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A STUDY OF ANTIMICROBIAL POTENCY OF DOXYCYCLINE HYDROCHLORIDE CAPSULES AVAILABLE IN KHULNA CITY

N.I. Siddiqui* and F. Sultana

Biotechnology Discipline, Khulna University, Khulna-9208, Bangladesh

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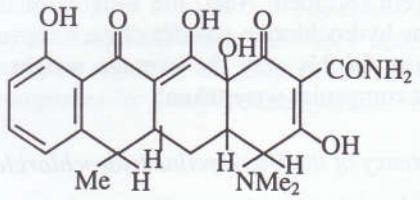
Abstract: Doxycycline hydrochloride is a broad spectrum antibiotic sensitive against a very large number of microorganisms. Since it causes less gastric irritation and can be administered once daily (initially some times twice daily), physicians are now choosing Doxycycline hydrochloride in place of tetracycline in many cases. Because of its dose advantage and effectiveness, it is being used very frequently worldwide. In Bangladesh, many pharmaceutical companies are manufacturing Doxycycline hydrochloride. This research project is aimed at investigating the antimicrobial potency of the Doxycycline hydrochloride capsules marketed by various pharmaceutical companies in Khulna city. Collected antibiotics were tested against E. *coli* and *S. aureus* through disc diffusion method. In this method paper disc were made to soak 30 µg of active ingredient and were placed on the agar plates previously seeded with *E. coli* and *S. aureus*. The drug diffused onto the media and killed the bacteria they encounter. The diameter of the clear zone formed by the paper disk as well as the standard antibiotics disk were measured which reflects the potency of the Doxycycline hydrochloride powder.

Key words: Doxycycline hydrochloride, antimicrobial potency.

Introduction:

Doxycycline hydrochloride is a broad-spectrum antibiotic and has a wide range of activity. It is both bacteriostatic and bacteriocidal. It is active against a large number of microorganisms including many strains of *S.aureus*, *E coli*, and many strains of *haemophilus, influenza, Vibrio cholerae, plasmodium falciparum* etc. Doxycycline hydrochloride is an antibiotic of Tetracycline group. These are a class of antibiotics having a nucleus of four cyclic rings. All are obtained from soil actinomycetes. All tetracyclines are slightly bitter solids, which are weakly water soluble, but their hydrochlorides are more soluble. Aqueous solutions are unstable. All have practically the same antimicrobial activity (with minor differences). The subsequently developed members have high lipid solubility, greater potency and some other differences. On the basis of chronology of development, as well as for convenience of description, they may be divided into 3 groups. Doxycycline hydrochloride is being used now a day mainly for dermatological, gynecological and urological indications as well as for special problems such as atypical pneumonias, brucellosis, cholera and multiresistant malaria. On the other hand tetracycline has presently lost some of its former significance. Very inexpensive preparations of Doxycycline hydrochloride are available. The Doxycycline hydrochloride is primarily bacteriostatic, inhibit protein synthesis by binding to 30s ribosome in susceptible organism. Subsequent to such binding, attachment of amynoacyl-t-RNA to the mRNA ribosome complex is interfered with. As a result the peptide chain fails to grow. In high doses it is also detrimental to the protein synthesis in human cells. The sensitive organisms have an energy dependent active transport process, which concentrates Doxycycline hydrochloride intracellular. In Gram-negative bacteria Doxycycline hydrochloride diffuse through porin channels as well. The more lipid soluble members (Doxycycline hydrochloride) enter by passive diffusion also.



Structure: Doxycycline hydrochloride

*Corresponding author: Tel: +88 (041) 721791, 720171-3, Fax: +88 (041) 731244, email: siddiquibiotech@yahoo.com

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Materials and methods

Collection and storage of the antibiotics: For the study, 250 mg capsules containing 100mg active ingredient were collected from local pharmaceutical shops in the Khulna city. Around 17 companies (local and multinational) were found to be currently marketing Doxycycline hydrochloride capsules in Khulna region. Two strips of capsule (bearing the same batch number and expire date) of each of the companies were purchased. One strip was used for assay purpose and the other was kept as a reference. Collected antibiotics were kept in refrigerator at 4 °C to maintain standard condition of storage.

Table 1. The name of the companies and trade name, batch number, and expire dates of the capsules

Name of the companies	*Trade name*	*Batch. No.*	*Expire date*
Incepta Pharmaceuticals	Ipadox	H11A53	November, 2002
JEMs Pharmaceuticals	Doxycycline	CM20	July, 2003
Noverties Ltd.	Servidoxyne	137	June, 2004
Nipa Pharmaceuticals	Vitrocin	06702	February, 2002
Chemist Pharmaceuticals	Doxy-C	1107	July, 2002
Ziska Ltd.	Doxiciline	K030	September, 2002
Beximco Pharmaceuticals	Megadox	119	June, 2003
Square Pharmaceuticals Ltd.	Doxacil	011039	November, 2003
Opsonin Chemical Industries	Doxin	CoKO16	November, 2003
Ibn Sena Pharmaceuticals	Doxysina	25E	May, 2003
Aristopharma.	Aristodox	17	May, 2001
Pacific Pharmaceuticals	Dopac	9904TL	April, 2001
Navana Pharmaceuticals	Navadox	020400	April, 2003
General Pharmaceuticals	Doxygen	010	March, 2002
ACI Pharmaceuticals.	Impedox	RK 25	July, 2002
The Acme laboratories Ltd.	Doxy-A	CH 1004	February, 2004
Rephco limited.	Reomycin	299	July, 2002

Determination of the average weight of the powder: Firstly, weight of the intact capsule was taken by an ultra-sensitive fine balance. Then, the powders of the capsule were taken out completely on a tracing paper and the weights of the powder were recorded. Next, the weights of the empty shell were recorded. The average weights of the Doxycycline hydrochloride powder of each capsule were calculated from the powder weight of 10 capsules of one strip. In this way the average weight of the powder of 17 Doxycycline hydrochloride capsules of different companies were taken.

Determination of antimicrobial potency of the Doxycycline hydrochloride by Disc Diffusion Method:

Preparation of the Doxycycline hydrochloride solution: Doxycycline hydrochloride is soluble in water. The antibiotics powders were dissolved in distilled water to the concentration of 3mg/ml.

Preparation of antibiotic discs: Whatman 41 filter paper was used to prepare the antibiotic disc. By using punching machine the Whatman paper was punched and small circular disc of diameter of 5mm were made. These discs were then autoclave and dried in oven to prevent contamination. Each dried disk was made to soak 10µl of the antibiotic solution so that each disc contains 30µg of Doxycycline hydrochloride. One disc was soaked with 30µg of water and was used as control disc. At the same time commercially available prepared Doxycycline hydrochloride disc (30µg Doxycycline hydrochloride\ disc) were used as standard.

Test organisms for the examination: The bacterial stains used in the sensitivity test were -*Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram –positive).

Preparation of Bacteriological Media: Two types of media were used for the test- Luria Bertani (LB) broth and LB agar.

Table 2. Media composition

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

For both of 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 inocula: For inocula preparation liquid media was used. Two conical flasks containing 50 ml liquid media each were taken. The liquid media was sterilized by autoclave at 121°c for 15 min. and was cooled at room temperature. One conical flask containing 50ml liquid media was inoculated with *E. coli*. Another 50 ml liquid media was inoculated with *S. aureus*. Both flasks were at 37°C for 12-14 hours with continuous shaking. After 12-14 hours the growth was completed, and it was confirmed by changing the colour of the liquid media. Another two conical flask each containing 100 ml of fresh liquid media were taken. Then, 3 ml *E. coli* inoculated liquid culture was taken and mixed with 100 ml fresh liquid media. Again 3 ml *S. aureus* inoculated liquid culture was taken and mix with another 100ml fresh liquid media. Then the two new inoculated liquid culture media were kept in the incubator for about 6 hours with continuous shaking. These liquid culture media were used for preparing the culture plate.

Preparation of monolayer with liquid culture media (both E. coli and S. aureus inoculated): At room temperature *E.coli* inoculated liquid culture media was spreaded gently on the solidified agar plates with the help of Pasteur pipette. The excess liquid culture media must be removed from the plate. A smooth (not over grown) monolayer is necessary for the test. In the same way monolayer agar plate with monolayer of *S.aureus* were prepared.

Application of Antibiotic discs on the monolayer and incubation: The sample impregnated discs and standard antibiotic discs were placed gently on the monolayer created on the agar plate to ensure contact with the media, with help of sterile forceps. The plates were then kept in refrigerator at 4°C for 15 minutes, so that the materials absorbed on to discs could get sufficient time to diffuse into the media. Finally, the plates were incubated at 37°C for 12-16 hours.

Determination of degree of antimicrobial activity by measuring the zone of inhibition: After 12-16 hours incubation the antibiotic discs produced clear zone on the agar plates. The antimicrobial potency was determined by measuring the zone of inhibition (in mm) in both horizontal and vertical direction at the out side of the bottom of the petridishes by a transparent scale. Inhibitory zone obtained by the samples were compared with that of the standard discs. The results of the zone of inhibition against *E. coli* and *S. aureus* produced by the capsules of different companies for Doxycycline hydrochloride are given in the table- 04 & table- 05 respectively.

Results

The weight of Doxycycline hydrochloride powder: The weight of Doxycycline hydrochloride powder for each capsule were found as in the following Table

Table 3. Weight of Doxycycline hydrochloride powder

Name of pharmaceutical Companies	*Trade name*	*Weight of intact Capsules (gm)*	*Weight of powder (gm)*	*Weight of empty shell (gm)*
Incepta pharmaceutical	Ipadox	0.2458	0.1745	0.0677
JEMs pharmaceutical	Doxycycline	0.3448	0.2781	0.0649
Noverties limited	Sarvidoxyne	0.3530	0.2882	0.0639
Nipa pharmaceutical	Vitrocin	0.2978	0.2271	0.0650
Chemist pharmaceutical	Doxy-C	0.3864	0.3044	0.0713
Ziska pharmaceutical	Doxiciline	0.3659	0.2917	0.0727
Beximco Pharma	Megadox	0.3697	0.3010	0.0686
Square pharma. Limited	Doxacil	0.4000	0.3353	0.0631
Opsonin pharmaceutical	Doxin	0.3832	0.3120	0.0691
IBNSINA pharmaceutical	Doxysina	0.3154	0.2448	0.0690
Aristopharma	Aristodox	0.3399	0.2726	0.0665
Pacific pharmaceutical	Dopac	0.3539	0.2893	0.0628
Navana pharma. Limited	Navadox	0.3672	0.2908	0.0758
General pharmaceutical	Doxygen	0.3232	0.2557	0.0666
ACI pharma. Limited	Impedox	0.3235	0.2541	0.0690
Acme laboratories limited	Doxy-A	0.3606	0.2934	0.0669
Rephco pharma. Limited	Reomycin	0.3282	0.2603	0.0664

The table shows that Doxycycline hydrochloride capsule of Nipa Pharmaceuticals and Incepta Pharmaceuticals contain lower amount of powder and therefore these capsules may have active ingredients in low amount.

Diameter of zone of inhibition of Doxycycline hydrochloride capsule against E. coli: Diameter of zone of inhibition of Doxycycline hydrochloride capsule against *E. coli* were found as in Table-4.

The zone of inhibition of the standard Doxycycline hydrochloride was found to be 20 mm against *E. coli.* Here we find capsules of some companies with zone of inhibition which are far below 20 mm. The rest of the capsules are found to be satisfactory in our examination.

Diameter of zone of inhibition of Doxycycline hydrochloride capsule against *S. aureus:* Diameter of zone of inhibition of Doxycycline hydrochloride capsule against *S. aureus* were found as in Table-5

Result against *S. aureus*: The zone of inhibition of the standard Doxycycline hydrochloride was found to be 30.3 mm. From the table we can categorize the capsules giving the zone of inhibition that are very close to 30.3 mm However for safety purpose the capsules giving smaller zone of inhibition e.g. below 27.5 mm (approximately) should be avoided.

Discussion

Antibiotics are very common & essential drugs. In our country more than fifty pharmaceutical companies are producing various kinds of antibiotics. But many of these companies do not maintain the quality of the antibiotic products and release impure and low quality antibiotic products into the market without proper

Table 4. Diameter of zone of inhibition of doxycycline hydrochloride capsule against *E. coli*

Name of the Pharmaceutical Companies	Trade name	Diameter of zone of inhibition									
		Result of the 1st observation: R1 (mm)			Result of the 2nd observation: R2 (mm)			Result of the 3rd observation: R3 (mm)			Final result: (mm)
		Horizontal	Vertical	Average	Horizontal	Vertical	Average	Horizontal	Vertical	Average	Average of R1, R2 and R3
Incepta Pharmaceuticals	Ipadox	20	20	20	18	17	17.5	18	20	19	18.8
JEMs Pharmaceuticals	Doxycycline	21	20	20.5	20	19	19.5	19	20	19.5	19.8
Noverties Limited	Sarvidoxyne	20	20	20	19	20	19.5	20	20	20	19.8
Nipa Pharmaceuticals	Vitrocin	17	16	16.5	17	19	18	18	19	18.5	17.6
Chemist Pharmaceuticals	Doxy-C	20	20	20	21	23	22	21	22	21.5	21.1
Ziska Pharmaceuticals	Doxiciline	20	21	20.5	21	22	21.5	21	21	21	21
Beximco Pharma	Megadox	19	18	18.5	2o	19	19.5	19	18	18.5	18.8
Square Pharma. Limited	Doxacil	20	20	20	18	19	18.5	20	18	19	19.16
Opsonin	Doxin	17	17	17	19	18	18.5	19	17	18	17.8
Ibn Sina	Doxysina	19	20	19.5	20	20	20	20	20	20	19.8
Aristopharma	Aristodox	20	19	19.5	22	21	21.5	19	21	20	20.3
Pacific Pharmaceuticals	Dopac	20	21	20.5	19	20	19.5	20	21	20.5	20.1
Navana Pharma. Limited	Navadox	19	18	18.5	22	21	20.5	19	20	19.5	19.5
General Pharmaceuticals	Doxygen	18	18	18	20	19	19.5	18	19	18.5	18.6
ACI Pharma. Limited	Impedox	17	16	16.5	19	21	20	16	17	16.5	17.6
Acme laboratories Ltd.	Doxy-A	22	21	21.5	19	20	19.5	21	20	20.5	20.5
Rephco Pharma. Limited	Reomycin	21	20	20.5	19	19	19	19	20	19.5	19.6
Renata Limited	Doxycap	21	21	21	20	19	19.5	20	20	20	20.16
Standard		20	21	20.5	19	20	19.5	20	20	20	20

This evaluation for the antimicrobial potency is restricted to the capsules of the particular batch mentioned in Table 1.

quality control surveillance. Doxycycline hydrochloride is one of the frequently prescribed antibiotics. It is normally prescribed in the case of atypical pneumonias, brucellosis, cholera, multi-resistant malaria, traveler's diarrhea and many sexual diseases. In our study we have tried to verify the antimicrobial potency of the market available of Doxycycline hydrochloride capsules by disc diffusion method. The findings may be helpful for the physicians and concerned users to choose good quality Doxycycline hydrochloride from the market. From the result, we found that Ziska, IBN Sina Pharmaceuticals, ZEMs Pharmaceuticals, Novertis Pharmaceuticals gave excellent result against both *E. coli* and *S. aureus*.

It is important that each Doxycycline hydrochloride capsule contains right amount of powder (in this case 100 mg per capsule). The amount of powder is very important to maintain the quality of the drug. Capsules with inappropriate amount of powder may contain low amount of active ingredient that cannot fulfill the prescribed dose and is a significant cause of developing drug resistance. However, from the result we found that Nipa Pharmaceuticals and Incepta Pharmaceuticals supplied capsules with lower amount of powder. Day by day different resistant form of bacteria is developing and the therapeutic indication for many drugs is being modified in different part of the world. So physicians should have the knowledge of the most recent therapeutic profile of the drugs. Extreme precaution should be taken before using the drug in the cases like pregnancy, breast-feeding, surgery etc. More over, the patient must follow the prescribed dose pattern.

Conclusion

All the drugs including Doxycycline hydrochloride must have a very good quality and potency for their proper action. In this study we have investigated the antimicrobial potency of Doxycycline hydrochloride of 17 pharmaceutical companies commonly available in the market in Khulna city. From the result, we found that Doxycycline hydrochloride capsule of Ziska Pharmaceuticals, Ibn Sina Pharmaceuticals, ZEMs

Table-5: Diameter of zone of inhibition of doxycycline hydrochloride capsule against *S. aureus*

Name of the Pharmaceutical Companies	Trade name	Diameter of zone of inhibition									
		Result of the 1st observation: R1 (mm)			Result of the 2nd observation: R2 (mm)			Result of the 3rd observation: R3 (mm)			Final result: (mm)
		Horizontal	Vertical	Average	Horizontal	Vertical	Average	Horizontal	Vertical	Average	Average of R1, R2 and R3
Incepta Pharmaceuticals	Ipadox	27	26	26.5	28	27	27.5	27	26	26.5	26.8
JEMs Pharmaceuticals	Doxycycline	30	29	29.5	29	29	29	29	30	29.5	29.3
Noverties limited	Sarvidoxyne	29	28	28.5	30	29	29.5	28	29	28.5	28.8
Nipa Pharmaceuticals	Vitrocin	32	30	31	28	27	27.5	28	30	29	29.1
Chemist Pharmaceuticals	Doxy-C	30	31	30.5	27	26	26.5	27	28	27.5	28.1
Ziska Pharmaceuticals	Doxiciline	31	31	31	28	29	28.5	31	30	30.5	30
Beximco Pharma	Megadox	28	27	27.5	30	29	29.5	28	30	29	28.6
Square Pharma. Limited	Doxacil	29	28	28.5	30	30	30	29	30	29.5	29.3
Opsonin Pharmaceuticals	Doxin	28	29	28.5	29	28	29.5	28	29	28.5	28.8
Ibn Sina	Doxysina	30	29	29.5	29	28	28.5	29	30	29.5	29.1
Aristopharma	Aristodox	27	28	27.5	30	29	29.5	27	29	28	28.3
Pacific Pharmaceuticals	Dopac	24	25	24.5	28	27	27.5	26	27	26.5	26.1
Navana Pharma. Limited	Navadox	27	26	26.5	28	29	28.5	27	28	27.5	27.5
General Pharmaceuticals	Doxygen	27	27	27	28	28	28	28	27	27.5	27.5
ACI Pharma. Limited	Impedox	26	27	26.5	28	29	28.5	26	28	27	27.3
Acme Laboratories Ltd.	Doxy-A	27	26	26.5	25	26	25.5	25	27	26	26
Rephco Pharma. Limited	Reomycin	27	28	27.5	28	29	28.5	27	27	27	27.6
Renata Limited	Doxycap	28	30	29	30	27	28.5	31	30	30.5	29.3
Standard		30	31	30.5	30	30	30	30	31	30.5	30.3

This evaluation for the antimicrobial potency is restricted to the capsules of the particular batch mentioned in Table 1.

Pharmaceuticals and Novertis given excellent result against both E. coli and S. aureus. Capsule of that Nipa Pharmaceuticals and Incepta Pharmaceuticals were found to contain lower amount of powder. This type of investigative study should be performed by non-government initiative at a regular interval to help the physicians and people use good quality drugs for maintaining a healthy life. It should be mentioned that the results of antimicrobial test is greatly influenced by many factors like pH of environment, components of medium, stability of drug, length of Incubation, metabolic activity of microorganisms etc. Since working laboratory was not up to standard level where uninterrupted electricity supply was not ensured, we cannot claim our findings 100% error free.

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