5. YERSINIOSIS: TAXONOMY, PHYLOGEOGRAPHY, POLYMORPHISM OF PATHOGENICITY FACTORS AND SELECTIVE VIRULENCE

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YERSINIA PESTIS VOLE'S STRAINS: TAXONOMY, PHYLOGEOGRAPHY, POLYMORPHISMS OF PATHOGENICITY FACTORS AND SELECTIVE VIRULENCE

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Strains of *Yersinia pestis*, the causative agent of plague, can be divided into those possessing universal virulence and isolates conditionally pathogenic for guinea pigs and humans.

Purpose and objectives of our study were intraspecific classification, analysis of phylogeography, determination of polymorphism of pathogenicity factors and identification of selective virulence factors using postgenomic technologies.

More than 100 isolates from natural plague foci of the former USSR and Mongolia were studied with (i) DFR-, IS*100*-, CRISPR-, MLVA25-, and SNP-typing, (ii) sequencing of genes coding for several pathogenicity factors, (iii) 50 of the strains were underwent the whole genome sequencing, (iv) the whole genome sequences, mRNA or protein spectra synthesized at 37°C *in vitro* or in a dialysis chambers placed into guinea pig peritoneum cavities were compared in the bv. ulegeica strains subcultures differing in subcutaneous virulence for guinea pigs by more than five orders of magnitude, (v) knockout mutagenesis and subsequent complementation were used to assess the significance of identified potential selective virulence factors.

Y. pestis division into two subspecies differing in epidemiological significance is justified. DFR-, IS*100*-, CRISPR-, MLVA25-, and SNP-typing had very similar clustering ability. Polymorphism of the classic pathogenicity factors was not associated with selective virulence. One or several genotypes were isolated only in certain natural foci. Strain subcultures dramatically differing in their subcutaneous virulence for guinea pigs had identical nucleotide sequences in their genomes but fluctuated in mRNA and protein spectra. Most of the observed differences in the spectra of synthesized mRNA and proteins did not affect virulence for both mice and guinea pigs. According to our research, the main candidates for the role of selective virulence factors are methionine ABC transporter lipoprotein (WP_038931127.1) and class II fructose-bisphosphate aldolase (WP_002209962.1).

A rational variant of bringing the taxonomy of the plague microbe in accordance with the rules of the International Code of Bacterial Nomenclature and evolutionary taxonomy is proposed. Two new potential molecular targets for the prevention and treatment of plague are found.

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CREATING A SPECTRA LIBRARY FOR SPECIFIC MALDI-TOF MASS SPECTROMETRY IDENTIFICATION OF *YERSINIA ENTEROCOLITICA*-LIKE SPECIES

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Phenotypic identification of *Y. enterocolitica*-like species by biochemical tests is laborious and not always reliable. Among these species, identification of *Y. bercovieri*, *Y. mollaretii*, *Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia* is a problem for clinical microbiologists, and usually misidentify them as *Y. enterocolitica*, an important food-borne pathogen. In last study, 1138 *Yersinia* strains isolated in Russia were analyzed by MALDI ToF mass spectrometry (MALDI ToF MS) using Microflex™ LT (Bruker Daltonics, Germany). We demonstrated the accurate MALDI ToF MS genus identification, and *Y. pseudotuberculosis* and *Y. enterocolitica* species identification (100% of strains).

However only 22.1% of strains 217 *Y. enterocolitica*-like species were further reliably identified to the species level (Score Value ≥ 2.3 correspond to highly probable species identification). Other *Y. enterocolitica*-like strains were misidentified as *Y. enterocolitica*.

The aim of this study was to create in-house spectra library for correct identifying *Y. enterocolitica*-like species.

MALDI ToF MS identification of the *Y. enterocolitica*-like species is limited by small number of their reference spectra available in the MALDI Biotyper database. The mass-spectra from 60 well-characterized *Y. enterocolitica*-like strains (53 were isolated in Russia, 7 are Institute Pasteur reference strains) were used to generate a project library: *Y. kristensenii* (n = 20), *Y. intermedia* (n = 15), *Y. frederiksenii* (n = 14), *Y. aleksiciae* (n = 5), *Y. bercovieri* (n = 4), *Y. mollaretii* (n = 2). To select strains, the obtained spectra of 263 *Y. enterocolitica*-like strains were used for cluster analysis. It was shown that *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii* spectra forms a few subclusters. So for library creation we selected the strains of spectra different subclusters.

For applying in-house spectra library additional verified strains *Y. intermedia* (n = 20), *Y. frederiksenii* (n = 15), *Y. kristensenii* (n = 20) and *Y. enterocolitica* (n = 20) were used. All strains were accurate identified to the species level with Score Values ≥ 2.3.

Thus the creating in-house spectra library allows to increase the specificity of *Y. enterocolitica*-like species MALDI ToF MS identification.

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EPIDEMIOLOGICAL AND CLINICAL PECULIARITIES OF PSEUDOTUBERCULOSIS OUTBREAKS

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Retrospective analysis of 29 pseudotuberculosis outbreaks in Siberia and at the Far East demonstrated the following patterns:

– outbreaks mainly belonged to short-term type I lasting no more than one incubation period;