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Antibiotic Susceptibilities of 96 Isolates of *Bacillus anthracis* Isolated in France between 1994 and 2000

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Ninety-six isolates of *Bacillus anthracis* recovered in France between 1994 and 2000 were tested for their susceptibilities to 25 different antibiotics. Resistance to penicillin G and amoxicillin was 11.5%. All of the isolates were resistant to cotrimoxazole and susceptible to doxycycline, ciprofloxacin, pefloxacin, levofloxacin, teicoplanin, vancomycin, clindamycin, imipenem, and rifampin.

Bacillus anthracis is an aerobic rod-shaped organism that is the causative agent for anthrax. Humans can be infected after contact with infected animals or their waste products. When anthrax affects humans, it is usually from an occupational exposure, and the cutaneous form represents the majority of cases (95%) (6). Infections usually respond well to prompt antibiotic treatment. The intestinal form is rare and follows the ingestion of food contaminated by spores (14). This form is characterized by an acute inflammation of the intestinal tract, and initial symptoms include nausea, vomiting, and fever. These symptoms are followed by abdominal pain, vomiting of blood, and severe diarrhea. Intestinal anthrax results in death in 25 to 60% of cases. The respiratory form follows the inhalation of spores and is characterized by an abrupt clinical onset with mild fever and malaise. The first stage of infection, lasting from hours to a few days, involves flu-like symptoms including fever, coughing, weakness, and chest pain. The second stage of inhalation infection usually ends in death within a period of days (1, 13). Despite their gravity, both intestinal and respiratory infections can be treated by early intravenous administration of antibiotics and intensive supportive care. Without immediate treatment, inhalation anthrax is usually fatal.

Recent events have demonstrated that the major threat of *B. anthracis* is connected to bioterrorism and biological warfare. Spores produced in dry form can be spread by means of letters or aerosols (2). As all incidents must be treated as real until proven otherwise, there is a necessity for a rapid and effective antibiotic prophylaxis. Doxycycline and ciprofloxacin are effective antibiotic choices for treatment; however, resistance to these antibiotics has been previously described (4, 10). Moreover, the inappropriate use of these drugs may result in the emergence of antibiotic-resistant strains in naturally acquired disease.

The aim of this study was to determine the in vitro susceptibilities of 96 isolates of *B. anthracis* recovered in France to 25 different antibiotics in order to expand the choices for effective antibiotic treatment.

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These 96 isolates were provided by (i) Le Centre d'Etudes du Bouchet, Vert-le-Petit, France; (ii) Unité des Toxines et pathogénie bactérienne, Institut Pasteur, Paris, France; and (iii) Laboratoire de l'Afssa Lerpaz, Unité des Zoonoses Bactériennes, Maisons Alfort, France.

One strain was isolated from a human source, 28 were isolated from animal sources, and 67 were isolated from environmental sources. Phenotypic identification was done by a routine laboratory technique: Gram staining, motility, and hemolysis on blood agar. Biochemical identification was performed by using the Biolog (Hayward, Calif.) system with the dangerous pathogens database. Genotypic characterization was performed by using PCR analysis to detect virulence factor genes located on two plasmids: pXO1 (*lef*) and pXO2 (*capA*) (12).

The antibiotics tested were penicillin G (Aventis-Pasteur), amoxicillin (Pan-Pharma), piperacillin (Lederle), cephalothin (Pan-Pharma), cefoxitin (Merck-Sharp-Dome-Chibret), ceftriaxone (Roche), aztreonam (Sanofi-Winthrop), imipenem (Merck-Sharp-Dome-Chibret), chloramphenicol (Sanofi-Winthrop), cotrimoxazole (Roche), vancomycin (Lilly), teicoplanin (Roussel-Diamand), erythromycin (Abbott), azithromycin (Pfizer), clindamycin (Pharmacia-Upjohn), doxycycline (Pfizer), rifampin (Roussel-Diamand), streptomycin (Sigma), gentamicin (Dakota), ciprofloxacin (Bayer), gatifloxacin (Grunenthal), pefloxacin (Aventis-Pasteur), ofloxacin (Aventis-Pasteur), and norfloxacin (Merck-Sharp-Dome-Chibret). The presence of β-lactamase was indicated by the nitrocefin test (Biomerieux) performed in liquid medium. For this test, a single colony, obtained after an overnight culture on Trypticase soy agar medium, was diluted in 0.5 ml of distilled water. The disk of nitrocefin was then added directly to the distilled water containing the bacteria. A light pink coloration of the medium could be observed in cases of positive reaction. The positive control used was a strain of *Bacillus cereus* isolated in our laboratory.

The MICs were determined by the agar dilution method with Mueller-Hinton medium. Three colonies of each strain were grown overnight in Mueller-Hinton broth. The final inoculum (10^4 CFU per spot) was obtained after dilution of this

TABLE 1. Antibiotic susceptibilities of 96 isolates of *B. anthracis*[a]

Antibiotic	MIC_{50}	MIC_{90}	Range	Breakpoints	% S/I/R[b]
Penicillin G	0.125	8	0.125–16	≤0.12–≥0.25	88.5/0/11.5
Amoxicillin	0.125	4	0.125–16	≤0.25–≥0.5	88.5/0/11.5
Piperacillin	1	1	0.25–32	≤8–≥16	99/1/0
Cephalothin	0.5	16	0.125–32	≤8–≥32	83.2/12.2/4.6
Cefoxitin	8	32	1–64	≤8–≥32	74/15.3/10.7
Ceftriaxone	32	32	4–64	≤8–≥64	0/100/0
Aztreonam	128	1–>128	1–>128	≤4–>32[c]	0/0/100
Imipenem	0.125	0.125	0.125–2	≤4–>16	100/0/0
Chloramphenicol	2	2	1–4	≤8–>32	100/0/0
Cotrimoxazole	>4/76	>4/76	<4/76	≤2/38–≥4/76	0/9/91
Vancomycin	1	1	0.25–2	≤4–≥32	100/0/0
Teicoplanin	0.25	0.5	0.125–0.5	≤8–≥32	100/0/0
Erythromycin	1	1	0.5–4	≤0.5–≥8	95.4/4.6/0
Clindamycin	0.125	0.25	0.125–1	≤0.5–≥4	100/0/0
Doxycycline	0.125	0.25	0.125–0.25	≤4–≥16	100/0/0
Rifampin	0.125	0.125	0.125–0.5	≤1–≥4	100/0/0
Streptomycin	1	1	0.5–2	≤8–>16[c]	100/0/0
Gentamicin	0.25	0.5	0.125–0.5	≤4–≥16	100/0/0
Nalidixic acid	4	8	0.125–32	≤8–>16[c]	94.8/4.2/1
Ciprofloxacin	0.06	0.25	0.03–0.5	≤1–≥4	100/0/0
Gatifloxacin	0.125	0.125	0.125–0.125	≤1–>4[c]	100/0/0
Pefloxacin	0.125	0.5	0.03–1	≤1–>4[c]	100/0/0
Levofloxacin	0.125	0.25	0.03–1	≤2–≥8	100/0/0
Ofloxacin	0.25	0.25	0.06–2	≤1–>8	99/1/0

[a] All parameters according to the NCCLS antimicrobial susceptibility standards for staphylococci, or to the French Comité de l'antibiogramme of the Société française de microbiologie if unavailable from the NCCLS.

[b] S/I/R, susceptible/intermediate/resistant.

[c] Breakpoints not available for staphylococci in the NCCLS antimicrobial susceptibility testing standards. Breakpoints presented are defined in the general recommendation of the French Comité de l'antibiogramme of the Société française de microbiologie.

overnight culture in distilled water. Inoculation of the plates was done by using a multiple automatic inoculator (Dynatech). The results were determined after incubating the plates for 18 h at 37°C. To check the validity of the antimicrobial test susceptibility method, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were tested in parallel with each series of determination. Interpretative criteria for staphylococci were used, because there are no National Committee for Clinical Laboratory Standards (NCCLS) guidelines for interpreting the results of susceptibility tests for *Bacillus* spp. When interpretative criteria were not available, the breakpoints used were those determined by the general recommendation of the Comité de l'antibiogramme de la Société française de Microbiologie (http://www.sfm.asso.fr/Wuk.html). The results, expressed as the MIC at which 50% of the isolates tested were inhibited (MIC_{50}), the MIC_{90}, the range, and the percentage of susceptibility, are presented in Table 1.

B. anthracis is usually susceptible to a broad range of antibiotics (5, 9). Among the 96 isolates tested in this protocol, 11 (11.5%) were categorized as resistant to penicillin G and amoxicillin. They were distributed among environmental and animal strains. As the nitrocefin test was positive for all of these isolates, the resistance could be due to a low level of production of the usually silent β-lactamase (3). A similar percentage of resistance to penicillin G has been previously described in South Africa in Kruger National Park (9). As resistance to penicillin G occurs frequently and should be suspected after a terrorist attack or in biological warfare, we advise that penicillin G and amoxicillin should not be used in prophylaxis or in the medical management of anthrax without previous susceptibility testing. Among the 11 isolates resistant to penicillin G, 10 were susceptible to piperacillin. We observed an identical pattern of resistance in a *Bacillus thuringiensis* isolate from a human infection (7). All of the isolates were resistant to the cephalosporins of the third generation, and 26% were intermediate or resistant to cefoxitin. This resistance is also present in *B. cereus* and *B. thuringiensis* (6). All of the isolates were susceptible to imipenem, but there is no report of the use of this antibiotic for the treatment of anthrax in the literature. Resistance to cotrimoxazole was found in all of the isolates; this intrinsic resistance has been previously reported (9). The susceptibilities to erythromycin and clindamycin were 95 and 100%, respectively. A resistance determinant to macrolide-lincosamine-streptogramin B has, however, been described (8), and it would be easy to produce resistant strains for use in biocrimes. Rifampin was also active, but resistance is easily produced in vitro (11). This antibiotic, as well as gentamicin and streptomycin, could be used in association with a fluoroquinolone for the treatment of *B. anthracis* infection. There was no resistance to vancomycin or teicoplanin. These antibiotics could be reserved for treatment in cases of infection due to multiresistant isolates. Resistance or decreased susceptibility to nalidixic acid was present in 5.2% of the isolates. Ciprofloxacin and doxycycline were determined to be effective against all of the isolates. Resistance to these antibiotics, however, has been reported (4). The other fluoroquinolones (gatifloxacin, pefloxacin, ofloxacin, and levofloxacin) also had good activities and could be used as replacements for ciprofloxacin if needed.

In conclusion, *B. anthracis* remains susceptible to many antibiotics, including doxycycline and fluoroquinolones. Resistance to penicillin G and amoxicillin was present in 11.5% of

the isolates, suggesting that these antibiotics should not be used in prophylaxis or in the treatment of humans without susceptibility testing. The good activity of doxycycline against all of the isolates demonstrates that this antibiotic is an appropriate choice for prophylaxis of *B. anthracis*. All of the fluoroquinolones tested have good activities, and ofloxacin, pefloxacin, gatifloxacin, and levofloxacin could be used as good alternatives in the replacement of ciprofloxacin.

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