SENSITIVITY OF LOCAL ISOLATES OF *BACILLUS ANTHRACIS* AGAINST SEVERAL ANTIBIOTICS

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ABSTRACT

Poerwadikarta, M.B., S. Hardjoutomo and K. Barkah. 1993. Sensitivity of local isolates of *B. anthracis* against several antibiotics. *Penyakit Hewan* 25 (46): 133-136.

Fourten local isolates of *Bacillus anthracis* were tested against several antibiotics using the procedure of disc diffusion. The result showed that all tested isolated were sensitive against all antibiotics. However, some were moderately sensitive to penicillin, streptomycin and erythromycin.

Key words: Sensitivity test, antibiotics, *B. anthracis*

ABSTRAK

Poerwadikarta, M.B., S. Hardjoutomo dan K. Barkah. 1993. Uji kepekaan isolat lokal *B. anthracis* terhadap beberapa antibiotika. *Penyakit Hewan* 25 (46): 133-136.

Empat belas isolat lokal *Bacillus anthracis* diuji kepekaanya terhadap beberapa antibiotika dengan menggunakan metode difusi cakram. Hasilnya menunjukkan bahwa seluruh isolat yang di uji peka terhadap seluruh antibiotika yang digunakan. Sekalipun demikian, beberapa isolat yang di uji menunjukkan kekurang pekaan terhadap penicillin, streptomycin dan erythromycin.

Kata kunci: Uji kepekaan, antibiotika, *B. anthracis*

INTRODUCTION

Bacillus anthracis has been recognized as a cause of anthrax, which is known as radang limpa in Indonesia (Soemanagara, 1958). Anthrax causes clinical disease in human beings and animals and has worldwide distribution (Bhat and Mohan, 1989; Bozzano *et al,* 1989; Doganay, 1989; Fujikura, 1989; Hardjoutomo, 1989; Jones, 1989; Pugh and Devies, 1989; Turnbull, 1989; Whitford, 1989). In Indonesia, the occurence of anthrax has been reported since 1885 (Soemanagara, 1958).

B. anthracis is reported to be sensitive to penicillin and some broadspectrum antibiotics (Kauffmann, 1981; Ezzel, 1986). The use of antibiotics for treatment of anthrax in animals has been reported by Kaufmann (1981) and Ezzel (1986). In suspected outbreaks, treatment with antibiotics should be initiated for all animals as soon as possible, after a correct diagnosis has been made. In human beings, penicillin, tetracycline or chloramphenicol can be effectively used as a treatment for anthrax (McKeadrick, 1980). Success of alternative therapy with derivatives of tetracycline, erythromycin, penicillin and streptomycin have also been reported by Kaufmann (1981) and Lightfood *et al.* (1989).

Although penicillin-G is commonly used as the antibiotic of choice for treating the disease in certain condition of outbreaks, penicillin resistant isolates of *Bacillus anthracis* have been reported by Lightfood *et al.* (1989). Treatment failures with penicillin have also been recorded by Gold (1967).

In Indonesia, there is no information of antimicrobial susceptibility test of local isolates of *B. anthracis* has been reported.

The purpose of this study is to examine suspectibility of some local isolates of *B. anthracis* against several antibiotics and to determine whether natural resistance against antibiotics exists.

MATERIALS AND METHODS

Source of *Bacillus anthracis* isolates

Fourteen local isolates of *B. anthracis* were obtained from infected animals in contaminated areas of 5 provinces in Indonesia during the past ten years from 1982-1992 (Table 1).

Table 1. Original source of Isolates of *B. anthracis*

No.	Species Affected	Province	Year Isolated
1.	Cattle	DKI Jakarta	1982
2.	Swine1	Irian Jaya	1984
3.	Swine2	Irian Jaya	1984
4.	Goats	West Java	1985
5.	Goats	West Java	1985
6.	Cattle	West Nusa Tenggara	1986
7.	Buffalo	West Java	1986
8.	Cattle	West Nusa Tenggara	1986
9.	Cattle	West Nusa Tenggara	1989
10.	Buffalo	West Nusa Tenggara	1989
11.	Cattle	Central Java	1990*)
12.	Cattle	West Java	1990
13.	Cattle	West Nusa Tenggara	1991
14.	Lion	DKI Jakarta	1992

*) Hardjoutomo *et al.* (1990)

Medium

Mueller Hinton agar was prepared, sterilized and poured into sterile petri dishes, 90 mm diameter, to a depth of 5 mm and was used within 7 days of preparation. The composition of the medium was adapted from the formula of Cowan and Steel's (1974).

Antibiogram discs

Antibiogram discs from Oxoid with diameter of 7 mm which contained chloramphenicol (30 ug), enrofloxacin (5 ug), erythromycin (30 ug), kanamycin (30 ug), neomycin (30 ug), novobiocin (30 ug), penicillin (10 iu), streptomycin (25 ug) and tetracycline (30 ug) were used for the sensitivity test. The discs were placed on the surface of inoculated agar plate.

Determination of organism number

Each inoculum was prepared from a pure culture of *B anthracis* and was grown overnight on a blood agar plate (10 % sheep blood tryptose agar). The next morning, 2-3 colonies were taken and inoculated into brain heart infusion (BHI) broth and grown at 37°C under aerobic condition for about 18 hours.

The number of viable organisms inoculated onto Mueller Hinton agar plate was estimated using the method of drop count after comparing to a Brown Opacity tube number 3.

Antibiotic sensitivity test method

Antibiotic sensitivity test of *B. anthacis* was determined using the disc diffusion procedure as the basic assay. This test was standardized according to the guide lines given in report of an International collaborative study on the testing for antibiotic sensitivity (Ericsson and Sherris, 1971).

Suspencis of *B. anthracis* wich has been inoculated for 24 hours subculture onto the surface of Mueller Hinton agar plate using a cotton wool swab and then allowed to dry for 15 minutes at 32-37°C. The discs containing antibiotics were applied with light pressure onto the surface of the agar using sterile forceps until the whole surface of the disc was flattened on the agar and incubated at 37°C for overnight.

Zones of inhibition were measured using a transparant ruler and the inhibition zone was compared to the standard zones of standard culture of *Staphycoccus aureus* NCTC 6571. All tests were repeated three times.

Interpretation of result

The inhibition zone of each isolate was evaluated for every antibiotic and compared to the standard zone of *Staphylococcus aureus* NCTC 6571 reference strain. The isolate was regarded as a **sensitive** strain if the inhibition zone was larger than the inhibition zone made by the standard reference strain by at least 3 mm. The

Table 2. *In-vitro* sensitivity test of 14 local isolates of *B. anthracis* to some antibiotics

Type of Antibiotics	Sensitive*)	Moderately Sensitive	Resistance
Chloramphenicol (30 ug)	14/14	0/14	0/14
Enrofloxacin (5 ug)	14/14	0/14	0/14
Erythromycin (30 ug)	10/14	4/14	0/14
Kanamycin (30 ug)	14/14	0/14	0/14
Neomycin (30 ug)	14/14	0/14	0/14
Novobiocin (30 ug)	14/14	0/14	0/14
Penicillin (10 iu)	8/14	6/14	0/14
Streptomycin (25 ug)	12/14	2/4	0/14
Tetracycline (30 ug)	14/14	0/14	0/14

*) = number of sensitive isolates/number of samples tested

isolate was considered to be moderately sensitive if the inhibition zone was smaller beyond 3 mm than the inhibition zone of the standard strain. The isolate as considered to be **resistant** if the inhibition zone was maximum 2 mm in diameter or no inhibition at all.

RESULTS

Sensitivity of *B. anthracis* isolates to several antibiotics is shown in Table 2. Fourteen isolates were completely sensitive to enrofloxacin, chloramphenicol, novobiocin, neomycin, tetracycline and kanamycin. Six isolates were moderately sensitive to penicillin and 4 isolates were moderately sensitive to erythromycin and 2 isolates were moderately sensitive to streptomycin. None of the isolates was resistant against the antibiotics used in the study.

DISCUSSION

This study shows the result of an *in vitro* study on the sensitivity test of local isolates of *B. anthracis* to antibiotics. Sensitivity of *B anthracis* to penicillin (Kauffmann, 1981; Ezzell, 1986 and Odendaal *et al.*, 1991) and to erythromycin (Odendaal *et al.*, 1991 and Lightfoot, *et al.*, 1991) has been described and was found to be sensitive to these antibiotics.

As it is reported by Pesti (1990) that *B. anthracis* and *B. cereus* were almost similar organism, therefores to distinguish between these organisms sensitivity test to penicillin at the concentration of 10 iu/ml was used. Anthrax organisms were found to be sensitive to penicillin at the dose rate of 10 iu/ml where *B. cereus* was resistant. This method is much simpler than other method to distinguish both organisms.

Although all local isolates of *B. anthracis* used in this study seem to be sensitive to antibiotics, some of them were only moderetaly sensitive. The isolate that moderately sensitive to antibiotics could probably indicate that the microorganisms have been exposed to the same antibiotics for several times. moderate sensitive organisms could probably become sensitive with higher dose rate of the antibiotic. However, further search need to be investigated. Fourteen isolates were found to be completely sensitive to enrofloxacin, neomycin, novobiocin, tetracycline, chloramphenicol and kanamycin. This indicates that these antibiotics are effective for killing the anthrax organism under *in-vitro* condition. This study shows in line with the study reported by Odendaal *et al.* (1991). However, this does not guarantee success of treatment of the disease under *in-vivo* condition. Therefore, the effectiveness of antibiotics should be taken into account carefully. At least, this test can be used to assist veterinarians in the field in selecting the antibiotics for the treatment of animals suspected suffering from anthrax. Although antibiotics used in this study, except for enrofloxacin, all have been introduced for long times in the field, their sensitivity are still maintained sensitive. This could probably be due to some relationship to the nature of the antibiotics especially the aminogycoside such as kanamycin, neomycin and streptomycin that have large molecular size of antibiotics which makes it difficult to develop resistance against the microorganisms (Lin, 1987).

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