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Full Article

Antibiotic resistance / susceptibility of 30 isolates of *Bacillus anthracis* isolated in Iran between 2007 and 2008

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ABSTRACT

Thirty isolates of *Bacillus anthracis* recovered from animal carcasses, soil and human in different localities in Iran between 2007 and 2008. They were tested by standard disc diffusion susceptibility method for their resistant/ susceptibility to different kinds of antibiotics. According to American National Committee of Clinical Laboratory Standards (NCCLS) guidelines all isolates were sensitive to levofloxacin (100%), cefixime (100%). Other isolates showed different kinds of sensitivity to: doxycycline (96.7%), cephalothin (95.6%), ampicillin (95.6%), nitrofurantoin (95.6%), tetracycline (94.4%), ofloxacin (89.9%), gentamycin (77.8%), nalidixic acid (72.2%), kanamycin (75.6%), erythromycin (71.1%), piperacillin (78.9%), tobramycin(64.4%), choramphenicol (59.9%), cefotaxime (33.3%), ciprofloxacin (1.1%), cefuroxime (33.3%), azithromycin (44.4%), streptomycin (55.6%), ticacillin (35.6%), rifampicin (34.4%), clindamycin (74.4%), ceftriaxon (26.7%), methicillin (55.6%), trimethoprim (8.9%), cloxacillin (31.1%) and penicillin (74.4%). One of the isolates was complete resistance to penicillin.Therefore, preventive and therapeutic strategies involving the use of antibiotics should take the possibility of resistance and/or susceptibility of the isolates into account and not decided without antibiotic sensitivity testing.

Keywords: Bacillus anthracis, Antibiotics, Resistance/susceptibility, Iran

INTRODUCTION*

Bacillus anthracis, the causative agent of anthrax, is a large, non–motile, aerobic rod- shaped and spore forming Gram-positive organism. A glutamic-polypeptide capsule is present that inhibits phagocytosis, and has a major role in the agent's pathogenic capabilities. Anthrax toxin is well known the major toxic component of the bacillus, but non-virulent toxin–producing strains have also been isolated. These mutant strains fail to produce the polyglutamic acid capsule. Anthrax is a zoonotic disease usually found in herbivorous animals with high mortality rate and is known to affect those humans who are occupationally exposed. Humans can be infected after contact with infected animals or their meat and

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waste products. Infections usually respond well to prompt antibiotic treatment. (Reinser & wood, 2001, Bryskier 2002, Sirisanthana *et al* 2002, Turnbull 2008).

Recent events have demonstrated that the major threat of *B. anthracis* is connected to bioterrorism and biological warfare. During the Persian Gulf war, it was clearly demonstrated that Iraq possesses anthrax as a biological weapon. Spores produced in dry form can be spread by means of letters of aerosols (Altas 1998, Bryskier 2002). As all incidents must be treated as real until proven otherwise, there is a necessity for a rapid and effective antibiotic treatment and prophylaxis. Doxycycline and ciprofloxacin are effective antibiotic choices for treatment, however, resistance to these antibiotics has been previously described (Cavallo *et al* 2002, Choe *et al* 2000). Moreover, the inappropriate use of these drugs may result in the emergence of antibiotic–resistance strains in naturally acquired disease. In control programs of anthrax in animals in Islamic Republic of Iran, the antibiotic susceptibility of local isolates for treatment of animals is also very important. In spite of the anthrax affecting human and animals in Iran (Moazeni Jula *et al* 2007, Tabei *et al* 2004, Parvizpour 1978, Babamahmoodi *et al* 2006). there is no information about antibiotic sensitivity and or resistant of Iranian B. anthracis isolates. The aim of this study was to determine the in vitro susceptibility of 30 isolates of B. anthracis recovered in Iran to 28 different antibiotics in order to expand the choices for effective antibiotic treatment.

MATERIALS AND METHODS

B. anthracis isolates. Two B. anthracis isolates were obtained from human infection cases acquired in Iran, 8 isolates were isolated from animal sources and 20 isolates were isolated from animal environmental sources during a research project from 2007–2008 in different parts of Iran (Moazeni Jula *et al* 2007). Isolates had been stored in lyophilized form in special ampules, purity of each isolates was checked by plating onto sheep blood agar.

Antibiotic sensitivity testing. All isolates of B. anthracis were tested for their susceptibility/resistance to 28 different antibiotic discs present in Iran, using the agar diffusion method according to the Kirby– Bauer disc–diffusion method (Astra 2008, Diaz *et al* 1992, Qin *et al* 2004). All works with the bacteria were done in a class 2 biosafety cabinet in the biosafety level 3 laboratory of Razi Vaccine and Serum Research Institute (RVSRI). A McFarland 0.5 standard suspension of each isolate in 5 ml of phosphate–buffered saline was prepared and swabbed over the entire surface of 5% sheep blood agar medium plates with a sterile cotton swab. With the aid of an automatic dispenser a set of 5 antibiotic discs with following concentration were delivered to the surface of medium: cephalothin (30µg), ampicillin (10µg), nitrofurantoin (300µg), gentamycin (10µg), penicillin G(10µg), nalidixic acid (30µg), kanamycin (30µg), erythromycin (15µg), cloxacillin (5µg), piperacilllin (100µg), trimethoprim (5µg) tobramyclin (10µg), chloramphenicol (30µg), cefotaxime (30µg), cefixime (5µg), ciprofloxacin (5µg), cefuroxime sodium (30µg), azithromycin (15µg), oflaxacin (5µg), stroptomycin (10µg), doxycycline(30µg), ticacillin (75µg), tetracycline (30µg), levofloxacin (5µg), rifampicin (5µg), clindamycin (2µg), ceftriaxone (30µg), methicillin (5µg). The discs were gently pressed onto the medium with sterile forceps to ensure firm contact. The inoculated plates were left at room temperature to dry for 5-10 minutes. Following overnight incubation at 37 ^{o}C clear zones produced by antimicrobial inhibition of bacterial growth were measured.

The size of zones of inhibition of growth around the discs indicated the different susceptibilities of the isolates to the antibiotics. These zones were checked carefully with the American NCCLS guidelines to determine the sensitivity of the isolates. The NCCLS advocates the use of "percent susceptibility" for each data box, clarification of where the isolates come from and description of collection period (NCCLS 2002) which was carried it out between 2007-2008. Reporting the susceptibility data as percent susceptible for each

organism-antimicrobial agent combination is the most commonly used method.

RESULTS

Antibiograms of Iranian isolates of B. anthracis showed that susceptibility/resistance of these isolates to 28 antibiotics were completely different. According to American NCCLS, some of the isolates were shown different susceptibility to different antibiotics for example highly sensitive (100% sensitivity), some were mild sensitive (60-70%), some were to some extend sensitive (30-40%) and the others were resistant (0-10%) to different antibiotics (NCCLS 2002, Zapantis *et al* 2005).

The results of the antimicrobial susceptibility testing are shown in Table 1. Among the 30 isolates tested in this study 13 isolates were 100% sensitive, 12 isolates were 66.7% sensitive, 4 isolates were less sensitive (about 33.3% sensitive) and one isolate was completely resistant (0% sensitive) to penicillin G.

Cloxacillin, trimethoprime, tobramycin, chloramphenicol, cefotaxime, ciprofloxacin, cefuroxime, azithromycin, streptomycin, ticacillin, tetracycline, rifampicin, ceftriaxon, and methicillin showed less activity against most of the isolates (55.6%≤ sensitivity percent ≤1.1%), in other words most isolates were less susceptible to them , and some isolates showed complete resistant to some of them, e.g., four isolates showed resistant to cloxacillin, 6 isolates to cefotoxime, all isolates to ciprofloxacin, 4 isolates to cefuraxime, 2 isolates to ticacillin, but only one isolate to clindamycin, 9 isolaes to ceftriaxon and 2 isolates to methicillin were completely resistant. All isolates were completely resistant to trimethoprim and ciprofloxacin (Table 1). One of the isolates which showed resistant to penicillin G, was also resistant to cloxacillin, trimethoprim, ciprofloxacin, cefuroxime, clindamycin, and methicillin, but highly susceptible to erythromycin, cefuroxime and levofloxacin. Moderate sensitivity occurred with cloxacillin (31.1% of the isolates just were sensitive) except 4 isolates which were resistant.

Table1. Antibiogram test results as sensitivity percent of the 30 isolates using American NCCLS for analysis and presentation of cumulative susceptibility data**.**

Antibiotics	**CF**	**AM**	**FM**	**GM**	**P**	**NA**	**K**	**E**	**CX**	**SXT**	**PIP**	**TOB**	**C**	**CTX**
Isolates sensitivity percent	95.6	95.6	95.6	77.8	74.4	72.2	75.6	71.1	31.1	8.9	78.9	64.4	59.9	33.3
Antibiotics	CP	CFM	XM	AZM	OFX	S	D	TIC	TE	LOM	RA	CC	CRO	ME
Isolates sensitivity percent	100	1.1	33.3	44.4	89.9	55.6	96.7	35.6	94.4	100	34.4	74.4	26.7	55.6

CF: cephalothin, AM: ampicillin, FM: nitrofurantoin, GM: gentamycin, P: penicillin-g, NA: nalidixic acid, K: kanamycin, E: erythromycin, CX: cloxacillin, SXT: trimethoprim, PIP: piperacillin, TOB: tobramycin, C: chloramphenicol, CTX: cefotaxime, CP: cefixime, CFM: ciprofloxacin, XM: cefuroxime, AZM: azithromycin, OFX: ofloxacin, S: sterptomycin, D: doxycycline, TIC: ticacillin , TE: tetracycline, LOM: levofloxacin, RA: rifampicin, CC: clindamycin, CRO: ceftriaxon, ME: methicillin

The results also showed that all isolates had a range of sensitivity from 100% to 70% for cephalothin, ampicillin, nitrofurantoin, gentamycin, nalidixic acid, kanamycin, erythromycin, piperacillin, cefixime, ofloxacin, doxycycline, levofloxacin and clindamycin.

While all of the isolates had a sesnsitivily rate about 44.4% to azithromycin, 34.4% to rifampicin, 31.1% to cloxacillin, 33.3% to cefotaxime and 26.7% to ceftriaxon, four, six and also nine isolates were resistant to last three above mentioned antibiotics respectively.

DISCUSSION

B. anthracis is usually susceptible to a broad range of antibiotics (Doganay & Aydin, Turnbull 2008, Yamamoto *et al* 2001, Odendaal *et al* 1991). Many factors have contributed to the development of resistance in the bacteria, including misuse, overuse, quality and potency of the antimicrobial agents (Turnbull 2008).

Among the 30 isolates tested in this study, one strain (3.3%) was unexpectedly found to be resistant to penicillin and 4 isolates (13.2%) were less sensitive to it. Penicillin-resistant *B. anthracis* strains are rare (about 3% of anthrax strains) but have been reported in various countries (Lightfoot *et al* 1990, Choe *et al* 2000). A similar or more percentage of resistance to penicillin G has been previously described in south Africa in Keruger National park and in some other countries (Bryskier 2002, Odendaal *et al* 1991, Bradaric & Punda–Polic 1992, Frean *et al* 2003). However, to our knowledge, it is the first report of such a strain in Iran. So, preventive strategies involving the use of antibiotics should take the possibility of resistance and sensitivity into accounts.

As all or some of the isolates are less sensitive or resistant to some of the antibiotics tested, since the less sensitive isolates may loss their sensitivity and develop resistance in the future, we advise that these drugs should not be used in prophylaxis or in the medical and veterinary management of anthrax without previously susceptibility testing. On the other hand some of antibiotics have shown acceptable sensitivity to the isolates so it is possible to them to prescribe against clinical cases. These antibiotics are included: levofloxacin (100% with sensitivity percent), cefixime (100%), doxycycline (96.6%), cephalothin (95.6%), ampicillin (95.6%), nitrofurantoin (95.6%), tetracycline (94.4%), and ofloxacin (90.0%).

The reason for this resistance and less susceptibility of some isolates to penicillin or other antibiotics is not known. However, it could be contributed to the naturally emergence of resistance in these isolates due to misuse or overuse and or other factors. The antibiotic susceptibility patterns showed that all isolates have different sensitivity to different drugs as shown in Table 1. Differences in susceptibility patterns were considered to be due to the variation in virulent plasmids (Venkatesh *et al* 2007).

Since resistant and/or less sensitive isolates to penicillin, trimethoprime, ciprofloxacin and ceftriaxon occurs, therefore these drugs do not advise to be used in prophylaxis or in medical and veterinary management of anthrax without previous susceptibility testing. Multidrugs resistance (2 or more antibiotics) was observed in some isolates. The only isolate resistant to penicillin, is also resistant to cloxacillin, trimethoprim, cefixime, cefuroxime, clindamycin, and methicillin, and less sensitive to cephalothin, ampicillin, chloramphenicol, cefotaxine, azithromycin, rifampicin and ceftriaxon. Cavallo (2002) has reported that ciprofloxacin and doxycycline were to be effective against all of their 96 isolates of B. anthracis isolated in France between 1994 and 2000, and the United States government recommended the use of these 2 drugs for treatment of inhalation and cutaneous anthrax (Cavallo *et al* 2002, CDC 2001). However, resistance to these 2 antibiotics has been reported elsewhere (Choe et al 2000). In our study it was found that these drugs have completely different patterns of activity against our B. anthracis isolates. As it is shown in Table 1 all isolates showed high sensitivity to levofloxacin, cefixim, cephalothin, ampicillin, doxycycline, nitrofuranthoin and tetracycline but all of them were resistant to ciprofloxacin. Since in this study cephalothion, ampicillin, nitrofurantoin, gentamycin, cefixime, ofloxacin, doxycycline, tetracycline and levofloxacin which showed relatively good in vitro activity, could be alternative drugs for treatment and prevention of B. anthracis infections in human and animals. The isolates with less antibiotic sensitivity may have been the potential for the emergence of resistance,

and so may develop resistance in future to some drugs.

The susceptibility of B. anthracis isolates to penicillin has changed considerably over time. In the past, penicillin has been the drug of choice for treatment of B. anthracis infections, and also has been used in laboratories as a means for differential diagnosis of B. anthracis isolates. In conclusion, B. anthracis remains susceptible to many antibiotics, including cephalothin, ampicillin, etc. The good activity of such drugs against all isolates demonstrates that these antibiotics are appropriate choice for prophylaxis and treatment of B. anthracis infection and could be used as good alternatives in the replacement of other antibiotics which have less or no activities. At last, periodic isolation and evaluation of the susceptibility/resistance patterns of new isolates could be particularly useful.

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