LIFE SCIENCES

Khulna University Studies 2(1): 159-165

AN INVESTIGATION FOR ANTIMICROBIAL POTENCY OF SOME COMMONLY USED ANTIBIOTICS (CAPSULE) MANUFACTURED BY VARIOUS PHARMACEUTICAL COMPANIES IN BANGLADESH

N.I. Siddiqui[a*] and M. Shahjahan[b]

[a] *Biotechnology Discipline, Khulna University, Khulna 9208, Bangladesh*
[b] *Department of Biochemistry, University of Rajshahi, Rajshahi 6205, Bangladesh*

Manuscript received: July 09, 1999; Accepted: March 25, 2000

Abstract: Antibiotics are indispensable drugs for the treatment of infectious diseases. For proper treatment, antibiotics as well as all drugs should have a standard level of purity. But in our country some pharmaceutical companies are producing impure and substandard antibiotics as have been reported in several newspapers. Consumption of these antibiotics may lead to the development of serious complexities. For this reason, we took the initiative to investigate the biopotency of three widely used antibiotics- ampicillin trihydrate, amoxycillin trihydrate and oxytetracycline hydrochloride. Antimicrobial potency of the antibiotics was determined by disc diffusion method (Beur *et al*, 1993) in nutrient agar and also by minimum inhibitory concentration (MIC) (Annon, 1965). It is evident from the study that some of the pharmaceutical companies are producing substandard antibiotics and physicians and consumers should be careful about the antibiotics.

Key words: Biopotency; Ampicillin trihydrate; Amoxycillin trihydrate; Oxytetracycline hydrochloride

Introduction

Quality of antibiotics depends on some parameters e.g. quantity of active ingredients, degree of antimicrobial activity, purity, disintegration time, quality of the shell (in case of capsule), etc. In our present study we examined the biopotency of the capsules of Ampicillin trihydrate, Amoxycillin trihydrate and oxytetracycline hydrochloride produced by various pharmaceutical companies. Antibiotics are available in various trade names and it is difficult for general people to choose antibiotics of the best quality. More over it is not always possible for the physicians also to know quality of antibiotics. So the best way is to perform qualitative tests for the antibiotics at a regular interval, say, twice a year. It can be performed by government initiative or by the scientists. This type of study may help to detect the companies producing low quality drugs so that people and physicians can be careful about their products.

* Corresponding author. Tel.: 880-41-721791, 720171-3 Ext. 228; Fax: 880-41-731244, 731521; E-mail: <ku@bdonline.com>
DOI: https://doi.org/10.53808/KUS.2000.2.1.159-165-Ls

Materials and Methods

Collection and Storage of the Antibiotics: We have tried to collect each antibiotic of as many company as available, specially those produced by local pharmaceutical companies. We collected ampicillin trihydrate capsules of 16 companies, amoxycillin trihydrate capsules of 17 companies and oxytetracycline hydrochloride capsules of 7 companies from the local pharmaceutical shops in the northern region of Bangladesh e.g. Rajshahi, Nawabgange, Naogaon, Rabgpur and Kushtia. After collection, the antibiotics were kept in the refrigerator at 4°C. Two strips (10 + 10) of antibiotics of each company bearing the same batch number and expiration date were purchased. Capsules of one strip were used for assay and the other strip was stored as a reference so that the authenticity of the assays can be verified in future, if necessary. Table 1 shows the name of the companies with their products, batch number and expiry dates.

In-vitro antimicrobial screening is a useful technique for the detection of biopotency of the antibacterial substances. In general, antibacterial screening is undertaken in two phases: a primary qualitative assay to detect the presence or absence of activity and a secondary assay which quantitates the relative potency, expressed as the minimum inhibitory concentration (MIC) values of active ingredients.

Bioassay: The primary biopotency assay, as normally done by disk diffusion method categorizes organisms into susceptible (intermediate or moderately susceptible) or resistant categories. The secondary assay is the broth dilution assay, which quantitates the antimicrobial activity of a compound by providing the MIC value of the compound for specific susceptible test organisms.

Diffusion assay is based on the ability of antibiotics to diffuse from a confined source through the nutrient agar gel and create a concentration gradient. If the agar media is seeded with a sensitive organism, a zone of inhibition results where the concentration exceeds the MIC for that particular organism. The disc diffusion technique involves the utilization of paper disc on which a known amount of drug has been absorbed and dried. A number of simultaneous events occur during this time.

- Initially, the dried disc absorbs water from the surrounding test medium and the drug dissolves in it.
- Then the drug migrates through the adjacent test medium due to concentration gradient.
- This results into a gradual change of drug concentration in the agar surrounding each disc.

The plates are then incubated in an incubator at 37°C for 16-24 hours. As the antibiotic's diffusion progresses, microbial multiplication also proceeds. After an initial lag phase a logarithmic growth phase is initiated and at that point of time, bacterial multiplication proceeds more rapidly than the drug can diffuses. The bacterial cells, which are not inhibited by the antibacterial agent, will continue to multiply until a layer of growth can be visualized. The activity of antibiotic is evidenced by the presence of a clear zone of inhibition surrounding the disc where the drug is present in inhibitory concentration. Disc-diffusion method is highly effective for rapidly measuring the diameter of the zone

of inhibition. The principal factors, which determine the size of the zone of inhibition, are:

- Intrinsic antimicrobial activity of the drug;
- Growth of the organisms;
- Diffusion rate of the drug which is related to its water solubility;
- Concentration of test organism inoculated in the medium and
- Thickness of the test medium in the petridish.

Table 1. Name of the companies with their products, batch number and expire dates.

Name of the companies	Ampicillin trihydrate		Amoxycillin trihydrate		Oxytetracycline hydrochloride	
	Batch No.	Exp. date	Batch No.	Exp. date	Batch No.	Exp. date
Opsonin Chemical Industries	B02994	Aug 1997	--	--	--	--
Jayson Pharmaceuticals Ltd.	56	Aug 1997	F-193U	Jun 1998	--	--
The Acme Laboratories	409014	Sep 1997	602005	Feb 1999	603005	Mar 1999
Fison's Bangladesh Ltd.	05	Nov 1995	0596	Nov 1999	--	--
Square Pharmaceuticals	409206	Sep 1997	605020	May 1999	401190	Jan 1997
Glaxo Bangladesh Ltd.	23	Oct 1996	133	July 1997	--	--
Ibn Sina Pharmaceuticals	83	Jan1995	605-208	Nov 1998	--	--
ACI Pharmaceuticals	K030	Jul 1996	066	Oct 1997	28	Jan 1997
Navana Pharmaceuticals	0295	Nov 1998	0896	Apr 1998	--	--
Ganashasthya	1019	Sep 1997	28011	Nov 1998	--	--
Pharmaco International Ltd.	010395	Nov 1998	--	--		
Renata Ltd.	539-001	Feb 1998	639-028	May 1999	651-006	Feb 1999
Peoples Pharma	130401	Mar 1997	--	--	--	--
Chemico Laboratories Ltd.	500102	Jan 1998	--	--	--	--
Therapeutics Bangladesh Ltd.	90	Sep 1997	76	May 1999	--	--
Delta Pharma Ltd..	1114	Jul 1997	--	--	--	--
Doctors Chemical Works Ltd.	--	--	166	Feb 1999	76	Apr 1999
Refco	--	--	01	Jan 1999	--	--
Sonear Laboratories Ltd.	--	--	103	Apr 1999	--	--
Central Pharmaceutical Ltd.	--	--	13	May 1999	--	--
Pharmadesh	--	--	215	Apr 1999	--	--
Nipa Pharmaceutical Ltd.	--	--	--	--	51734	Oct 1998
Chemist Laboratories Ltd.	--	--	--	--	--	Jul 1996

Powder of ampicillin trihydrate capsules (250 mg/capsule) manufactured by 16 different companies: The antibiotics were dissolved in dilute (0.1N) HCl to the concentration of 1 mg/ml. Then 10 µl of the solution was soaked per disk so that each disk with a diameter of 5 mm contains 10 µg of ampicillin trihydrate. One disc was soaked with 10 µl of 0.1 N HCl and was used as control disc. At the same time commercially available [British Drug House (BDH)] prepared ampicillin trihydrate disc (10µg/disc) was used as standard. Powder of amoxycillin trihydrate capsules (250 mg/capsules) manufactured by 16 different companies: The antibiotic powder was dissolved in 0.1 N HCl and the paper discs were made to contain 10 µg of amoxycillin trihydrate. One disc was soaked with 10 µg of 0.1N HCl and was used as control disc and commercially available prepared (BDH) amoxycillin trihydrate (10µg/disc) was used as standard. Powder of oxytetracycline hydrochloride capsules (250 mg/capsule) manufactured by 7 different companies: The powder was dissolved in water and the discs were made to contain 25 µg oxytetracycline hydrochloride. One disc was soaked with water to use as control disc and a prepared (BDH) oxytetracycline hydrochloride disc (25 µg/disc) was used as standard.

Escherichia coli was used as test organism in the study. Two types of bacteriological media were used for the test - Luria Bertani (LB) broth and LB agar. The composition of the media is shown in Table 2.

Table 2. Composition of Luria Bertani (LB) media.

Composition	LB agar media	LB broth media
	Amount (gm/litter)	Amount (gm/litter)
Bacto trypton	10.0	10.0
Bacterial yeast extract	5.0	5.0
Sodium Chloride	10.0	10.0
Agar	15.0	--

For both the media, the components were dissolved in distilled water and the volumes were adjusted to 1000 ml. The pH of the media was adjusted to 7.5 by 5N NaOH. The media were sterilized by autoclaving at 15 lbs pressure and 121 C for 15 minutes and stored at 4 C. In the case of agar media, the media was poured in the petridishes (20-25 ml/petridish) at about 50 C and was allowed to solidify for 30 minutes at room temperature.

Preparation of continuous culture of *E. coli* for seeding onto the agar plates: Freshly grown pure colonies of *E. coli* were picked from the culture plate with sterile loop and poured into 50 ml LB broth media in a conical flask. The broth culture was next placed in an orbital shaker and allowed to grow at 37 C with continuous shaking for about 12 to 15 hours. After incubation, the liquid culture of *E. coli* was used for seeding the agar plates. A few drops of *E. coli* culture were spread over the agar media in the petridish with the help of spreading rod (glass-made). Extra amount of liquid culture was removed by Pasteur pipette.

The sample impregnated discs and standard antibiotic discs were placed gently on the solidified agar plates with the organisms to ensure contact with the media, with the help

of sterile forceps. The plates were then kept in a refrigerator at 4 C for 15 minutes so that the materials absorbed on to the discs could get sufficient time to diffuse into the media. Finally, the plates were incubated at 37.5 C for 12-16 hours. After incubation, the antibacterial activity of the antibiotics was determined by measuring the zone of inhibition (in mm) at the outside of the bottom of the petridishes by a transparent scale.

Determination of Minimum Inhibitory Concentration (MIC) of the Antibiotics: The minimum inhibitory concentration of ampicillin trihydrate, amoxycillin trihydrate and oxytetracycline hydrochloride powder was determined against *E.coli*. The test was done by serial dilution technique using broth media.

For ampicillin trihydrate- a solution of 1 mg/ml of ampicillin trihydrate in 0.1N HCl. For amoxycillin trihydrate- a solution of 1 mg/ml of amoxycillin trihydrate in 0.1N HCl. For oxytetracycline hydrochloride- a solution of 1 mg/ml of oxytetracycline hydrochloride in water.

The test bacteria were grown in nutrient broth media for 6 hours. The optical density (OD) of the culture was noted at every hour at 680 nm. When the OD was found to be 1.0, then the bacterial solution was taken for the use as inoculum. At that stage the bacteria are in their log phase and had a concentration of 10^6 cfu/ml. To each of the antibiotics of each company, 7 sterile conical flasks were used, 5 of which were marked 1,2,3,4 and 5 and the rest two were assigned as C_M (blank media) and C_1 (antibiotic free inoculum). To each of the seven flasks, 50 ml of nutrient broth media was added and mixed well. 1 ml of properly diluted inoculum was added to each of the five flasks and mixed well. One ml of the inoculum was added to the control flask- C_1 to observe the growth of the organism in the medium. The control flask- C_M containing the media only was used to confirm the sterility of the media. All the flasks were incubated at 37.5 C for 16 hours with continuous shaking. OD of the content of each of the flask was taken for 8 consecutive hours at 680 nm. The MIC is the lowest drug concentration at which there was no bacterial growth. Experimental design for MIC test is summarized in Table 3.

Table 3. Experimental design for MIC test.

Ingredient	Blank	Control (antibiotic free)	Experimental	Standard
Media (50 ml)	√	√	√	√
Bacteria (1 ml)	×	√	√	√
Antibiotic (experimental)	×	×	√	×
Standard antibiotic (BDH)	×	×	×	√

Results and Discussion

The results of the antimicrobial potency tests for the capsules of ampicillin trihydrate, amoxycillin trihydrate and oxytetracycline hydrochloride are given below in tubular form. According to United State Pharmacopoeia, standard MIC value for ampicillin trihydrate and amoxycillin trihydrate is 10 µg/ml and for oxytetracycline hydrochloride 8 µg/ml. It should be noted that MIC values for ampicillin and amoxycillin trihydrate are

Table 4. Results of the antimicrobial potency tests for ampicillin trihydrate.

Name of the companies	Parameters		
	Diameter of zone of inhibition (mm)	% of antimicrobial activity according to the diameter of zone of inhibition	MIC (μg)
Opsonin Chemical Industries	19	95	5
Jayson Pharmaceuticals Ltd.	19	95	10
The Acme Laboratories	18	90	10
Fison's Bangladesh Ltd.	20	100	5
Square Pharmaceuticals	20	100	10
Glaxo Bangladesh Ltd.	20	100	5
Ibn Sina Pharmaceuticals	19	95	10
ACI Pharmaceuticals	20	100	10
Navana Pharmaceuticals	20	100	10
Ganashasthya	15	75	20
Pharmaco International Ltd.	15	75	20
Renata Ltd.	19	95	10
Peoples Pharma	19	95	10
Chemico Laboratories Ltd.	18	90	10
Therapeutics Bangladesh Ltd.	19	95	10
Delta Pharma Ltd., Dinajpur	19	95	10
Standard	20	100	5
Control	00	00	

Table 5. Results of the antimicrobial potency tests for amoxycillin trihydrate.

Name of the companies	Parameters		
	Diameter of zone of inhibition (mm)	% antimicrobial activity according to the diameter of zone of inhibition	MIC (μg)
Jayson Pharmaceuticals Ltd.	15	75	20
The Acme Laboratories	20	100	10
Fison's Bangladesh Ltd.	20	100	10
Square Pharmaceuticals	19	95	10
Glaxo Bangladesh Ltd.	20	100	5
Ibn Sina Pharmaceuticals	20	100	10
ACI Pharmaceuticals	20	100	10
Navana Pharmaceuticals	19	95	10
Ganashasthya	19	95	10
Renata Ltd.	20	100	5
Therapeutics Bangladesh Ltd.	17	85	20
Doctors Chemical Works Ltd.	15	75	20
Refco	16	80	10
Sonear Laboratories Ltd.	18	90	20
Central Pharmaceutical Ltd.	20	100	20
Pharmadesh	15	75	10
Standard	20	100	5
Control	00	00	

rising day by day since these old generation antibiotics are being prescribed for avery long time. Currently, average MIC value for ampicillin trihydrate has reached up to 1.5 mg/ml for some organisms in some region of the world (Gilman *et al*., 1996). Now a day ampicillin trihydrate is hardly prescribed.

From the test results it is evident that the antibiotics produced by some pharmaceutical companies do not give satisfactory MIC value. Likewise antibiotics produced by a few companies are not 100% potent against test organism used.

Table 6. Results of the antimicrobial potency tests for oxytetracycline hydrochloride.

Name of the companies	Parameters		
	Diameter of zone of inhibition (mm)	% antimicrobial activity according to the diameter of zone of inhibition	MIC (μg)
The Acme Laboratories	20	100	10
Square Pharmaceuticals	19	95	5
ACI Pharmaceuticals	19	95	20
Renata Ltd.	20	100	10
Doctors Chemical Works Ltd.	18	90	20
Nipa Pharmaceutical Ltd.	18	90	20
Chemist Laboratories Ltd.	19	95	20
Standard	20	100	10
Control	00	00	

Conclusion

Although Bangladesh Drug Administration Authority monitors the quality of the pharmaceutical products, it is evident from this study that many pharmaceutical companies escape the observation and supply impure products. Therefore, it is necessary to investigate the quality of the pharmaceutical products by non-government initiative at a regular basis. This type of study will create awareness among the people and physicians about using quality antibiotics.

Reference

Annon, 1965. *United States Pharmacopoeia* . 17th edition, 4th Park Avenue, New York, pp. 522-562.

Annon, 1993. *British Pharmacopoeia, Vol. I and II*. pp. 113-115, 930-934.

Beur, A.W., Kirby, Q.M.M., Sherris, J.C. and Turck, M., 1993. Antibiotic susceptibility test by a standard single disc method. *American Journal of Clinical Pathology*, 44: 493-496.

Burger, A., 1970. *Medicinal Chemistry, Part I and II*. 3rd edition, Wiley-Interscience, New York, p. 1070.

Gilman, A.G., 1996. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 9th edition, McGraw-Hill, New York, pp. 1078-1085.

Huq, I. and Aziz, K.M.S. 1977. Changing antibiotic sensitivity patterns of the commonly occurring organisms causing dysentery. *Bangladesh Medical Journal*, 5:119-125.

Miles, A.A., Misra, S.S. and Irwin, J.O., 1938. The estimation of bactericidal power of the blood. *J. Hyg*. 38: 732-749.