

Preliminary Results on a Study of Antimicrobial Susceptibility Testing of *Bacillus anthracis* Strains Isolated during Anthrax Outbreaks in Italy from 2001 to 2017

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Abstract : Anthrax is a zoonotic disease that affects a wide range of animal species (primarily ruminant herbivores), and can be transmitted to humans through consumption or handling of contaminated animal products. The etiological agent *B.anthraxis* is able to survive in unfavorable environmental conditions by forming endospore which remain viable in the soil for many decades. Furthermore, *B.anthraxis* is considered as one of the most feared agents to be potentially misused as a biological weapon and the importance of the disease and its treatment in humans has been underscored before the bioterrorism events in the United States in 2001. Due to the often fatal outcome of human cases, antimicrobial susceptibility testing plays especially in the management of anthrax infections an important role. In Italy, animal anthrax is endemic (predominantly found in the southern regions and on islands) and is characterized by sporadic outbreaks occurring mainly during summer. Between 2012 and 2017 single human cases of cutaneous anthrax occurred. In this study, 90 diverse strains of *B.anthraxis*, isolated in Italy from 2001 to 2017, were screened to their susceptibility to sixteen clinically relevant antimicrobial agents by using the broth microdilution method. *B.anthraxis* strains selected for this study belong to the strain collection stored at the Anthrax Reference Institute of Italy located inside the Istituto Zooprofilattico Sperimentale of Puglia and Basilicata. The strains were isolated at different time points and places from various matrices (human, animal and environmental). All strains are a representative of over fifty distinct MLVA 31 genotypes. The following antibiotics were used for testing: gentamicin, ceftriaxone, streptomycin, penicillin G, clindamycin, chloramphenicol, vancomycin, linezolid, cefotaxime, tetracycline, erythromycin, rifampin, amoxicillin, ciprofloxacin, doxycycline and trimethoprim. A standard concentration of each antibiotic was prepared in a specific diluent, which were then twofold serial diluted. Therefore, each wells contained: bacterial suspension of $1-5 \times 10^4$ CFU/mL in Mueller-Hinton Broth (MHB), the antibiotic to be tested at known concentration and resazurin, an indicator of cell growth. After incubation overnight at 37°C, the wells were screened for color changes caused by the resazurin: a change from purple to pink/colorless indicated cell growth. The lowest concentration of antibiotic that prevented growth represented the minimal inhibitory concentration (MIC). This study suggests that *B.anthraxis* remains susceptible *in vitro* to many antibiotics, in addition to doxycycline (MICs $\leq 0,03 \mu\text{g/ml}$), ciprofloxacin (MICs $\leq 0,03 \mu\text{g/ml}$) and penicillin G (MICs $\leq 0,06 \mu\text{g/ml}$), recommend by CDC for the treatment of human cases and for prophylactic use after exposure to the spores. In fact, the good activity of gentamicin (MICs $\leq 0,25 \mu\text{g/ml}$), streptomycin (MICs $\leq 1 \mu\text{g/ml}$), clindamycin (MICs $\leq 0,125 \mu\text{g/ml}$), chloramphenicol (MICs $\leq 4 \mu\text{g/ml}$), vancomycin (MICs $\leq 2 \mu\text{g/ml}$), linezolid (MICs $\leq 2 \mu\text{g/ml}$), tetracycline (MICs $\leq 0,125 \mu\text{g/ml}$), erythromycin (MICs $\leq 0,25 \mu\text{g/ml}$), rifampin (MICs $\leq 0,25 \mu\text{g/ml}$), amoxicillin (MICs $\leq 0,06 \mu\text{g/ml}$), towards all tested *B.anthraxis* strains demonstrates an appropriate alternative choice for prophylaxis and/or treatment. All tested *B.anthraxis* strains showed intermediate susceptibility to the cephalosporins (MICs $\geq 16 \mu\text{g/ml}$) and resistance to trimethoprim (MICs $\geq 128 \mu\text{g/ml}$).

Keywords : *Bacillus anthracis*, antibiotic susceptibility, treatment, minimum inhibitory concentration

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