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A STUDY OF THE TOXIC EFFECTS ON *BACILLUS ANTHRACIS* BY DRUGS OF PENICILLIN GROUP OF ANTIBIOTICS

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ABSTRACT

Bacillus anthracis is gram positive, rod shaped bacterium frequently found in the environment and causes Anthrax disease which is common in animal but rare in human. In the present research work the favorable condition for the growth of *Bacillus anthracis* and confirmed it by Gram's staining and the growth in different media was observed, the effect of antibiotic was also observed.

Key-words: *Bacillus anthracis*, toxic effect, penicillin, *Bacillus anthracis*

INTRODUCTION

Bacillus anthracis are large gram positive rod with square ends, frequently found in soil, water, dust and air. Spore formation occurs when nutrients such as source of carbon and nitrogen are depleted. The spore forms inside the cell which contains cytoplasm, bacterial DNA, cell membrane, peptidoglycans, little water and importantly a thick keratin like coat which is responsible for the remarkable resistance of the spore to heat, dehydration, radiation and chemicals. When *Bacillus anthracis* enters in the body it starts working rapidly and within 12 to 48 h it causes disease. In animals it causes anthrax disease like Intestinal anthrax, in which portal of entry is the mouth and intestinal tract (Levinson *et al*., 1992). Pulmonary anthrax is caused by the inhalation of spores. Humans are infected by spores on animals products such as hides, bristles and wool or by contact with sick animals. The portals of entry are the skin, mucous membrane and respiratory tract. In human they cause mainly two types of diseases (a) wool salter disease and (b) painless ulcer. Penicillin and streptomycin are effective for the treatment; they inhibit the growth of the bacteria. Anthrax spore vaccine is produced, it contains the protective antigen purified from the organism . It is given to the person, who is associated with the spores of anthrax (Clark *et al*., 1992). Penicillin is highly effective with an extremely wide margin of safety. It is an organic acid obtained from the culture of mold *Penicillium chrysogenum*. Penicillin has common central core called β lactam ring fused to a thiazolidine ring (Katzung 1995; Tipper 1979). Certain bacteria can produce β lactamase penicillinase which break the lactam ring. Thus penicillin is hydrolysed to harmless form. These bacteria are resistant to penicillin. The main site of penicillin action is at the cell wall of bacterium. The cross links of peptidoglycan are the main target of drug action (Wendel *et al.,* 1985, Glaueri *et al.,* 1969). Toxicity of penicillin is extremely low but very high dose can cause serum sickness, skin rash, fever, gastrointestinal disturbance, abnormal platelet functioning and hemolytic anemia (Pelezer *et al.*, 1993; Rolinson *et al.*, 1989) .The aims of research were: to study the growth of *Bacillus anthracis* in different media, to find out the favorable conditions for growth whether aerobic or anerobic and to screen the susceptibility of this organism against penicillin group of antibiotics.

MATERIALS AND METHODS

Growth of bacteria in Different Media

For making BHI broth Materials are Calf Brain Infusion solid 12.5 g (Oxoid); Brain Heart Infusion solid 5.0 g (Oxoid); Protease peptone 10.0 g (Oxoid); Glucose 2.0 g (Oxoid); NaCl 5.0 g (Oxoid) and Di-Sodium Phosphate 2.5 g (Oxoid). A 1.85g BHI media was put in the flasks and 50 ml distilled water added to prepare media.

For making CYS media materials are Casein Hydrolysate 0.9g (Gibco); Sucrose 1.8 g (Merck); Yeast Extract 1.8 (Merck); NaCl 1.5g (Merck); Di Pot. Hyd Phosphate 2.58 g (Merck) and Pot. Hydrogen Phosphate 0.39 g (Merck). This CYS 1.5 g was placed in a flask and 50 ml distilled water added.

NB 0.4 g was put in a flask and added 50 ml distilled water and prepared 50 ml media. Now these three flasks sterilized in a autoclave for 50 min at 120^{o}C . Then these flasks were placed in the incubator at 37^{o}C. In the next step solidified the petri plate with nutrient agar (Oxoid). When it was solidified the bacteria *Bacillus anthracis* was inoculated with the help of inoculation wire loop and was placed in the incubator at 37^{o}C for 24 h for the growth of the colonies. Next day took 2 to 4 well grow colonies from the petri dish was inoculated in the nutrient broth in the test tube, shacked well and placed in the incubator for 2 h. So next day bacteria was cultured in the test tube.

Growth of *Bacillus anthracis* in aerobic and anaerobic conditions

After conformation, 9 sterilized universal bottles were taken and added all three media (NB, BHI, CYS) in them. Which were already prepared in equal quantity. Then added 15 ml media in each universal bottle .Now three bottles were separated for the control and were marked.

C 1:- Control for NB, C 2:- Control for BHI,
C 3:- Control for CYS, In other 6 test tubes were inoculated the culture bacteria for the growth and divided these 6 universal bottles in to 2 parts, one for aerobic condition and other for anaerobic condition.

Effect of antibiotic on *Bacillus anthracis*

To check the effect of different antibiotics, the sensitivity disks were impregnated with known amount of chemotherapeutic agents, placed up on the surface of inoculated plates. After incubation of 24 h the plates were observed for the zone of inhibition.

Table 1. Growth of *bacillus anthacis* in aerobic and anaerobic conditions.

	(Absorbance At 540 nm)	
MEDIA	**AEROBIC**	**ANAEROBIC**
CYS	0.32	0.30
BHI	0.32	0.30
NB	0.32	0.30

Table 2. Mean absorbance of different media at 540 nm .

S.NO.	**BHI**	**R & I**	**NB**	**CYS**
1.	1.5	1.0	0.85	1.4
2.	1.6	1.2	0.88	0.46
3.	1.63	1.23	0.86	1.5
4.	1.65	1.26	0.90	1.55
5.	1.68	1.29	0.92	1.57
6.	1.68	1.55	0.92	1.57

NB= Neutrient Broth; BHI= Broth; CYS=Cystine

Table 3. Antimicrobial susceptibility testing: Effect of pencillin group of antibiotic on the growth of *Bacillus anthracis*.

	DIAMETER IN (mm) w.r.t. DISK DIAMETER			
S.NO.	SYMBOL	NA	BHI	DST
1.	P	2.7	2.3	1.6
2.	AML	3.1	2.9	3.1
3.	AMC	3.4	2.8	3.6
4.	AMP	2.9	2.9	2.7
5.	ATM	NO	NO	2.3
6.	**MEM**	**3.6**	**3.8**	**3.8**

P= Pencillin 10µg; AML = Amoxicillin 25µg; AMC = Amoxicillin 30µg; AMP = Ampicillin 10µg; ATM = Aztreonam 30µg; MEM = Meropenam 10µg.

RESULTS AND DISCUSSION.

The aim of research were to study the growth of *Bacillus anthracis* in different media, to check the favourable conditions for growth whether it is aerobic or anaerobic and to screen the susceptibility of penicillin group of antibiotic (Rolinson *et al.*, 1989). Table 1 and Fig.1 show the growth of *Bacillus anthracis* in aerobic and anaerobic conditions. It is quite obvious from the results of Table 1 that *Bacillus anthracis* is an aerobic bacterium, which grows rapidly in aerobic conditions compared to anaerobic condition. In gram staining, it stained purple which confirmed that species is gram positive. Table 2 and Fig. 2 show the growth comparison between different media. The absorbence of different media at 540 nm of Table 2 show that CYS agar is not good for the growth of B. anthracis while BHI and NA are good for the growth of B. anthracis. Therefore the results of CYS agar are deleted. Table 3 shows the susceptibility of penicillin group of antibiotic on the growth of B. anthracis. Six drugs were employed. The strains were considered either sensitive or resistant according to the diameter of zone size that corresponds to the control *B. anthracis* standard. The used drugs were Penicillin 10µg (P), Amoxicillin 25µg (AML), Amoxicillin 30µg (AMC), Ampicillin 10µg (AMP), Aztreonam 30µg (ATM) and Meropenam 10µg (MEM). The highest frequency of resistant was with ATM in NA and BHI media but in DST media it shows some sensitivity. The MEM shows highest sensitivity among these antibiotics. These antibiotics interact microbe cells through the inhibition of cell wall synthesis. The cell wall contain a chemical distinct complex cross-linked polymer, peptidoglycan (murein, mucopeptide) consisting of polysaccharides and polypeptides. The polysaccharides regularly contain the amino sugars N-acetylglucosamine and acetylmuramic acid. The latter is found only in bacteria, to the amino sugars are attached short peptide chains. The final regidity of the cell wall is imparted by cross-linking of the peptide chains (eg. through pentaglycine bonds) as a result of transpeptidation reactions catalyzed by several enzymes.The peptidoglycan layer is much thicker in the cell wall of gram positive than gram negative bacteria . Penicillin group of antibiotics are selective inhibitors of cell wall synthesis. Susceptibility of becteria to these antibiotics depends on various structural and functional characteristic (Luis *et al.*, 1997, Neu *et al*., 1975).The results showed that penicillin has lower sensitivity then other derivatives such as ampicillin which is D-amino benzylpenicillin,addition on side chain increases the sensitivity of penicillin.The mechanisms of resistance may include changes occurring within the cell of the bacteria to affect the receptivity to the antibiotic, which make it more difficult for the antibiotic to attack (Ypung *et al.*, 1986; Baun *et al.*, 1975). From the above performed experiments it can be concluded that *Bacillus anthracis* is gram positive bacteria which grow in aerobic condition and penicillin group of antibiotics can be used to inhibit the growth of this organism, among these antibiotics the resistance was observed against Aztreonam.

REFERENCES

Baun, V. (1975). Anti microbial Resistance. *Biochem, Biophys. Acta*, 415: 335-377.

Clark, W. G. (1992). *Brater, D.C. and John, A.R. Medical Pharmacology.* 13th Ed. Mosby year Book. p.628.

Glaueri, A. M and M. J. Thornel (1969). *A. Rev. Microbial.,* 23: 159-198.

Katzung, B.G. (1995). *Basic & Clinical Pharmacology*, 6th Ed. Prentice Hall International, USA. p.169.

Levinson, W. E. (1992). *Jewatz, Medical Microbiology & Immunology*. Prentice Hall Internation, 2nd Ed. USA. p.177.

Luis, M . and de la Maza (1997). *Pezzlo . M. T . Baron . J.E. 'Color atlas of diagnostic Microbiology.* Vice president publisher, Don Lading Mosby . 107.

Neu, H.C. (1975). Cherubin, E . Longo .B . Flouton and J.Winter. Antimicrobial resistance and R-factor transfer among isolates of Salmonella in the North eastren of the USA . A comparison of human and animal isolates . *J . Infect . Dis* ., 132: 617-622.

Pelezer (1995). *Chan, Kriec Microbiology* 5th ed. Vice Presiedent publisher.

Rolinson, G.N. (1989). beta-lactamase induction and resistance to lactam antibiotics . *J antimicrob chemother,* 23: 1.

Tipper, D.J. (1979). Mode of action of beta-lactam antibiotics. *Rev. infect.Dis., 1:39.*

Wendel, G. D. (1985). Penicillin allergy and desensitization in serious infections during pregnancy. *N. Engl. Med*., 312: 1229.

Ypung, L. (1986). Antimicrobial resistance implication for antimicrobial use. *Am .J .Med*., 80: 35-39.

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