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THE ANTIBIOTIC SENSITIVITY PATTERNS OF *BACILLUS ANTHRACIS* ISOLATED FROM THE KRUGER NATIONAL PARK

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ABSTRACT

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Forty-four isolates of *Bacillus anthracis* made from carcasses and soil in different localities of an endemic anthrax area in the Kruger National Park, South Africa, were tested by standard disc diffusion for their susceptibility to 18 different antibiotics. These were ampicillin, penicillin G, sulphatriad, streptomycin, clindamycin, gentamicin, fusidic acid, trimethoprim, sulphamethoxazole, chloramphenicol, erythromycin, methicillin, tetracycline (2 different concentrations), novobiocin, cefotaxime, netilmicin, cefamandole and cefoxitin.

All the isolates were susceptible to ampicillin, streptomycin, chloramphenicol, erythromycin, tetracycline, methicillin and netilmicin. More than 90 % of the isolates were sensitive to clindamycin, gentamicin and cefoxitin, whereas only 84,1 % of the isolates were sensitive to penicillin G, 86,4 % to novobiocin and 68,18 % to cefamandole. Complete resistance in 100 % of the isolates was encountered with trimethoprim and sulphamethoxazole, with 95,45 % for sulphatriad. Moderate sensitivity occurred with penicillin G (15,9 % of the isolates), clindamycin (6,8 %), novobiocin (13,6 %), fusidic acid (84,1 %), cefotaxime (100 %), cefamandole (31,8 %) and cefoxitin (6,8 %). The relevance of the findings to the therapeutic uses of different types of antibiotic in human clinical cases referred to in the literature is discussed.

INTRODUCTION

Anthrax as a disease caused by *Bacillus anthracis* is still encountered clinically in humans and animals. It is enzootic in the animal population of the 3rd world countries in Africa, Asia and South America (Bhat, Mohan & Lalitha, 1989; Doganay, 1989; Hardjoutomo, 1989; Polydorou, 1989; Pugh & Davies, 1989; Whitford, 1989). Recently it was the cause of an acute outbreak of active disease amongst the indigenous human population in Zimbabwe (McKendrick, 1980; Turner, 1980; Levy, Baker, Meyer, Crosland & Hampton, 1981) and Turkey (Doganay, 1989) and it was the cause of some mortality in humans in Russia, resulting from the ingestion of contaminated meat (Turnbull, 1986). In South Africa and Namibia, it is an important cause of mortality amongst wildlife (McConnell, Tustin & De Vos, 1972; Turnbull, Hofmeyr, McGetrick & Oppenheim, 1986; De Vos, 1989).

The widespread use of the avirulent Sterne spore vaccine in South Africa has dramatically minimized the number of outbreaks in cattle and other domestic animals over the last 3 decades. Furthermore, the use of antibiotics as a therapeutic control measure in animals has probably limited value owing to the peracute/acute nature of the disease and the extensive nature of farming operations. Outbreaks in cattle feedlots can be treated with the parenteral administration of penicillin G or with the oral ingestion of oxytetracycline or chlortetracycline in feed or water for a period of 7–10 days (Jensen & Mackey, 1971). In humans, all forms of anthrax could be treated effectively with penicillin, streptomycin, tetracycline and chloramphenicol, if a correct and timely diagnosis was made (McKendrick, 1980; Turner, 1980; Chiodini, 1988; Doganay, 1989). Under normal situations penicillin G is used as the antibiotic of choice for treating the usual clinical manifestations, although penicillin resistant isolates of *Bacillus anthracis*, producing beta-lactamase, have been reported (Lightfoot, Scott & Turnbull, 1989).

Antibiograms are usually done for 2 reasons; firstly, to establish the incidence of susceptible and resistant organisms and, secondly, to make a prediction on the therapeutic treatability of the organism. Studies of this nature have been described for *Escherichia coli* (Buys, Coetzee & Van der Walt, 1978), *Salmonella* (Cox, 1980; Mills & Kelly, 1986), *Pseudomonas aeruginosa* (Cox & Luther, 1980), and *Bacillus anthracis* (Lightfoot *et al.* 1989).

The isolation and identification of the organism is usually a relatively simple procedure, provided certain basic laboratory requirements are met. Understandably, it seems that not many laboratories are capable or willing to handle this pathogenic organism, let alone perform antibiotic susceptibility tests. This could be the reason for the absence of published reports on antimicrobial sensitivity of the anthrax organism. A recent report by Lightfoot *et al.* (1989) is the only specific reference found on the subject. The isolates used in this study did not have any prior exposure to antibiotics, as the Kruger National Park has been an animal sanctuary since 1898, with the result that no farming activities were established there. This situation is perpetuated by the policy of the National Parks Board that therapeutic intervention with regard to animal diseases is avoided.

The purpose of this study was to examine some of the *Bacillus anthracis* isolates, made from the Kruger National Park for their antibiotic susceptibility patterns against a variety of antibiotics, and to determine whether natural resistance against antibiotics exists. It could also serve to document useful information, pertinent to therapeutic intervention of human and veterinary anthrax cases. No such information has been recorded for isolates collected from a natural African ecosystem.

MATERIALS AND METHODS

Source and identification of Bacillus anthracis *isolates*

All the isolates were isolated from an endemic-contaminated area in the Kruger National Park from

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animal carcasses and soil specimens. Initial identification was made by using a selective medium, colony morphology and ability to cause mouse mortality. This was later confirmed with additional biochemical and morphological tests (Odendaal, Pieterson, De Vos & Botha, 1991).

Antibiogram discs

Mastring-S[1] M5 rings, containing ampicillin (10 µg), chloramphenicol (25 µg), penicillin G (1 unit), streptomycin (10 µg), sulphatriad (200 µg) and tetracycline (25 µg), M11 rings, containing chloramphenicol (25 µg), erythromycin (5 µg), fusidic acid (10 µg), methicillin (10 µg), novobiocin (5 µg), penicillin G (1 unit), streptomycin (10 µg) and tetracycline (25 µg), and M43 rings, containing penicillin G (1 unit), clindamycin (2 µg), gentamicin (10 µg), fusidic acid (10 µg), erythromycin (5 µg), trimethoprim (1,25 µg), sulphamethoxazole (25 µg) and tetracycline (10 µg), were used. Each ring was placed on a separate Mueller Hinton[2] agar plate. Separate discs containing cefotaxime (30 µg), netilmicin (10 µg), cefamandole (30 µg) and cefoxitin (30 µg) were placed together on a Mueller Hinton agar plate.

Disc diffusion assay for antimicrobial sensitivity

The basic assay was standardized according to the guidelines given in a report of an International Collaborative Study on the testing for antibiotic sensitivity and adapted to suit our need (Ericsson & Sherris, 1971).

Mueller Hinton agar[2] was prepared, sterilized and poured in sterile plastic Petri dishes 90 mm diameter to a depth of 4 mm and was used within 7 days of preparation. The inoculum was prepared from a pure culture of *Bacillus anthracis,* grown overnight on 10 % sheep blood tryptose agar[2]. From this, 2–3 colonies were taken and inoculated into Tryptone Soy Broth[2], and grown for approximately 14 h at 37 °C under aerobic conditions. An appropriate dilution was made with sterile physiological saline to give a density comparable to a McFarland standard opacity tube No 5. This suspension was diluted further with saline to 10^{-2}. A cotton swab was dipped into this final bacterial suspension, transferred to the Mueller Hinton agar plate and streaked in 3 directions. Uniform seeding of the plate resulted in a "just short of confluent" growth or an "apples in a box" growth pattern. The agar surface was allowed to dry for 15 min at 37 °C. The antibiotic containing discs were applied to the surface with sterile forceps and by applying light pressure, ensuring all round contact of the disc to the agar surface. A pre-incubation period of 20–30 min was allowed before overnight incubation took place aerobically at 37 °C. The inhibition zones were measured in mm with a pair of callipers and the susceptibility compared to the inhibition zones of *Staphylococcus aureus* NCTC 6571 (Ericsson & Sherris, 1971). All the above-mentioned procedures were performed in a class II Biohazard laminar flow cabinet. All the spent cultures were autoclaved for 1 h at 121 °C, immediately after use.

Evaluation of measurements

The inhibition zone of each isolate was evaluated for every antibiotic in comparison with the zones obtained with the control strain of *Staphylococcus aureus* NCTC 6571. The isolate was regarded as sensitive when its inhibition zone was larger than, equal to or <2 mm smaller than the inhibition zone of the standard reference strain. The strain was moderately sensitive when its inhibition zone was more than 2 mm smaller than the inhibition zone of the standard reference strain but >10 mm. A resistant strain had an inhibition zone of <10 mm, the diameter of the disc being 7 mm.

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RESULTS

All the isolates were sensitive to ampicillin, streptomycin, chloramphenicol, erythromycin, tetracycline, methicillin and netilmicin. More than 90 % of the isolates were sensitive to clindamycin, gentamicin and cefoxitin (Table 1). Sensitivity to penicillin G, novobiocin and cefamandole was encountered in 84,1 %, 86,4 % and 68,18 % of the isolates. A number of isolates were moderately sensitive to penicillin G (15,9 %), sulphatriad (2,2 %), clindamycin (6,8 %), fusidic acid (84,1 %) and novobiocin (13,6 %), cefamandole (31,8 %) and cefotaxime (100 %). Complete resistance was encountered with all isolates to trimethoprim (100 %) and sulphamethoxazole (100 %) and in 95,45 % of isolates to sulphatriad. No resistance occurred with ampicillin, penicillin G, streptomycin, clindamycin, fusidic acid, gentamicin, chloramphenicol, erythromycin, tetracycline, novobiocin, methicillin, cefotaxime, cefoxitin, netilmicin and cefamandole.

TABLE 1 The antibiotic susceptibility patterns of 44 different isolates of *Bacillus anthracis*

Type of antibiotic	% of isolates		
	Sensitive	Moderately sensitive	Resistant
Ampicillin	100	0	0
Cefotaxime	0	100	0
Cefamandole	68,18	31,80	0
Cefoxitin	93,18	6,80	0
Chloramphenicol	100	0	0
Clindamycin	93,18	6,80	0
Erythromycin	100	0	0
Fusidic acid	15,90	84,1	0
Gentamicin	97,80	2,20	0
Methicillin	100	0	0
Netilmicin	100	0	0
Novobiocin	86,40	13,60	0
Penicillin G	84,10	15,90	0
Streptomycin	100	0	0
Sulphatriad	2,27	2,27	95,45
Sulphamethoxazole	0	0	100
Tetracycline (10 µg)	100	0	0
Tetracycline (25 µg)	100	0	0
Trimethoprim	0	0	100

DISCUSSION

It is traditionally assumed that *Bacillus anthracis* is sensitive to penicillin G and that it will be effective as a first line of defence for the treatment of all the clinical forms of anthrax in both humans and in animals. Sensitivity to penicillin G has also been used as a diagnostic aid to distinguish between *Bacillus anthracis* and *Bacillus cereus,* the former being susceptible and the latter resistant. According to the first report of *Bacillus anthracis* isolates producing beta-lactamase (Lightfoot *et al.*, 1989), it would seem that this is not the case any more. In this study none of the isolates were resistant to penicillin G, ampicillin or methicillin. All the isolates were sensitive/moderately sensitive to the 2nd generation

cephalosporins, cefamandole and cefoxitin and to one of the 3rd generation cephalosporins, cefotaxime. Although these antimicrobial susceptibility patterns cannot predict the therapeutic success of the specific antibiotic under all circumstances, it can give a subjective indication as to the probable effectiveness.

The 2nd choice of drugs recommended includes erythromycin, tetracycline, any of the cephalosporins, and chloramphenicol, with the 3rd choice being lincomycin (Goodman & Gilman, 1975; Turner, 1980; McKendrick, 1980; Chiodini, 1988). In some human clinical cases, penicillin G, tetracycline, "Bactrim", or streptomycin were described as the antibiotics of choice (Turner, 1980; Pugh & Davies, 1989). Apart from the use of "Bactrim", these findings concur with the results of this study where it is evident that *Bacillus anthracis* is sensitive/moderately sensitive to erythromycin (100 % of isolates), chloramphenicol (100 %), tetracycline (100 %), and the aminoglycosides including streptomycin (100 %), gentamicin (97,8 %) and netilmicin (100 %), and clindamycin (93,18 %). It is assumed that these antibiotics can be used with a reasonable measure of success alone or even possibly in combination with each other, when an early clinical case of anthrax is presented. The use of any sulphonamide alone or in combination would not be effective as a treatment. The fact that these isolates originated from an area where previous exposure to any type of commercial antibiotic preparation was and is impossible implies that the resistance encountered to trimethoprim (100 % of isolates), sulphamethoxazole (100 %) and sulphatriad (95,45 %) is part of the genetic constitution of these isolates. In effect, this would mean that the organism would not respond to therapy with any of these sulphonamides, and they probably could be used to constitute a selective medium to suppress bacterial contaminants during primary isolation procedures.

The difference or resemblance of the antibiotic susceptibility patterns for the different isolates of *Bacillus anthracis* could possibly be of assistance as epidemiological markers in an endemic region such as the Kruger National Park.

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