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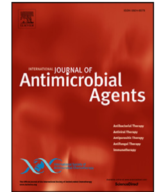

Short Communication

Comparative genome analysis of global and Russian strains of community-acquired methicillin-resistant *Staphylococcus aureus* ST22, a 'Gaza clone'

Vladimir Gostev[a,b], Ksenia Ivanova[a,c], Alexander Kruglov[d,1], Olga Kalinogorskaya[a], Irina Ryabchenko[d], Sergey Zyryanov[e,f], Ekaterina Kalisnikova[a], Daria Likholetova[a,g], Yuri Lobzin[a,b], Sergey Sidorenko[a,b,*]

[a] *Pediatric Research and Clinical Center for Infectious Diseases, Saint Petersburg, Russian Federation*
[b] *North-Western State Medical University named after I.I Mechnikov, Saint Petersburg, Russian Federation*
[c] *ITMO University, Saint Petersburg, Russian Federation*
[d] *National Agency for Clinical Pharmacology and Pharmacy, Moscow, Russian Federation*
[e] *Peoples' Friendship University of Russia (RUDN University), Moscow, Russian Federation*
[f] *City Hospital № 24, Moscow, Russian Federation*
[g] *Saint-Petersburg State University, Saint Petersburg, Russian Federation*

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ABSTRACT

In this study, we identified the relationship between the genetic lineage of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) sequence type 22 (ST22) from Russia and other regions. Sixty ST22 isolates from Russia were characterised through whole-genome sequencing. To evaluate the phylogenetic relationship of Russian isolates with the global ST22 population, we analysed 1283 genomes obtained from NCBI's GenBank. The phylogenetic tree of the ST22 global population consisted of three main clusters (A, B and C). The first (cluster A) was represented by EMRSA-15 isolates, the second (cluster B) by heterogeneous isolates from different regions harbouring different sets of virulence genes, and the third (cluster C) by isolates from the Middle East previously recognised as 'Gaza clone' and similar isolates from Russia. Presence of the toxic shock syndrome toxin (*tsst*) and elastin-binding protein S (*ebpS*) genes as well as the hypothetical proteins NCTC13616_00051 and NCTC13616_00047 were the most useful factors in discriminating ST22 lineages. Although the CA-MRSA 'Gaza clone' was mainly recovered from carriers, its widespread occurrence is a cause for concern. Differentiation of the 'Gaza clone' from other MRSA lineages is necessary for planning infection control measures.

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1. Introduction

Asymptomatic *Staphylococcus aureus* colonisation of the nasal mucosa is detected in ~30% of the human population; carriage is considered to be one of the risk factors for subsequent development of hospital- and community-acquired staphylococcal infections [1]. Carriage of methicillin-resistant *S. aureus* (MRSA) poses a great threat. The clonal structure of the global MRSA population is unstable; periodically, new genetic lineages displace their predecessors. Determination of differences in epidemiological features, virulence, antibiotic susceptibilities and clinical presentations of community-acquired MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA) infections caused by individual genetic lines requires differentiation and typing of isolates.

Staphylococcus aureus sequence type 22 (ST22) is important for the healthcare system because the genetic lineage EMRSA-15, a typical HA-MRSA, belongs to this group. EMRSA-15 was originally isolated from England in 1991 [2] and rapidly spread throughout the UK, Europe, Australia and some Asian countries [3,4]. Genomic analysis of the *S. aureus* ST22 population revealed that EMRSA-15 forms a separate lineage (ST22-A clade); its main characteristics are the presence of staphylococcal cassette chromosome *mec* (SCC*mec*) type IVh, a single nucleotide deletion within the *ureC* gene, a 2268-bp deletion in the fibronectin-binding protein (FnBP) locus, and resistance to fluoroquinolones and macrolides [4].

* Corresponding author. Present address: Pediatric Research and Clinical Center for Infectious Diseases, Professor Popov Str. 9, Saint Petersburg, Russia. Tel.: +7 963 316 0808.
E-mail address: sidorserg@gmail.com (S. Sidorenko).
[1] Present address: City Hospital № 40, Moscow, Russian Federation.

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In 2012, an unusually high prevalence of CA-MRSA carriage was reported in the Gaza Strip; the most predominant strain, ST22-MRSA-IVa, was later called the 'Gaza clone' [5]. A detailed study of the genomic epidemiology of this lineage revealed its heterogeneity and distant relationship with the EMRSA-15 clone [6]. However, the problem of differentiation of staphylococcus genetic lineages within the ST22 group has not been completely resolved.

Recently, we described the emergence of ST22-MRSA-IVa isolates similar to the 'Gaza clone' among healthy carriers in Russia [7]. In the present study, whole-genome sequencing was used to characterise ST22-MRSA that colonises healthy adults and children in Russia and analysed their relationship with different ST22 lineages.

2. Methods

2.1. Bacterial isolate selection and antibiotic susceptibility testing

Non-duplicate ST22-SCC*mec* IV-MRSA isolates were selected from 7093 *S. aureus* isolates recovered in Moscow (2015–2016) and Saint Petersburg (2017–2019) from healthy adults and children during routine screening for nasal carriage. *Staphylococcus aureus* was identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using a Microflex LT system (Bruker Daltonik GmbH, Bremen, Germany) according to the manufacturer's instructions. The presence of *mecA* was confirmed by PCR [7]. Multilocus sequence typing (MLST) and *spa* typing were performed as described previously [8,9]. Multiplex PCR was used for genotyping SCC*mec* (according to the recommendations of the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements) [10]. Minimum inhibitory concentrations (MICs) of antibiotics were determined by microdilution in cation-adjusted Mueller–Hinton broth according to ISO standard 20776-1 using appropriate control strains, and the results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) v.10.0 (2020).

2.2. Whole-genome sequencing and data analysis

Genomic DNA was extracted using a PureLink^{TM} Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Preliminary lysis of the cells was performed using 1 mg/mL lysostaphin (Sigma-Aldrich, St Louis, MO, USA). DNA libraries were prepared using a Nextera Flex Kit (Illumina Inc., San Diego, CA, USA) and were sequenced on a MiSeq system (Illumina Inc.). All genomic data were deposited in the NCBI Sequence Read Archive (SRA) and are available in BioProject PRJNA609231.

De novo contigs were assembled using SPAdes [11]. Genomes were annotated with Prokka [12] and the pangenome was analysed with Roary [13]. Core genes of the genomes included in the study were aligned and subsequently clustered using Bayesian analysis of population structure (BAPS) with rhierBAPS [14]. The resulting alignment was used to construct a maximum-likelihood phylogenetic tree using IQ-TREE [15]. Whole-genome single nucleotide polymorphism (wgSNP) analysis was performed with Snippy (github.com/tseemann/snippy), followed by filtration of recombinations in selected genomes from the detected BAPS clusters using Gubbins [16].

The gene content matrix of the pangenome was used for defining genes associated with rhierBAPS clusters using the XGBoost classification algorithm (https://xgboost.readthedocs.io/). Principal component analysis (PCA) and unweighted pair-group method with arithmetic mean (UPGMA) analysis were used for defining the accessory genes associated with rhierBAPS clusters (R package factoextra).

2.3. Inclusion of genomes from publicly available databases

Using the list of ST22 *S. aureus* genomes from the PATRIC database [17] (update November 2019), sequence data were downloaded from NCBI's GenBank. The genomes that were represented by raw reads were assembled using the approaches mentioned above.

3. Results

3.1. Genomic characterisation of the global ST22 population

Genomes of *S. aureus* ST22 from NCBI GenBank ($n = 1283$) and genomes of ST22-SCC*mec* IV isolates from Russia ($n = 60$) were included in the analysis. Among the genomes from GenBank, 724 belonged to EMRSA-15 [2] and 59 to 'Gaza clone' [6]. The pangenome of ST22 consists of 10 555 genes. Maximum-likelihood phylogeny of ST22 was constructed using 19 693 core SNPs from 1528 core genome loci from 1343 genomes. Using Bayesian hierarchical clustering, three main BAPS clusters (A, B and C) were determined (Fig. 1, top). Intergroup median pairwise core SNP difference between the clusters varied from 27 to 33 SNPs [interquartile range (IQR) 24–37].

The first cluster A was represented by highly similar EMRSA-15 genomes with a median (IQR) pairwise SNP difference of 8 (6–10) SNPs. The EMRSA-15 cluster mainly consists of isolates from the UK; significantly fewer isolates come from Europe, and only a single isolate from Australia, Asia and the Middle East. Almost all of the isolates of this lineage carried type IVh SCC*mec* cassettes, with types IVa and V found in single isolates.

Maximum-likelihood phylogeny of the second (cluster B) and third (cluster C) clusters was constructed based on wgSNP alignment (Fig. 1, bottom). Cluster B included isolates from Asia, Europe, the UK, Australia and other regions, and one isolate each from the Middle East and Russia. Of these, 35% of isolates were *mecA*-negative. Subtypes IVa and IVc SCC*mec* cassettes were prevalent in MRSA isolates of this lineage. Genomes in this cluster demonstrated a median (IQR) pairwise core SNP difference of 29 (23–34) SNPs. The majority of cluster C isolates originated from the Middle East and Russia; a small number of isolates were from Europe, the UK, Asia and other regions. Isolates in this cluster were more homogeneous in comparison with cluster B, with a median (IQR) pairwise core SNP difference of 13 (9–17) SNPs. All isolates previously characterised as 'Gaza clone' were localised in cluster C. A low level of recombination events was typical for all clades with a mean rho/theta value of ~0.02.

The PCA and UPGMA analyses based on binary distribution of accessory genes identified three clusters that coincided with Bayesian clustering. However, clusters corresponding to BAPS clusters B and C had a huge overlap (Supplementary Fig. S1A). A comparative XGBoost analysis (confirmed by BLAST analysis) revealed differences in the gene content between the three clusters (Table 1). The presence of genes for hypothetical proteins NCTC13616_00047 and NCTC13616_00051 according to the locus tag of genome NCTC13616 (**NZ_LR134193.1**), the presence of *ermC*, deletion in *ureC* and mutations in *gyrA* were characteristic of cluster A (EMRSA-15). Presence of the toxic shock syndrome toxin (*tsst*) was a characteristic sign of cluster C, while absence of the elastin-binding protein S (*ebpS*) gene was a characteristic sign of cluster B (absent in 55% of genomes). Cluster B was also associated with a high prevalence of *lukSF* (Panton–Valentine leukocidin) genes. The three clusters also differed in the number of fibronectin-binding protein (*fnb*) genes. All clusters contained *fnbA*. However, *fnbB* was mainly detected in cluster C; *fnbB* was absent in cluster A isolates and was detected only in 5% of cluster B isolates. Cluster C consisted of two highly similar subclusters C1 and C2; differences be-

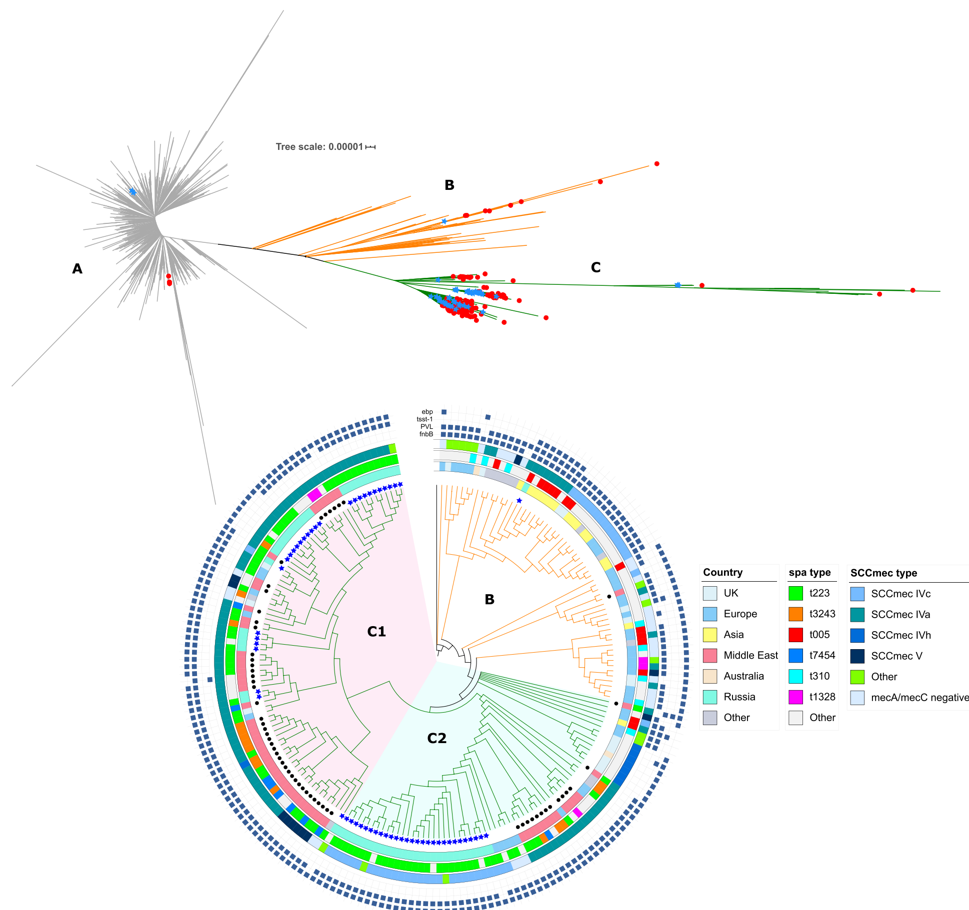

Fig. 1. (Top) Maximum-likelihood phylogeny of *Staphylococcus aureus* ST22 population ($n = 1343$) based on core SNP alignment with mapped data of distribution of *tsst* genes (red circles). According to BAPS clusterisation, three clusters were determined: grey, cluster A (represented by EMRSA-15 clone, mostly from the study by Reuter et al. [2]); orange, cluster B; and green, cluster C (represented by 'Gaza clone'). (Bottom) Phylogenetic reconstruction (cladogram) of clusters B and C ($n = 207$) based on wgSNP alignment of 21 922 SNPs with recombination filtering. C1 and C2 subclusters are marked by background colour fill. Data on the origin of genomes, *spa* types, SCC*mec* types and presence of virulence genes (*ebpS, tsst, lukFS* and *fnbB*) are mapped on the phylogenetic tree (from inner to outer circle). In both phylogenetic trees, blue stars indicate Russian ST22 genomes and black circles indicate genomes from the study by Chang et al. [6]. SNP, single nucleotide polymorphism; *tsst*, toxic shock syndrome toxin; BAPS, Bayesian analysis of population structure; wg, whole-genome; SCC*mec*, staphylococcal cassette chromosome *mec; ebpS*, elastin-binding protein S gene; *lukFS*, Panton–Valentine leukocidin gene; *fnbB*, fibronectin-binding protein B gene.

Table 1
Prevalence of genetic markers among community-acquired methicillin-resistant *Staphylococcus aureus* ST22 clusters

Marker	Cluster A ($n = 1137$)	Cluster B ($n = 60$)	Cluster C ($n = 146$)
SCC*mec* type(s)	IVh	IVa, IVc, V	IVa, IVc
Dominant *spa* types	t032 (53%), t379 (9%)	t005 (22%), t310 (12%)	t223 (55%), t3243, (11%)
NCTC13616_00051* gene (%)	98	0	5.5
NCTC13616_00047* gene (%)	98.6	37	20
fnbB (%)	0	5	92
ebpS (%)	100	55	96.6
mecA (%)	99	65	94.5
blaZ (%)	99	90	86
ermC (%)	67	27	18.5
aac(6′)-aph(2′′), aadD (%)	2	50	6
tetK (%)	1.6	2	9.5
folA (snp)+*fusB/C* (%)	8	8	2
ureC 69delT (%)	99.3	0	4.8
tsst (%)	0.35	13	87
Recombination level, (rho/theta)	Low (0.027 ± 0.022)	Low (0.012 ± 0.008)	Low (0.02 ± 0.016)
GyrA (S84L) (%)	98.1	31.6	6.8
RpoB (A477V, A477D, H481N) (%)	2	0	0
lukFS (%)	0	60	3.4

ST, sequence type; SCC*mec*, staphylococcal cassette chromosome *mec*.
* According to NCTC13616 reference genome (NZ_LR134193.1)

tween the two subclusters were related to the type of SCC*mec* elements. Isolates of the C1 subcluster predominantly contained cassettes of IVa type, whilst C2 isolates were characterised by a significant variety of types (IVc, IVa and IVh).

3.2. Characterisation of Russian isolates

All Russian ST22 isolates had a median (IQR) pairwise core SNP difference of 25 (14–35) SNPs. Among the 60 isolates, 2 isolates (ST22-t032-SCC*mec* IVh) belonged to cluster A (EMRSA-15), 1 (ST22-t005-SCC*mec* IVa) to cluster B and the rest ($n = 57$) to cluster C (ST22-t223-SCC*mec* IVa/IVc). Within cluster C, the isolates were almost evenly distributed between the C1 and C2 subclusters (Supplementary Fig. S2). All isolates of subcluster C1 harboured SCC*mec* Iva and were resistant to cefoxitin and 55% of them were phenotypically susceptible to oxacillin (MIC < 2.0 mg/L). Isolates of subcluster C2 harboured SCC*mec* IVc and were resistant to cefoxitin and only 13% of them demonstrated susceptibility to oxacillin. Isolates from this subcluster also carried *lnuA*. Other genes determining antimicrobial resistance (*aac-aph, cat, ermC* and *tetK*) were detected only in single isolates of both subclusters. All isolates were fully susceptible to linezolid, tigecycline, daptomycin, ceftaroline and vancomycin (Supplementary Table S1).

4. Discussion

In 2012, an unusually high incidence of ciprofloxacin-susceptible CA-MRSA-t223-SCC*mec* IVa was reported among healthy individuals in the Gaza Strip [5]. The authors concluded that the ‘Gaza clone’ evolved from the local ST22-MSSA-t223, rather than from EMRSA-15. Later, another characteristic of this ‘Gaza clone’, that is a high frequency of *tsst*, was recognised [18]. The genomic approach used in recent studies made it possible to localise the ‘Gaza clone’ in a group separate from EMRSA-15 (in a non-ST22-A clade); moreover, it was shown that the ‘Gaza clone’ is represented by two divergent clones (clade A and clade B), probably resulting from two independent acquisitions of SCC*mec* elements [6]. The authors of the study also expressed their concerns over the expansion of this genetic lineage outside the Gaza Strip. It was found that the MRSA isolates similar to the ‘Gaza clone’ had spread to other regions of the Middle East [3]. In addition, ST22-MRSA-IVa, *spa* t223, *tsst*-positive isolates were also reported in Italy [19].

Analysis of 60 genomes from Russian isolates and 1283 genomes from GenBank revealed that the global ST22 population is quite homogeneous; however, we cannot exclude that the level of homogeneity is exaggerated due to underrepresentation of methicillin-susceptible *S. aureus* (MSSA) in the population under study. Bayesian clusterisation of the ST22 population in the current study partially corresponded to the maximum-likelihood phylogenetic trees from previous studies [4,6]. Cluster A was represented by highly similar EMRSA-15 isolates and corresponded to ST22-A lineage from Chang et al. [6]. We identified new properties for differentiating cluster A from other ST22 lineages, including the presence of *ebpS* and two hypothetical protein genes. Genes of both proteins are located in the J-region of SCC*mec* and can be used to differentiate the subtypes of SCC*mec* IV elements. NCTC13616_00047 shares an amino acid similarity with Lp14313 protein. This small protein interacts with single-stranded DNA and could have a potential role in the mobilisation of SCC*mec* elements [20]. Despite differences in the age of the host, geographic region and time of isolate recovery, only a limited number of SNPs and a low level of recombinations were observed in the genomes of ST22 isolates and particularly in the ‘Gaza clone’, which indicates homogeneity of the genetic lineage.

Cluster B was the most heterogeneous within the ST22 lineage; it included MSSA and MRSA from different regions and of different *spa* and SCC*mec* types. Individual sublineages within this cluster contained *lukFS, ebp* or *tsst-1* genes. A common feature of the isolates of this lineage was the presence of *fnbB*. The majority of MRSA lineages contain two fibronectin-binding proteins (FnbA and FnbB), and the absence of intact *fnbB* is a characteristic of ST22 and CC5 [21]. A subcluster carrying *fnbB* was identified within ST22 in a previous work [4].

Cluster C included all ST22 isolates originated from Gaza Strip and recognised in the previous studies as ‘Gaza clone’ [6] as well as 57 isolates from Russia and a small number of isolates originating from other geographical regions. Despite the fact that isolates of cluster C were highly similar, we managed to divide them into two subclusters C1 and C2. This clusterisation differed from that (clusters A and B) presented previously [6]. Cluster C1 of this study corresponded to cluster A of the previous study; however, cluster C2 did not correspond to cluster B of that study. Isolates of cluster B harboured SCC*mec* V, whilst isolates of cluster C2 carried SCC*mec* types IVa, IVc, IVh and others. Differences in clustering of ‘Gaza clone’ isolates are most likely related to different numbers of isolates included in the studies and different methods of analysis.

Since all of the isolates from Russia were grouped in subclusters C1 and C2 together with the ‘Gaza clone’ isolates, we can assume that in the Middle East region the ‘Gaza clone’ differentiated into two closely related lineages and that these lineages were imported into Russia independently. Currently, we have no epidemiological explanation for this expansion. Hence, one of the study’s limitations is the absence of information about possible risk factors for the acquisition of CA-MRSA. It also seems unlikely that the ‘Gaza clone’ has spread only in two distant geographic regions. Most likely, its distribution in other regions of the Middle East and Southeast Europe is not well studied.

In a vast majority of cases, the ‘Gaza clone’ is recovered from healthy carriers; however, in a study of a hospital on the Gaza Strip, isolation of *tsst*-positive ST22 MRSA from different sources, including blood, was described [18]. The presence of *tsst* in the genome poses a potential threat to the development of severe infections.

In conclusion, the danger of the ‘Gaza clone’ spreading even further seems plausible and requires caution in regions where this genetic lineage has not been previously described. The detection of *tsst* may raise suspicions about the clinical MRSA isolate that belongs to the ‘Gaza clone’; however, whole-genome sequencing would be necessary for accurate identification. Additional studies to evaluate the clinical significance of the ‘Gaza clone’ and to reveal the reasons for the successful colonisation of the nasal mucosa are needed in the future.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2020.106264.

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