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# Whole-genome analysis of Staphylococcus aureus isolates from ready-to-eat food

Mikhaylova Y.*, Shelenkov A., Chernyshkov A., Kulikova N., Tyumentseva M., Saenko S., Egorova A., Manzeniuk I., Akimkin V.

*Central Research Institute of Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Moscow, Russia*
** mihailova@cmd.su*

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*Motivation and Aim*: The urgency of the food safety issue is increasing every year, since it is one of the main risk factors for public health and the gene pool maintenance [1]. *Staphylococcus aureus* is a versatile opportunistic pathogen, which can survive in diverse environments, grow in many types of foods and can cause food poisoning [2]. Thus, it is important to identify the origin of RTE food-related *S. aureus* strains and to evaluate the pathogenic potential of these isolates. Thirty-five isolates were characterized using the whole genome sequencing (WGS) analysis in terms of population structure, the presence of resistance and virulence determinants, as well as plasmid replicon sequences and CRISPR-Cas systems. To the best of our knowledge, this is the first WGS-based surveillance of Russian RTE food-associated *S. aureus* isolates.

*Methods and Algorithms*: During one year period (2019-2020) RTE food samples were collected from cafes and restaurants of different geographic regions in Russia (from Moscow to Kamchatka), corresponding to eight Russian Federal Centers of Hygiene and Epidemiology. All isolates were identified down to a species level by time-of-flight mass spectrometry (MALDI-TOF MS) using the VITEK MS system (bioMerieux, Marcy-l'Étoile, France). The isolates were tested for phenotypic antimicrobial resistance by the boundary concentration method on VITEK MS analyzer. WGS was performed on the Illumina NextSeq platform using Nextera DNA Sample Prep Kit (Illumina, USA). Genome assemblies and comparisons were performed using SPAdes, roary, dnadiff, and custom software.

*Results*: The analyzed 35 *S. aureus* samples belonged to 15 different MLST-based sequence types and to 20 SpaII types including four new SSR1 sequences of SpaII gene and three groups of AGR system (I-III). Worth noting, 11 *S. aureus* samples under investigation were MRSA and isolated from different types of products (bread, salad, meat products and rolls). We observed a large difference in the carriage of virulence and AMR genes between methicillin-resistant (MRSA) and sensitive (MSSA) isolates studied. We also revealed the isolates with high pathogenic potential including one multi-drug resistant MRSA and separate samples harboring different combinations of enterotoxin genes. By analyzing the isolates belonging to the same/single strain, we were able to identify the differences in their accessory genomes marking their dynamics and plasticity.

The analysis of plasmid sequences showed that six isolates from our set harbored multiple types (more than 2) of plasmid replicons simultaneously, while only four

isolates did not have any rep-sequences at all. It is well known that Staphylococci typically carry one or more plasmids per cell and these plasmids include varied gene content [3]. Bioinformatics analysis of CRISPR in foodborne pathogens is crucial for assessing their potential evolution in order to predict the outbreaks, which is of great importance for food safety [4]. In our RTE food *S. aureus* collection, we revealed three isolates bearing CRISPR-Cas systems of IE type. Additionally, we found the genes encoding potential Cas proteins (Cas3_0_I) similar to the CRISPR/Cas type I system in the genomes of five isolates.

At a first glance, the isolates from our collection mostly are non-MDR and do not seem to represent a significant danger to public health. On the other hand, however, given the fact that they were isolated from RTE food, the samples and their sources represent a "means of transfer and dissemination" of important pathogenicity determinants. Moreover, the pathogenic potential of separate isolates studied cannot be ignored, especially for those carrying enterotoxin genes. Our WGS results revealed that 14 samples (40 %) carried at least one enterotoxin gene (*sea, sec, sed* or *sel*). Additionally, major part of the isolates harboring the *tsst1* gene were MRSA and were assigned to CC22 (seven out of 10). Therefore, the presence of these pathogenic factors in *S. aureus* isolates from RTE food can pose a threat to public health. We can also suggest that such pathogenic potential, including an arsenal of mechanisms providing resistance to antimicrobial drugs, leads to the long-time circulation, transmission and dissemination of *S. aureus* in the community and, in particular, within the RTE food supply chains.

*Conclusion*: Therefore, the development of monitoring criteria for MRSA and MSSA, as well as for other selected foodborne bacteria, may provide a valuable option for controlling the dissemination of antimicrobial resistance and other pathogenic factors. Such criteria could be based on microbiological properties or antimicrobial resistance potential of the isolates studied. We believe that the WGS data obtained will greatly facilitate further studies of foodborne *S. aureus* epidemiology, as well as its genome plasticity, in terms of the acquisition of various genetic elements related to host adaption, antimicrobial resistance and virulence.

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