CONCISE COMMUNICATION

Antibiotic susceptibility of isolates of *Bacillus anthracis*, a bacterial pathogen with the potential to be used in biowarfare

M. E. Jones[1*], *J. Goguen*[2], *I. A. Critchley*[1], *D. C. Draghi*[1], *J. A. Karlowsky*[1], *D. F. Sahm*[1], *R. Porschen*[3], *G. Patra*[4] *and V. G. DelVecchio*[4,5]

[1]Focus Technologies, Herndon, VA, [2]Department of Molecular Genetics and Microbiology, University of Massachusetts, Worcester, MA, [3]Focus Technologies, Cypress, CA, [4]Medical School and Institute of Molecular Biology and Medicine, University of Scranton, Scranton, PA and [5]Vital Probes Inc., Mayfield, PA, USA

[*]Tel: +1 703 480 2500 Fax: +1 703 480 2654 E-mail: mjones@focustechnologies.com

Bacillus anthracis is a bacterial species that could be used in a bioterrorist attack. We tested a collection of isolates with a range of relevant antimicrobial compounds. All isolates tested were susceptible to ciprofloxacin and doxycycline. Penicillin and amoxicillin, with or without clavulanate, showed in vitro activity against all *B. anthracis* isolates. Ceftriaxone demonstrated lower-level in vitro activity compared to penicillin-related compounds against *B. anthracis*. In vitro data from this study are in keeping with available guidelines.

Keywords *Bacillus anthracis*, biowarfare, antibiotic susceptibility

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Recent events have demonstrated the need to improve our understanding of organisms such as *Bacillus anthracis*, a species that can be used as a bacterial warfare agent during a bioterrorist attack. For *B. anthracis*, disease following inhalation of aerosolized organisms is the likely course of events following a bioterrorist attack. *B. anthracis* is the causative agent of anthrax, a zoonotic disease most commonly acquired via cutaneous entry [1]. Inhalation anthrax is the most severe form and is usually fatal if not treated immediately. Antimicrobial resistance in this species has been reported [2–4], and inappropriate antibiotic usage may select for resistant isolates. Naturally occurring anthrax strains are susceptible to penicillin and doxycycline, and both drugs are approved by the Food and Drug Administration (FDA) for the treatment of anthrax disease. However, based on limited in vitro and animal data, ciprofloxacin has emerged as a recommended first-line agent for the treatment and prevention of anthrax, since expert groups recommend that anthrax strains should be assumed to be resistant to penicillin and doxycycline until laboratory results demonstrate otherwise [5]. Here we report susceptibility test results from a collection of *B. anthracis* strains and consider test data in the light of the treatment guidelines available.

The strain collection (Table 1) included 12 isolates of *B. anthracis* derived from various geographic locations in Europe and North America. These included *B. anthracis* Ba 7700, a French laboratory plasmid-less strain obtained by curing the *B. anthracis* 7702 strain by nalidixic acid treatment [6], and the strain *B. anthracis* Sterne, used as live vaccine. *B. anthracis* strains RA3R and 7611R were isolated from a recent French outbreak [7]. The Ames strain used in recent terrorist attacks in the USA was not included. All organisms were identified using standard laboratory techniques that included biochemical [8] and genotypic identification, which included analysis of plasmid content (i.e. pXO1, pXO2) by a multiplex PCR assay to confirm identity and virulence status [9]. For safety reasons, only avirulent isolates were included in this study (i.e. $pXO1^{+}/2^{-}$, $pXO1^{-}/2^{+}$, $pXO1^{-}/2^{-}$). Other methods, not used here, such as those utilizing antigen-specific ELISA [10] and various real-time PCR protocols [11], have also been developed to identify *B. anthracis*. All isolates were tested for susceptibility to amoxicillin, amoxicillin–clavulanate, azithromycin, ceftriaxone, ciprofloxacin, doxycycline, erythromycin, and penicillin. National Committee for Clinical Laboratory Standards (NCCLS) recommended standard

Table 1 Minimal inhibitory concentrations of antimicrobial agents for isolates of *B. anthracis*

Organism	MIC (mg/L)							
	Azithromycin	Amoxicillin	Amoxicillin–clavulanate	Ceftriaxone	Ciprofloxacin	Doxycycline	Erythromycin	Penicillin
B. anthracis Ba7700[a]	1	≤0.06	≤0.06/0.03	4	0.25	0.015	0.5	≤0.06
B. anthracis Ba1099	4	≤0.06	≤0.06/0.03	8	0.03	0.015	0.5	≤0.06
B. anthracis BaΔUM2311	2	≤0.06	≤0.06/0.03	4	0.06	0.015	1	≤0.06
B. anthracis Ba0074	4	≤0.06	≤0.06/0.03	4	0.03	0.015	1	≤0.06
B. anthracis BaA58	2	≤0.06	≤0.06/0.03	4	0.03	0.015	0.5	≤0.06
B. anthracis Ba0077	2	≤0.06	≤0.06/0.03	4	0.03	0.015	0.5	≤0.06
B. anthracis Ba0074	4	≤0.06	≤0.06/0.03	8	0.06	0.015	1	≤0.06
B. anthracis Ba1014	2	≤0.06	≤0.06/0.03	8	0.06	0.015	0.5	≤0.06
B. anthracis BaRA3R	4	≤0.06	≤0.06/0.03	4	0.03	≤0.008	1	≤0.06
B. anthracis Ba Sterne[b]	4	≤0.06	≤0.06/0.03	8	0.03	0.015	0.5	≤0.06
B. anthracis Ba 7611R	4	≤0.06	≤0.06/0.03	4	0.12	≤0.008	1	≤0.06
B. anthracis BaA3	1	≤0.06	≤0.06/0.03	16	0.06	0.015	2	≤0.06

[a]Nalidixic acid-resistant laboratory strain [6].
[b]Vaccine strain.

procedures have not been defined for these organisms [12]. Antimicrobial susceptibility testing was therefore conducted in accordance with NCCLS guidelines for testing of non-fastidious facultative aerobic organisms. As a guide to the interpretation of antibiotic MICs for *B. anthracis*, NCCLS-defined interpretive criteria for *Staphylococcus aureus* as a representative Gram-positive species were used; these are also commonly used by other authors, including the Centers for Disease Control (CDC) and Prevention, USA [13]. The distribution of antimicrobial data in *Bacillus* species tends to be in line with *Staphylococcus* MIC breakpoints used in this study. Antimicrobial agents were tested using broth microdilution on commercially prepared dried panels (Trek Diagnostics, Westlake, OH, USA), to determine the MIC of each agent after incubation at 35–37 °C for 18–24 h. *S. aureus* ATCC 29213 was used as a quality control strain. A recent study by Mohammed *et al*. [13] showed no variability between results produced by broth microdilution and those produced by Etest gradient strips.

For anthrax, in the USA at least, the consensus is that ciprofloxacin is effective in both prophylaxis and curative treatment [14]. Doxycycline, penicillin G and amoxicillin are also possible choices if susceptibility testing predicts their efficacy. From our collection, all *B. anthracis* strains (Table 1) were susceptible to ciprofloxacin, with MICs ranging from 0.03 to 0.25 mg/L, including the nalidixic acid-resistant laboratory mutant strain Ba7700, although for this strain the MIC of ciprofloxacin was raised to 0.25 mg/L, which leads to concern about the possibility of engineering ciprofloxacin-resistant strains. Similarly, all isolates were susceptible to doxycycline, penicillin and amoxicillin, with no increase in susceptibility provided through the addition of clavulanic acid. Recent data have also suggested that some strains of *B. anthracis* express a penicillinase unlikely to be inhibited by clavulanic acid, although we did not detect any [15]. Using the *S. aureus* breakpoint of 8 mg/L, all strains of *B. anthracis* tested were susceptible to ceftriaxone except for BaA3 which tested as intermediate, although previous reports and a recent report from France [2,7,15,16] show variable susceptibility in other strain collections tested, and consensus opinion does not recommend the use of non-penicillin β-lactams for the treatment of anthrax. All strains also showed susceptibility to erythromycin and azithromycin.

However, there are no clinical data available for this compound class to enable any recommendation for use. For these compounds and other protein inhibitor compounds not tested here that display in vitro activity, such as rifampin, chloramphenicol, and aminoglycosides, combination therapy might be useful in improving clinical outcome, although again there is a lack of clinical data.

The susceptibility data presented here support current recommendations for the use of ciprofloxacin or doxycycline as empirical agents for the treatment of anthrax, and penicillin as a first-line agent if susceptibility is confirmed. However, a recent report showed serially passaged isolates of *B. anthracis* Sterne to have raised MICs of doxycycline, macrolides, and ciprofloxacin, and thus clearly resistant isolates are fairly easy to engineer [4]. Additionally, another report also characterized a strain of *B. anthracis* expressing resistance to macrolides, lincosamides and streptogramins through a single gene locus *ermJ*, presumably making the in vitro genetic modification of a susceptible strain straightforward [16]. For children or pregnant women, susceptibility testing plays a crucial role in allowing a switch to agents such as penicillin (or amoxicillin) for anthrax. Websites published by the CDC (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5042a1.htm), the European Medicines Evaluation Agency (EMEA) (http://www.emea.eu.int/htms/human/bioterror/bioterror.htm) and the Public Health Laboratory Service (http://www.phls.org.uk/topics_az/deliberate_release/menu.htm), respectively, provide treatment recommendations. The susceptibility data reported in this study would support the recommendations published in these guidelines.

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REFERENCES

1. Dixon TC, Meselson M, Guillemin J, Hanna PC. Anthrax. *N Engl J Med* 1999; 341: 815–26.
2. Cavallo JD, Ramisse F, Girardet M, Vaissaire J, Mock M, Hernandez E. Antibiotic susceptibilities of 96 isolates of *Bacillus anthracis* isolated in France between 1994 and 2000. *Antimicrob Agents Chemother* 2002; 46: 2307–9.
3. Choe CH, Bouhaouala SS, Brook I, Elliot TB, Knudson GB. In vitro development of resistance to ofloxacin and doxycycline in *Bacillus anthracis* Sterne. *Antimicrob Agents Chemother* 2000; 44: 1766.
4. Brook I, Elliott TB, Pryor HI *et al*. In vitro resistance of *Bacillus anthracis* Sterne to doxycycline, macrolides and quinolones. *Int J Antimicrob Agents* 2001; 18: 559–62.
5. Inglesby TV, O'Toole T, Henderson DA *et al*. Anthrax as a biological weapon, 2002: updated recommendations for management. *JAMA* 2002; 1: 2236–52.
6. Patra G, Fouet A, Vaissaire J, Guesdon JL, Mock M. Variation of rRNA operon number as revealed by ribotyping of *Bacillus anthracis* strains. *Res Microbiol* 2002; 153: 139–48.
7. Patra G, Vaissaire J, Weber-Levy M, Le Doujet C, Mock M. Molecular characterization of *Bacillus* strains involved in outbreaks of anthrax in France in 1997. *J Clin Microbiol* 1998; 36: 3412–4.
8. Turnbull PC, Kramer JM. Bacillus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of clinical microbiology*, 6th edn. Washington, DC: ASM Press, 1995: 349.
9. Ramisse V, Patra G, Garrigue H, Guesdon JL, Mock M. Identification and characterization of *Bacillus anthracis* by multiplex PCR analysis of sequences on plasmids pXO1 and pXO2 and chromosomal DNA. *FEMS Microbiol Lett* 1996; 145: 9–16.
10. Sastry KS, Tuteja U, Santhosh PK, Lalitha MK, Batra HV. Identification of *Bacillus anthracis* by a simple protective antigen-specific mAb dot-ELISA. *J Med Microbiol* 2003; 52: 47–9.
11. Keim P, Price LB, Klevytska AM *et al*. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J Bacteriol* 2000; 182: 2928–36.
12. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing; Twelfth Informational Supplement*. M100-S12. Wayne, PA: NCCLS, 2002.
13. Mohammed MJ, Marston CK, Popvic T, Weyant RS, Tenover FC. Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. *J Clin Microbiol* 2002; 40: 1902–7.
14. Centers for Disease Control and Prevention. Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. *MMWR* 2001; 50: 909–19.
15. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. *Scand J Infect Dis* 1991; 23: 333–5.
16. Kim HS, Choi EC, Kim BK. A macrolide–lincosamide–streptogramin B resistance determinant from *Bacillus anthracis* 590: cloning and expression of *ermJ*. *J Gen Microbiol* 1993; 139: 601–7.