Phylogenetic analysis of Ukrainian *Bacillus anthracis* strains from various sources

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Objective

Due to the lack of information about the phylogenetic origins of Ukrainian *Bacillus anthracis* strains, the goal of this work was to make phylogenetic analysis of Ukrainian isolates obtained from various sources (soil, clinical material from infected humans and animal products) for better understanding of phylogenetic origins of this pathogen in Ukraine and Eastern Europe.

Introduction

Anthrax is a widely spread zoonotic disease with natural transmissive cycle involving wildlife, livestock and humans [1]. It is caused by *Bacillus anthracis*, a highly pathogenic gram-positive, spore-producing bacterium, which poses a serious threat to public and animal health due to its mortality both for animals and for humans [2-4]. The ability of B. *anthracis* spores to remain viable in soils for decades enables their isolation from freely accessible environment [5]. This unique feature to form highly resistant spores in the environment plays a major role in the ecology and evolution of this pathogen [6]. During the spore phase, evolution is greatly reduced in rate, which limits the amount of genetic diversity found among isolates of this species [1]. All these factors demonstrate the need for reliable anthrax diagnosis and trace-back methods. This comprises bio forensic capabilities including state-of-the-art methods for accurate genotyping of *B. anthracis* strains.

Methods

23 thermolysates of *B. anthracis* broth cultures isolated from various sources (vesicles from eleven different people infected with cutaneous anthrax when disease's sporadic outbreaks were detected in Ukraine in 1963-2002, as well as two samples from sheep wool, and eight soil samples) were obtained from the Central Epidemiological Station (Kyiv, Ukraine), as well as from I.I. Mechnikov Ukrainian Scientific and Research Anti-plaque Institute (Odessa, Ukraine). These anthrax cultures were confirmed with classical microbiological methods (microscopy, cultivation on solid and liquid media), "string of pearls" reaction, and using bioassay on living white mice (the mortality was observed two days after subcutaneous injection of 0,2-0,5 ml of cells' suspension). All these tests were carried out at the institutions where samples were obtained. Besides, one *B. anthracis* isolate was cultivated from soil sample of an animal grave site nearby Koviagy village, Valky district, Kharkiv region. All samples were analyzed at the Bundeswehr Institute of Microbiology (Munich, Germany). To confirm the presence of the anthrax genome and plasmids, we isolated genomic DNA (gDNA) from thermolysates and studied the presence of the genomic marker *dhp61* as well as the plasmid specific marker *pagA* (pXO1) and *capC* (pXO2) using qPCR. Quality of the isolated gDNA was tested using the Agilent bioanalyzer. To characterize regional and global phylogeographic patterns of these strains, canonical Single Nucleotide Polymorphisms analysis (canSNP) was conducted using high resolution melt (HRM). Three thermolysates of broth cultures isolated and soil sample isolated from animal grave site in Kharkiv region were analyzed using NewSeq Full genome sequencing.

Results

B. anthracis chromosomal DNA-marker *dhp61* as well as pXO1 marker *pagA* and pXO2 plasmid marker *capC* could be detected in all thermolysates. However, the soil isolate from the Koviagy grave site was positive for *dhp61* but contained only the pXO1 plasmid. The Bioanalyzer assay revealed that only 6 out of the 23 thermolysates had good enough DNA quality to be sequenced. So far only genomes of thermolysates of soil samples from Mykolaiv and Sumy regions, the thermolysate of sick patient's vesicle from Kherson region as well as the soil sample from the animal grave site in Kharkiv region have been sequenced. For the residual 3 thermolysates the full genome analysis is still in progress. The sequencing results showed that the *B. anthracis* strain isolated from Mykolaiv soil sample belongs to the Vollum linage group and other thermolysates from Sumy and Kherson regions are closely

clustering with isolates from Japan. Thus, human isolate from Kherson region is clustering with the Japanese isolate BA104 which was obtained from pig during sporadic anthrax incident in 1982 and soil isolate from Sumy region is clustering with the BA 103 isolate which was obtained from beef cattle in Japan in 1991. In contrast, we analyzed the genomic sequence of the pXO2-negative isolate from grave site in Kharkiv region using BioNumerics software and found that it has high similarity to STI strain.

Conclusions

The infrequent sporadic occurrence of anthrax in the country of Ukraine is likely caused by a heterogeneous population of *B. anthracis.* The found STI strain in the grave site of Kharkiv region is probably an environmental recovery of the Russian anthrax live vaccine which was commonly used for vaccination of animals in the former Soviet Union The sequencing result of the soil isolate from Mykolaiv region indicates the occurrence of another canSNP group, the Vollum group, which is quite untypical for Ukraine. The latter is mainly prevalent in the Asian regions (namely Pakistan) and therefore might have been introduced to Ukraine over the silk road. Other two thermolysates from Sumy and Kherson regions also showed unexpected results clustering with Japanese isolates. The further research of Ukrainian B. anthracis isolates will allow us to expand our knowledge about the population structure and evolution of anthrax in Ukraine.

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