouraging its excretion.

1.9 Drug Interactions

Doxycycline is a substrate of CYP3A4 enzymes and a moderate inhibitor of the same cytochrome P450 drug-metabolizing system. Therefore, its levels may be decreased by CYP3A4 inducers. Phenytoin, carbamazepine and barbiturates apparently induce the metabolism of and reduce the serum concentration of doxycycline. Other drugs that can reduce doxycycline levels by this mechanism include nafcillin, nevirapine, and rifampicin. Cholestyramine binds doxycycline. Doxycycline has been shown to increase methotrexate levels in one patient, precipitating neutropenia and gut toxicity (TortajadaIturen *et al.*, 1999). In patients treated for acne with both doxycycline and retinoic acid derivatives, the risk of benign intracranial hypertension is increased. Failure of the oral contraceptive pill (OCP) and increased risk of pregnancy has been suggested to be a consequence of doxycycline therapy. A pharmacokinetic study of women showed no reduction in serum ethinyl estradiol levels with concomitant doxycycline use. Furthermore, no progesterone rise was found, showing that breakthrough ovulation did not occur (Neely *et al.*, 1991). Reported failure of the OCP while taking doxycycline may be due to antibiotic-associated diarrhea or reduced compliance consequent on nausea and vomiting, all of which may reduce serum ethinyl estradiol levels. An observation suggesting clinically relevant antagonism between penicillin and an early tetracycline, aureomycin arose from analysis of the outcome of pneumococcal meningitis patients. The 79% mortality seen in patients treated with the penicillin–tetracycline combination was significantly higher than in patients treated with penicillin monotherapy. More patients had adverse prognostic features in the penicillin group, and, although the study involved only 57 patients, it was adequately powered to provide a statistically valid result (Lepper and Dowling, 1951). Doxycycline has more recently been shown experimentally to have synergistic or additive effect

with beta-lactams on clinical *Stenotrophomonas maltophilia* isolates (San Gabriel *et al.*, 2004) and C. trachomatis (How *et al.*, 1985) and on balance, the concerns of antagonism between penicillin and tetracyclines, raised in 1951, have not been borne out by subsequent in vitro data and clinical experience.

1.10 Resistance

The mechanism of resistance of doxycycline, and tetracyclines in general, involves the production of bacterial proteins that, even with the drug bound to the ribosomes, allows the continuation of protein biosynthesis. At this time, it is not understood exactly how this happens. Another mechanism of resistance is through the active efflux of the magnesium-chelated drug to the outside of the bacterial cell in exchange for protons (Medicinal Chemistry Project, 2012).

1.11 Precautions

All patients taking Doxycycline should be advised:

- To avoid excessive sunlight or artificial ultraviolet light while receiving Doxycycline and to discontinue therapy if phototoxicity (e.g., skin eruptions, etc.) occurs. Sunscreen or sunblock should be considered.
- To drink fluids liberally along with Doxycycline to reduce the risk of esophageal irritation and ulceration.
- That the absorption of tetracyclines is reduced when taken with foods, especially those which contain calcium. However, the absorption of Doxycycline is not markedly influenced by simultaneous ingestion of food or milk.
- That the absorption of tetracyclines is reduced when taking bismuth subsalicylate.
- Not to use outdated or poorly stored Doxycycline.
- That the use of Doxycycline might increase the incidence of vaginal candidiasis.

Diarrhea is a common problem caused by antibiotics which usually ends when the antibiotic is discontinued. Sometimes after starting treatment with antibiotics, patients can develop watery and bloody stools (with or without stomach cramps and fever) even as late as two or more months after having taken the last dose of the antibiotic. If this occurs, patients should contact their physician as soon as possible.

Patients should be counseled that antibacterial drugs including Doxycycline capsules should only be used to treat bacterial infections. They do not treat viral infections (e.g., the common cold). When Doxycycline capsules are prescribed to treat a bacterial infection, patients should be told that although it is common to feel better early in the course of therapy, the medication should be taken exactly as directed. Skipping doses or not completing the full course of therapy may

(1) Decrease the effectiveness of the immediate treatment and

(2) Increase the likelihood that bacteria will develop resistance and will not be treatable by Doxycycline capsules or other antibacterial drugs in the future. (Doxycycline, 2012)

1.12 Quality Control and Assurance

Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Ideal antibiotic therapy is based on determination of the aetiological agent and its relevant antibiotic sensitivity. Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed due to the seriousness of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic. Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host's immune system can overcome them.

Standardized susceptibility test procedures require the use of quality control microorganisms to control the technical aspects of the test procedures. Quality control (QC) microorganisms are specific strains of organisms with intrinsic biological properties. QC strains are very stable strains which will give a standard and repeatable susceptibility pattern. The specific strains used for microbiological quality control are not clinically significant. QC is performed to check the quality of medium, the potency of the antibiotic, to check manual errors. Quality control strains

should be included daily with the test. Not more than 1 in 20 results should be outside accuracy limits. No zone should be more than 4 standard deviations away from midpoint between the stated limits.

If, for reasons of expense or manpower constraints, it is not possible to include all strains on a daily basis, then the following guidelines should be followed.

The frequency can be decreased to once weekly if proficiency has been demonstrated by

1. Performing QC daily for 30 days with less than 10% inaccuracy for each drug
2. Proficiency testing is repeated for each new drug included in the testing
3. All documentation is maintained indefinitely
4. Proficiency testing is repeated for each new batch of media or reagents

All tests must be within accuracy limits if QC is done once weekly (Lalitha, M.K.; 2004).

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 (e.g., 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).

Doxycycline 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 tetracycline antibiotic that has a longer half-life (approximately 60 hours) and better pharmacokinetic properties compared to the tetracycline. 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).

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

Aim of the study

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

2.1 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 Doxycycline can be determined by disc diffusion method by following Standard Pharmacopeia's recommendation on antimicrobial susceptibility test (Donald *et al*, 2006).

2.2 Kirby-Bauer disc diffusion susceptibility test procedure

2.2.1 The Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate .If only one organism is to be tested e.g. *M. tuberculosis*, the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism. The dilutions are made in a small volume of water

and added to agar which has been melted and cooled to not more than 60^{o}C. Blood may be added and if 'chocolate agar' is required, the medium must be heated before the antibiotic is added (Lalitha, M.K., 2004).

2.3 Apparatus and reagents

1. Petri dish,
2. Autoclave,
3. Laminar Air Flow,
4. Hot Air Oven,
5. 1000ml Bottle,
6. Nutrient Agar (Media),
7. Normal Saline (0.9% NaCl),
8. Cotton Buds,
9. Micropipette,
10. Centrifuge Tube,
11. 100ml Volumetric Flask,
12. Beaker,
13. Distilled Water,
14. Forceps,
15. Bunsen Burner,
16. Inoculating Loop,
17. Electronic Balance,
18. Ruler,
19. Disc (Prepared by filter paper).

2.4 Sample Preparation

Seventeen 100mg Doxycycline capsule was uncovered. Then weighed and recorded in the Record Book. 200mg, 250mg, 300mg, 350mg and 400mg equivalent powder weighed and that kept in tube. Added distilled water with Doxycycline powder to make 10ml solution. Mixed the solution by shaking carefully where Doxycycline readily soluble with the distilled water. The solution was filtered by using filter paper then ready to antimicrobial test.

2.5 Preparation of dried filter paper discs

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

2.6 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.

2.7 Preparation of Agar plate

Agar plate was allowed to come to room temperature. If the surface of the agar has visible liquid present, the plate should be inverted, agar on its lid would allow the excess liquid to drain from the agar surface and to evaporate. The plates were placed in a laminar air flow. Each agar plate was appropriately labeled for each organism to be tested.

2.8 Placement of the antibiotic and blank disc

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

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

a) The Muller-Hinton agar plate was placed over the disc template.
b) One disc was removed from the cartridge using forceps that had been sterilized.
c) The plate was lifted and the disc was placed over one of the positioning marks.

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

2.9Incubation of the plates

A temperature range of 35°c ±2°c was maintained. Temperature above 35°c might not allow the detection of growth and zone of inhibition. The plates were not incubated carbon dioxide as this would decrease the pH of the agar and result in error due to incorrect pH of the media. Results were read after 24 hours of incubation.

2.10 Measuring zone sizes

Following incubation, the zone sizes were measured to the nearest millimeter using a ruler. The diameter of the disc was included in the measurement. When measuring zone diameter, it was round up to the next millimeter. The plate was held a few inches above a black, nonreflecting surface illuminated with reflected light. The plate was viewed using a direct, vertical line of sight to avoid any parallax that might result in misreading. The zone size was recorded on the recording sheet. If the placement of the disc or the size of the zone did not allow to read the diameter of the zone, then it was measured from the centre of the disc to a point on the circumference of the zone where a distinct edge was present(the radius) and the measurement was multiplied by 2 to determine the diameter.

2.11 Procedures

800ml media (Agar) was prepared with distilled Water, where Nutrient Agar was needed 22.4gm. Media and other equipments were sterilized in the autoclaving machine for 1 hour, where temperature range was 60°C To 121°C and pressure was 1 atm. Petridishes were cleaned and kept in the hot air woven to dry and sterilize. At least 1 day before the experiment the bacterial subculture was made. There were four types of bacterial solution prepared with 1ml normal saline (0.9% NaCl) and microorganisms each. Labeling was done before pouring the media into petridish. Media was poured into four petridish equally and wait for being solid the media. The bacterial solution was spreaded on solid media with very careful. After drying the antibiotic discs, they were placed into each petridish very carefully. The plates should be incubated soon after placing the disc. The temperature range of 35°c ±2°c is normally required for incubation and the incubation time was 24 hours which were considered as standard for this test. (Lalitha, M.K., 2004)

3. Results

Antimicrobial sensitivity test was performed and no zone of inhibition was found for the blank test.

3.1. Comparison of zone of inhibition of Doxin® & Doxysina® with standard Doxycycline

Table 3.1.1: Result of zone of inhibition for *Haemophilus influenzae.*

Serial no.	Concentration	Zone of Inhibition (mm)		
		Standard	Doxin®	Doxysina®
01.	200µ/Disc	22.83	25.67	26.16
02.	250µg/Disc	24.50	26.83	27
03.	300µg/disc	32.66	29.66	29.83

Table 3.1.2: Result of zone of inhibition for *Escherichia coli.*

Serial no.	Concentration	Zone of Inhibition (mm)		
		Standard	Doxin®	Doxysina®
01.	300µ/Disc	15	13.33	18.83
02.	350µg/Disc	18.66	16.66	19.33
03.	400µg/disc	24.33	18.66	20.33

Table 3.1.3: Result of zone of inhibition for *Staphylococcus aureus.*

Serial no.	Concentration	Zone of Inhibition (mm)		
		Standard	Doxin®	Doxysina®
01.	300µ/Disc	17.33	13	19.5
02.	350µg/Disc	18	15.66	20.33
03.	400µg/disc	18.66	17.66	21

Table 3.1.4: Result of zone of inhibition for *Sreptococcus pneumoniae.*

Serial no.	Concentration	Zone of Inhibition (mm)		
		Standard	Doxin®	Doxysina®
01.	300µ/Disc	25.33	24.33	26.67
02.	350µg/Disc	27.66	26.5	27.16
03.	400µg/disc	29.66	27.16	28.67

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.

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. Two antibiotics under different brand names have been chosen 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 (200, 250, 300µg/disc for *H. influenzae* & 300, 350, 400µg/disc for *E. coli, S. aureus, S. pneumoniae*) of both the brands of 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.

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.

In the current study, available two brands showed almost similar antimicrobial activity to that of standard, so it could be claimed that manufacturers using pure active pharmaceutical ingredients to prepare these antibiotics.

Furthermore, assay of these antibiotics need to be investigated to determine the biological effectiveness of the two brands of doxycycline *in vivo*.

Conclusion

After performing the antimicrobial susceptibility test for two different brands Doxin® 100mg and Doxysina® 100mg capsules manufactured by Opsonin Pharmaceutical Co. Ltd and IbnSina Pharmaceutical Co. Ltd, where three concentrations 200 µg, 250µg, 300 µg, 350 µg and 400 µg tested for each brand that met all the required specifications for all the quality control parameter. So it can be concluded that, all the two brands capsules were manufactured according to the specification given by the USP/BP and have almost activity like the standard Doxycycline.

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