Original article
https://doi.org/10.17816/2311-2905-17610

Recurrent Cases of Periprosthetic Joint Infection Caused by *Staphylococcus Aureus*: Reinfection or Reactivation of a Pathogen?

Andrey A. Kechin [1,2], Victoria S. Borobova [1,2], Taalaibek U. Sheraliev [3], Svetlana O. Chretien [3], Irina N. Tromenshleger [1], Vitaliy V. Pavlov [3], Maxim L. Filipenko [1]

[1] *Institute of Chemical Biology and Fundamental Medicine of Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia*
[2] *Novosibirsk State University, Novosibirsk, Russia*
[3] *Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics, Novosibirsk, Russia*

Abstract

Background. *Staphylococcus aureus* is one of the most common pathogens causing periprosthetic joint infection (PJI). Despite the high genetic diversity of *S. aureus* strains, determining phylogenetic relationships and, consequently, the source of infection is a challenging task that can only be addressed through detailed comparison of the genomes of the obtained isolates.

The aim of the study was to assess the feasibility of differentiating the cases of nosocomial periprosthetic joint infections, using whole-genome sequencing to identify genetic and phenotypic differences between isolates with the prospect of the application of evidence-based treatment strategies.

Methods. Genomes of 20 *S. aureus* isolates from 13 patients with PJI were sequenced. Standard microbiological tests and *in silico* analysis of genomes using ResFinder, KmerFinder, spaTyper, and SCC*mec*Finder programs were employed.

Results. Phylogenetic analysis was performed using core genome reconstruction and identified potential cases of nosocomial infections, as well as cases of recurrent infections. The relatedness of isolates collected between 2012 and 2019 was demonstrated, along with the evolution of their genomes, including the acquisition and loss of antibiotic resistance genes. In one case of recurrent infection, the loss of several genes was observed over a remission period of approximately 5 years. Comparison of phenotypic testing results using the disk diffusion method and resistance predictions based on genome analysis revealed discrepancies for three isolates containing the *aac(6')-aph(2'')* gene, which were resistant to tobramycin and gentamicin but susceptible to amikacin. Based on the treatment outcomes of several recurrent PJI cases, it was hypothesized that radical treatment might be more effective in cases of infections caused by multidrug-resistant nosocomial strains.

Conclusions. Whole-genome sequencing enables the identification of phylogenetically related isolates, with shared genetic and phenotypic properties confirming their relatedness. Against the backdrop of high-dose antibiotic therapy, *S. aureus* genomes accumulate changes that, through molecular genetic testing, may help to justify the choice of radical treatment strategy for periprosthetic joint infection, such as prosthesis removal.

Keywords: *Staphylococcus aureus*, periprosthetic joint infection, nosocomial infection, whole-genome sequencing, phylogenetic analysis, core genome.

Cite as: Kechin A.A., Borobova V.S., Sheraliev T.U., Chretien S.O., Tromenshleger I.N., Pavlov V.V., Filipenko M.L. Recurrent Cases of Periprosthetic Joint Infection Caused by *Staphylococcus Aureus*: Reinfection or Reactivation of a Pathogen? *Traumatology and Orthopedics of Russia*. 2025;31(2):18-32. (In Russian). https://doi.org/10.17816/2311-2905-17610.

✉ *Andrey A. Kechin*; e-mail: aa_kechin@niboch.nsc.ru

Submitted: 28.10.2024. Accepted: 20.02.2025. Published online: 18.03.2025.

Научная статья
УДК 616-002.3-08-039.4
https://doi.org/10.17816/2311-2905-17610

Случаи развития повторной перипротезной инфекции *Staphylococcus aureus*: реинфекция или реактивация патогена?

А.А. Кечин [1,2], В.С. Боробова [1,2], Т.У. Шералиев [3], С.О. Кретьен [3], И.Н. Троменшлегер [1], В.В. Павлов [3], М.Л. Филипенко [1]

[1] ФГБУН «Институт химической биологии и фундаментальной медицины» Сибирского отделения Российской академии наук, г. Новосибирск, Россия
[2] ФГАОУ ВО «Новосибирский национальный исследовательский государственный университет», г. Новосибирск, Россия
[3] ФГБУ «Новосибирский научно-исследовательский институт травматологии и ортопедии им. Я.Л. Цивьяна» Минздрава России, г. Новосибирск, Россия

Реферат

Актуальность. *Staphylococcus aureus* — один из наиболее частых возбудителей перипротезной инфекции (ППИ). Несмотря на высокое генетическое разнообразие штаммов *S. aureus*, определение филогенетических связей, а следовательно, и источника заражения является непростой задачей, которая может быть решена только при подробном сравнении геномов получаемых изолятов.

Цель — используя полногеномное секвенирование с выявлением генетических и фенотипических отличий между изолятами, изучить возможность дифференцирования случаев заражения пациентов внутрибольничной перипротезной инфекцией с перспективой обоснованного выбора тактики лечения пациентов.

Материал и методы. Были определены нуклеотидные последовательности геномов 20 изолятов *S. aureus*, полученных от 13 пациентов с перипротезной инфекцией. В работе были использованы стандартные микробиологические тесты и анализ геномов *in silico* программами ResFinder, KmerFinder, spaTyper и SCC*mec*Finder.

Результаты. Применив филогенетический анализ с построением корового генома, были идентифицированы потенциальные случаи внутригоспитальной инфекции, а также исследованы случаи повторного развития инфекции. Показано родство изолятов, выделенных на протяжении 2012–2019 гг., а также эволюция их геномов с приобретением и потерей генов антибиотикорезистентности. Так, в одном из случаев повторного развития инфекции была обнаружена потеря нескольких генов за период ремиссии около 5 лет. При сравнении результатов фенотипического тестирования изолятов диско-диффузионным методом и предсказаний резистентности по данным анализа генома было выявлено несоответствие для трех изолятов, содержащих ген *aac(6')-aph(2'')* и резистентных к тобрамицину и гентамицину, но чувствительных к амикацину. На основании результатов лечения нескольких случаев с повторным развитием ППИ было выдвинуто предположение, что в случае развития инфекции, вызванной мультирезистентным внутригоспитальным штаммом, более эффективным может быть проведение радикального лечения.

Заключение. Полногеномное секвенирование позволяет выявлять филогенетически родственные изоляты, общность генетических и фенотипических свойств которых подтверждает их родство. На фоне проводимой высокодозной антибактериальной терапии в геномах *S. aureus* накапливаются изменения, которые при молекулярно-генетическом тестировании могут помочь обосновать выбор радикальной тактики лечения перипротезной инфекции — удаление эндопротеза.

Ключевые слова: *Staphylococcus aureus*, перипротезная инфекция, внутригоспитальная инфекция, полногеномное секвенирование, филогенетический анализ, коровый геном.

Для цитирования: Кечин А.А., Боробова В.С., Шералиев Т.У., Кретьен С.О., Троменшлегер И.Н., Павлов В.В., Филипенко М.Л. Случаи развития повторной перипротезной инфекции *Staphylococcus aureus*: реинфекция или реактивация патогена? *Травматология и ортопедия России*. 2025;31(2):18-32. https://doi.org/10.17816/2311-2905-17610.

Кечин Андрей Андреевич; e-mail: aa_kechin@niboch.nsc.ru

Рукопись получена: 28.10.2024. Рукопись одобрена: 20.02.2025. Статья опубликована онлайн: 18.03.2025.

INTRODUCTION

Periprosthetic joint infections (PJI) are among the most severe postoperative complications following primary major joint arthroplasty, leading to significant functional impairment of the replaced joint and even fatal outcomes [1, 2]. Timely diagnosis and the assessment of risk factors for PJI can help to prevent such complications or facilitate the selection of an optimal treatment strategy. One of the key factors determining subsequent antibiotic therapy is the nature of the causative agent and its characteristics, including antimicrobial susceptibility. Among PJI pathogens, *Staphylococcus* spp. are the most frequently isolated, with *Staphylococcus aureus* being predominant [3, 4, 5]. Identifying the source of infection is clinically crucial, as contamination during operation, hematogenous spread, recurrence of a previous infection, or contact transmission from a local focus can all contribute to PJI. Each scenario influences the course of the disease, response to antibiotic therapy, and, ultimately, treatment outcomes [6]. In most studies, the source of recurrent infection remains undetermined, leaving the mechanisms of reinfection poorly understood. Many studies attribute recurrent infection to the same microorganism based solely on species identification through standard microbiological tests [7, 8]. However, given the high genetic diversity of *S. aureus* strains, accurate differentiation of two isolates requires more in-depth molecular genetic analysis of their genomes [9].

In recent years, whole-genome sequencing (WGS) has been increasingly applied to study *S. aureus* isolates responsible for PJI, enabling the identification of multiple clinically significant characteristics. WGS allows for the determination of phylogenetic relationships between two isolates, the detection of genetic determinants of antimicrobial resistance [10], and the prediction of strain virulence based on the presence of genes encoding various virulence factors [11]. Beyond these clinically relevant characteristics, which can affect the subsequent treatment of a patient, WGS data can also enhance our fundamental understanding of disease progression. For example, comparative genomic analysis of related isolates, such as those from reactivated primary infections, can provide insights into new strain characteristics acquired through horizontal gene transfer or point mutations. Achieving this requires both extensive statistical data linking molecular genetic characteristics with pathogen virulence and resistance properties, as well as detailed investigations of newly identified virulence and resistance factors. Such comprehensive study of PJI will ultimately contribute to the development of new strategies for managing and preventing this type of postoperative complication [12].

The aim of the study was to assess the feasibility of differentiating the cases of nosocomial periprosthetic joint infections, using whole-genome sequencing to identify genetic and phenotypic differences between isolates with the prospect of the application of evidence-based treatment strategies.

METHODS

Bacterial isolates

From 2012 to 2019, twenty *S. aureus* isolates were collected at the Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics for whole-genome analysis. These isolates were obtained from 13 patients undergoing treatment for PJI after hip arthroplasty. In five patients, two to three isolates were collected due to recurrent PJI following treatment. Standard culture methods using growth media (sheep blood agar, chocolate agar, and Schaedler agar) were applied to identify etiologically significant microorganisms. The isolated microorganisms were identified using the VITEK 2 Compact 30 analyzer (bioMérieux, France) with Vitek ID-GP identification cards, Vitek AST-GP67 susceptibility cards, and the API STAPH system (bioMérieux, France) for staphylococcal and micrococcal identification. In 2021, following an agreement with the Federal State Budgetary Institution “Novosibirsk TB Research Institute” of the Ministry of Health of the Russian Federation for microorganism identification via mass spectrometry, the species identity of the isolates was validated. Bacterial cultures were stored at -80°C in Luria-Bertani medium supplemented with 30% glycerol.

In addition to Vitek AST-GP67 cards, antibiotic susceptibility was assessed using the standard disk diffusion method [13]. Antibiotic susceptibility testing was performed using disks impregnated with cefoxitin (for methicillin resistance detection), rifampicin (rifampin), linezolid,

trimethoprim-sulfamethoxazole, levofloxacin, ciprofloxacin, tigecycline, gentamicin, amikacin, erythromycin, tetracycline, and clindamycin (Bio-Rad, USA). Susceptibility criteria were based on the Russian clinical guidelines on antimicrobial susceptibility testing*, prepared following EUCAST (European Committee on Antimicrobial Susceptibility Testing) standards. For each patient, data on admission dates, diagnosis, and antibiotics administered for PJI treatment were recorded.

DNA extraction

For DNA extraction, 50 ml of pure *S. aureus* cultures were centrifuged at 4000 g for 10 minutes. The pellets were resuspended in 180 μl of 0.15 M NaCl, followed by the addition of 200 μg of lysozyme (AppliChem, Germany) and 50 μg of RNase (SERVA, USA) and incubated at room temperature for 10 minutes. A 600 μl of lysis buffer (300 mM NaAc; pH = 5.4; 40% guanidine thiocyanate; 0.05% urea; 5% Triton X-100; 0.1% sodium dodecyl sulfate) was then added, mixed, and incubated at 65°C for 10 minutes. The lysate was transferred into spin columns for DNA and RNA Extraction (BioPharmExpert, Russia), centrifuged at 13.000 g for 5 minutes, and the DNA was precipitated on the column using 50% isopropanol with 10 mM Tris-HCl (pH = 8.0) and washed with 80% ethanol. The DNA was eluted by adding 100 μl of 10 mM Tris-HCl (pH = 8.0) to the center of the membrane, incubating at 65°C for 10 minutes, and centrifuging at 8000 g for 2 minutes. DNA purity and concentration were assessed spectrophotometrically using A260/A280 ratios and A260 values. The extracted DNA was stored at -20°C.

Whole-genome sequencing

For WGS, 100 ng of DNA was fragmented via ultrasound, and sequencing libraries were prepared according to a previously described protocol [14]. Fragment ends were repaired using 5 U of T4 polynucleotide kinase (SibEnzyme, Russia) and 1 U of T4 DNA polymerase (SibEnzyme, Russia) in 1 × ligation buffer (30 mM Tris-HCl; pH = 7.8; 100 mM MgCl2; 10 mM dithiothreitol; 1 mM ATP). Deoxyadenosine residues were then added to the fragments using 1 U of Klenow exo-enzyme (Thermo Scientific, USA), followed by NEB adapter ligation with 1 U of T4 DNA ligase (SibEnzyme, Russia). Sequencing was performed on a MiniSeq platform (Illumina) using the MiniSeq High-Output reagent kit (300 cycles, 2 × 150 bp) (Illumina) according to the manufacturer's instructions.

NGS data analysis

Adapter sequences were removed from demultiplexed reads using Trimmomatic v0.32 [15] with the following parameters: "leading" – 20, "trailing" – 20, "minimum length" – 30. The remaining reads were assembled into contigs *de novo* using SPAdes v3.9.0 with default settings [16]. Files in the scaffolds.fasta format were used for further analysis. Antibiotic resistance genes and mutations were identified using ResFinder [17], while species identity was confirmed using KmerFinder [18]. Multilocus sequence typing (MLST) was conducted using the MLST tool [19]. In addition to ResFinder, β-lactam resistance was assessed by detecting mutations in *gdpP* and *pbp4* genes, which may be associated with cephalosporin resistance [20]. Phylogenetic analysis was performed using core genome alignment across all isolates with Snippy (https://github.com/tseemann/snippy) and FastTree 2 [21], using *S. aureus* subsp. *aureus* NCTC 8325 (GenBank assembly NC_007795.1) as the reference genome. A total of 23.315 polymorphic core genome positions were included in the phylogenetic analysis. The number of mutations between genomes of isolates was estimated from this list. The phylogenetic tree was visualized using MEGA v10.2.4 [22]. Gene annotation in contigs was performed using RAST [23]. Spa typing was conducted using spaTyper (https://github.com/HCGB-IGTP/spaTyper) with the Ridom SpaServer database (http://spaserver.ridom.de/). SCC*mec* typing was performed using SCC*mec*Finder-1.2 [24].

Statistical analysis

Median and quartile calculations were performed using the standard Python math package. Bootstrap support values for phylogenetic analysis were obtained from FastTree 2.

* Russian clinical guidelines on antimicrobial susceptibility testing. Version 2004-02. Smolensk: IACMAC, NSMU; 2024. 192 p.

RESULTS

Patient sample and isolate characteristics

A total of 13 patients with PJI following hip arthroplasty were included in the study. Among them, eight patients had a single isolate collected, three had two isolates, and two had three isolates. A higher number of isolates from the same patient was associated with recurrent infections, leading to re-admission. The median time from primary arthroplasty to admission due to PJI was 5 months (ranging from 1 week to 10 years) (Table 1).

In 5 out of 11 primary admission cases for which antibiotic data were available, dual antibiotic therapy was administered. In three cases, a single antibiotic was used, and in another three, three antibiotics were used. For three of four recurrent infections, at least three antibiotics were prescribed. The most frequently used antibiotic was the first-generation cephalosporin cefazolin (11 out of 15 admissions, including recurrent cases), followed by the glycopeptide vancomycin (6 cases) (Table 2).

The primary objective of the examination of the obtained *S. aureus* isolates was to determine the cause of recurrence and the source of re-infection: whether it resulted from reactivation of the same bacterial strain after prior antibiotic therapy or from a new strain. However, to enable the identification of related isolates, the study also included isolates from patients having a single (primary) admission due to PJI who were successfully treated within one admission without recurrence over a follow-up period of at least two years.

Antibiotic resistance of isolates

The nucleotide sequence of all genomes, except one (isolate 264), was determined with sufficient accuracy (N50 value > 70.000 bp). Therefore, all read genomes were included in the subsequent analysis. Due to insufficient sequencing data, all types of analysis were conducted for isolate 264 except for *de novo* gene annotation. To assess the prevalence of antibiotic resistance genes in the studied sample and their phenotypic expression, all genomes were analyzed using the ResFinder program. Most isolates (17 out of 20) were predicted to be β-lactam-resistant due to the presence of the *blaZ* or *mecA* genes (Table 3). All *mec*-containing isolates (226, 318, 359, and 412) contained the *mecA* gene and belonged to the SCC*mec* IVc (2B) type. In five cases, the resistance predicted by the presence of the *blaZ* gene corresponded with the phenotypic data, whereas in one case, the isolate was found to be susceptible. The opposite discrepancy was also observed: some isolates exhibited phenotypic β-lactam resistance despite the absence of corresponding resistance genes. This could be attributed to either errors in phenotypic testing or the presence of unidentified resistance mechanisms.

Table 1

Characteristics of the patients included in the study

Parameter		Values
Age, Me [Q_1-Q_3]		45 [39-58]
Male (n, %)		8 (62)
Time from surgery to diagnosis	<30 days 91-365 days >365 days	4 6 2
Basic treatment strategy	DAIR Two-stage treatment Girdlestone procedure	2 6 7 with prosthesis replacement (4 cases) or without (3)

DAIR (Debridement, Antibiotics, and Implant Retention) — a surgical procedure for PJI that includes debridement of the infection site, antibiotic therapy, and prosthesis retention.

Table 2

Characteristics of *S. aureus* isolates indicating the time from primary arthroplasty to admission due to PJI and isolation of the corresponding isolate, and a list of antibiotics received by patients in the hospital, as well as the number of paired NGS reads by which genomes were sequenced

A patient´s number	An isolate´s number	Time from primary arthroplasty to admission due to PJI	Antibiotic therapy	Number of paired NGS reads
1	153	2 years	VCM; CPF	1658796
2	406	4 weeks	VCM; CFX	1355447
2	1063	4 years	MRP; CFX; CPF	3043924
5	379	10 months	N/D	2289340
5	212	1 year	VCM; CFZ	487278
5	264	17 months	VCM; CFZ; CTM; RFP	88448
6	359	4 months	N/D	385875
6	318	5 months	N/D	759876
6	412	7 months	N/D	1690858
7	310	1 year	AM; VCM; CFZ	887505
8	419	4 months	AM; CFZ	5248835
9	3716	1 week	CTM; CFZ; EPM	2264877
10	326	4 weeks	CFZ	1326171
10	348	1 month	CFZ	648313
11	226	3 weeks	VCM	564691
13	399	6 weeks	VCM; CFZ; CPF	1794654
13	3692	5 years	CTM; CFZ; CPF	2233867
14	217	11 months	AM; CFZ	2128946
17	3825	5 months	CFZ	322697
18	3808	10 years	N/D	3155607

AM — amikacin; VCM — vancomycin; CTM — co-trimoxazole; MRP — meropenem; RFP — rifampicin; CFZ — cefazoline; CFX — ceftriaxone; CPF — ciprofloxacin; EPM — ertapenem; N/D – no data on which antibiotics were used in therapy.

In addition to ResFinder, which predicts β-lactam resistance based on the presence of *blaZ* or *mecA* genes, manual searches for mutations in the *gdpP* and *pbp4* genes were conducted. Resistance to certain cephalosporins has previously been shown to develop via mechanisms independent of *blaZ* and *mecA* genes [25, 26]. Two mutations previously described in resistant strains were identified in the studied sample: c.-298C>T (in the promoter) and p.Asp98Glu in the *pbp4* gene of isolate 217, which was phenotypically susceptible to cefoxitin. This is likely due to the fact that for the above-mentioned *mec*A-independent mechanisms, only data on the presence of these mutations in resistant strains have been presented in the literature, but no experimental studies have confirmed that these mutations directly cause resistance. An exception is a 36 bp duplication and a 90 bp deletion in the *pbp4* promoter region, which have been linked to increased gene expression, leading to structural changes in the bacterial cell wall peptidoglycan and subsequent resistance [26, 27].

Another discrepancy was observed in three isolates (217, 226, and 412) that were phenotypically susceptible to amikacin according to the disk diffusion method but carried the *aac(6')-aph(2'')* gene, which confers resistance to gentamicin and tobramycin. These discrepancies indicate that WGS-based assessment of *S. aureus* antibiotic resistance cannot yet fully replace phenotypic testing due to gaps in understanding all possible resistance mechanisms. For all isolates resistant to ciprofloxacin (226, 318, 359, and 412), two mutations were detected in the *gyrA* (p.Ser84Leu) and *grlA* (also known as *parC*, p.Ser80Phe) genes.

Table 3

List of antimicrobial agents, to which the studied isolates were resistant or sensitive according to the results of the disk diffusion method, Vitek AST-GP67 susceptibility cards or genome analysis

Isolate	BPC	CFXT	VCM	GMC	ERM	CPF	CLP	TOB	AM
153	R/?	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
212	R/R	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
217	R/?	S/S	S/S	R/R	S/S	S/S	S/?	R/R	R/S
226	R/?	R/R	S/S	R/R	R/R	R/R	R/?	R/R	R/S
264	R/R	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
310	R/?	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
318	R/?	R/R	S/S	S/S	S/S	R/R	R/?	S/S	S/S
326	R/?	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
348	R/?	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
359	R/?	R/R	S/S	S/S	S/S	R/R	R/?	S/S	S/S
379	R/?	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
399	S/?	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
406	R/R	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
412	R/?	R/R	S/S	R/R	S/S	R/R	R/?	R/R	R/S
419	R/S	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
1063	S/R	S/S	S/S	S/S	S/S	S/S	S/?	S/S	S/S
3692	R/R	S/S	S/S	S/S	S/?	S/S	S/?	S/S	S/S
3716	R/R	S/S	S/S	S/S	S/S	S/S	S/S	S/S	S/S
3808	S/?	S/?	S/?	S/?	S/?	S/?	S/?	S/?	S/?
3825	R/R	S/S	S/S	S/S	S/S	S/S	S/S	S/S	S/S

For each isolate and antibiotic group, data from the genome analysis are listed first, with phenotypic testing data (R – resistant, S – sensitive, ? – the isolate was not tested) indicated across the slash. βL — β-lactam, CFXT — cefoxitin, VCM — vancomycin, GMC — gentamycine, ERM — erythromycin, CPF — ciprofloxacin, CLP — chloramphenicol, TOB — tobramycin, AM — amikacin. The color indicates results that matched by genome analysis and phenotypic testing (green and blue), those that did not match (yellow and orange), and results where resistance was shown only by bioinformatic methods without the disc diffusion test (purple). Resistance to doxycycline, trimethoprim, sulfometaxazole and fosfomycin was determined by *in silico* methods only: all isolates were sensitive to the listed antibiotics.

Phylogenetic analysis of genomes of isolates

To determine the genetic relationships among the PJI-associated isolates, a phylogenetic analysis was performed using core genome reconstruction. This approach includes most of the genome while excluding isolate-specific regions, simplifying the analysis. Additionally, MLST was conducted for each isolate. Identical MLST types indicate close genetic relationships between isolates and allow comparisons with strains described in the literature.

According to phylogenetic analysis, all isolates clustered into six distinct branches, which aligned with MLST results (Figure 1). The predominant MLST type was ST97 (9 out of 20 isolates), belonging to clonal complex 97 (CC97), frequently identified in livestock animals [28, 29, 30]. A separate branch included three isolates of MLST ST30, previously linked to foodborne infections [31].

Of particular interest were isolates from patients with recurrent PJI, marked in Figure 1 with identical colors. In four out of five patients, recurrent PJI appeared to be caused by the same strain (isolates highlighted in purple, red, blue, and orange), suggesting clinical recurrence. In one case (patient No 13), PJI was caused by a strain phylogenetically related to another patient's strain, who was admitted around the same time (green). This suggests that during the initial admission in December 2013, patient No 13 may have acquired the strain from patient No 8. This strain was circulating in the

hospital at that time (isolate 419) and was later isolated from patient No 13 (isolate 3962) upon admission in February 2014. The same strain (isolate 3692) caused a recurrent PJI five years later, leading to re-admission. Due to the severity of the recurrent infection, the hip prosthesis was removed (Girdlestone procedure), as the patient had already undergone 12 different surgeries for unrelated medical reasons.

For the remaining four patients (No 5, 13, 6, and 9) with recurrent infection caused by the same strain, two cases resulted in prosthesis

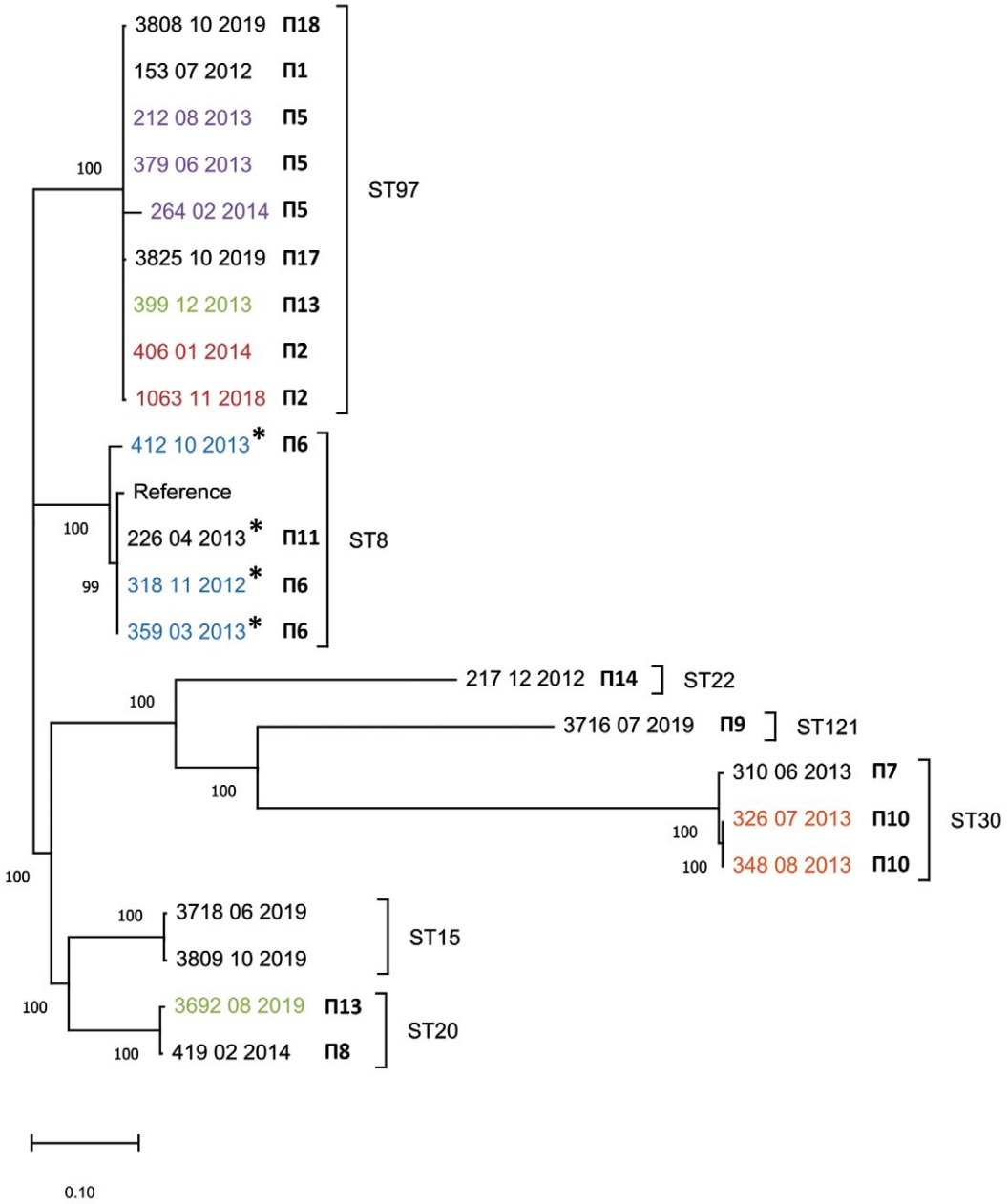

Figure 1. Results of phylogenetic analysis of genomes of the isolates performed using core genome reconstruction. Isolate numbers are indicated with the date of hospital admission (month and year) and patient numbers. Multidrug-resistant isolates are marked with an asterisk. The distances between nodes reflect genetic differences between the samples. Colors represent patients with recurrent infections: isolates from the same patient are shown in the same color, with the number in parentheses indicating the MLST. Numbers at the nodes of the tree indicate bootstrap support levels. Isolates 3718 and 3809 were used only in the phylogenetic analysis, as they were obtained from patients with infections caused by different microorganisms

removal due to treatment failure (Figure 2). For patient No 5 (isolates 379, 212, and 264), the primary infection was likely acquired nosocomially during admission in August 2012, as the isolate (153) was identical to that of another patient (patient No 1) admitted for PJI in July 2012. Two months after initial infection resolution, the same strain (isolate 212) reactivated, causing clinical recurrence. After antibiotic therapy, another recurrence (isolate 264) occurred six months later. The treatment of PJI resulted ineffective, ultimately leading to prosthesis removal. Additionally, patients No 2 (isolate 406) and No 13 (isolate 399) were likely infected by patient No 5, as their isolates differed by only 39 and 7 mutations, respectively.

Patient No 6 (isolates 318, 359, and 412) underwent two courses of antibiotic therapy. Four months after the first episode of PJI (isolate 359), reactivation of the same strain (isolate 318) occurred, with genomic differences amounting to four mutations between bacterial genomes. Clinically, this manifested as a recurrence of PJI. The prosthesis was immediately removed (Girdlestone procedure), followed by a course of antibiotic therapy. After a surgical pause and re-implantation with subsequent antibiotic therapy, another recurrence of the infectious process developed, caused by the same strain (isolate 412). The small number of mutations confirmed the identity of the strains. As a result, another course of antibiotic therapy was administered, leading to infection resolution. A new resistance to aminoglycosides emerged only in isolate 412 (resistance to gentamicin and tobramycin due to the *aac(6')-aph(2'')* gene). A phylogenetic relationship was identified between *S. aureus* isolates from patient No 6 and patient No 11, from whom isolate 226 was obtained. This isolate contained the same newly acquired antibiotic resistance genes as isolate 412, suggesting that all these isolates likely had a nosocomial origin.

In two other patients (No 2 and No 10) with recurrent infection, recovery was achieved after the second course of antibiotic therapy. The initial infection in patient No 2 (isolate 406) also appeared to have a nosocomial origin due to its close phylogenetic relationship with isolate 264 from patient No 5 (only 39 mutations between genomes). After the first phase of therapy with ceftriaxone (1.0 g twice daily) and vancomycin (1.0 g twice daily IV), reactivation of the infection occurred only after 4 years and 10 months (isolate 1063). However, given the time elapsed since operation, this case can be considered an independent hematogenous translocation infection. A combination therapy regimen

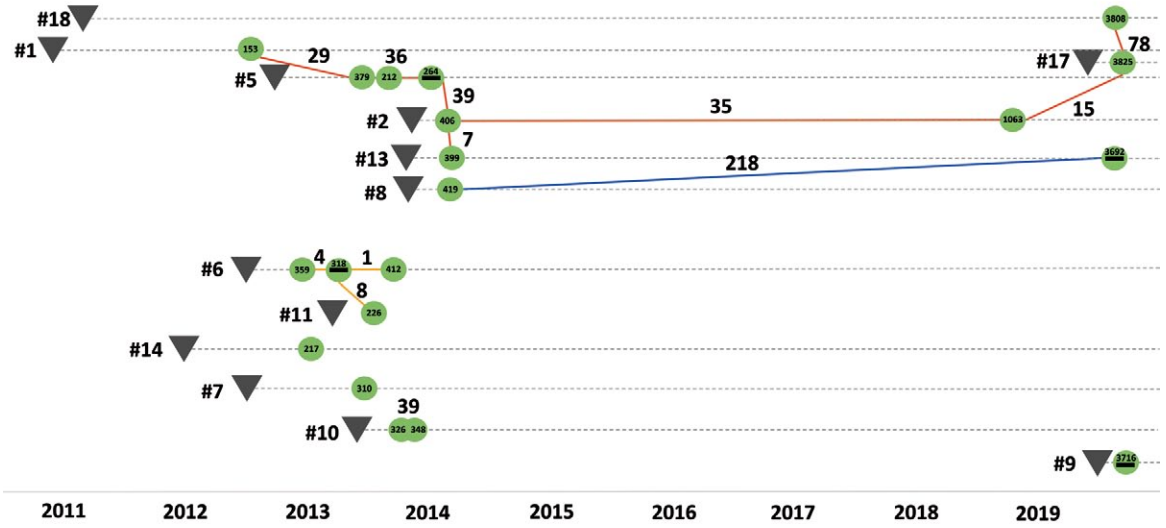

Figure 2. Genetic relatedness of the isolates, indicating the approximate date of primary arthroplasty for the corresponding patient (triangle) and hospital admission due to PJI (green circles). The dashed line represents the timeline for each patient. Orange and blue lines indicate phylogenetic relatedness of two isolates with fewer than and more than 100 genomic differences, respectively. Above each such line, the number of single nucleotide substitutions, insertions, and deletions distinguishing the two genomes is indicated. A minus sign in a circle denotes prosthesis removal, and the number represents the isolate number

consisting of ceftriaxone (1.0 g twice daily), vancomycin (1.0 g twice daily IV), meropenem (1.0 g three times daily), ceftaroline fosamil (600 mg twice daily), and ciprofloxacin (400 mg twice daily) during admission, followed by trimethoprim-sulfamethoxazole (960 mg twice daily for 3 weeks) and ciprofloxacin (500 mg twice daily for 3 weeks) in the outpatient phase, resulted in infection resolution while preserving the prosthesis. Notably, isolate 1063, compared to isolate 406, had lost the *blaZ* gene while retaining β-lactam resistance and becoming susceptible to cefoxitin. This suggests that over such a prolonged period, bacterial selection favored strains lacking this gene. For patient No 10, both episodes of PJI, caused by the same strain, were treated with cefazolin. Infection resolution was achieved after the second course of antibiotic therapy, most likely due to a more radical debridement, including necrotic tissue removal and friction pair replacement via the double-DAIR procedure. The relationships between surgical interventions, phylogenetic relatedness of isolates, and treatment outcomes are presented in Table 4.

Table 4

Relationships between the types of surgery and therapy performed, phylogenetic relatedness of isolates, and treatment outcomes

Patient	1st operation / number of isolate	Rec.	2nd operation / number of isolate	Rec.	3rd operation / number of isolate	Rec.	4th operation	Outcome
1	Spacer implantation / 153	Yes	Girdlestone procedure / 153	Yes				Chronic osteomyelitisт
2	Girdlestone procedure / 406	Yes	Debridement / 1063	No	Revision THA			Recovery
5	Spacer implantation / 379	Yes	Spacer re-implantation / 212	Yes	Girdlestone procedure / 264	Yes		Chronic osteomyelitisт
6	Spacer implantation / 359	Yes	Girdlestone procedure / 318	Yes	Debridement/ 412	No	Revision arthroplasty	Recovery
7	DAIR / 310	Yes	Spacer implantation / 310	Yes	Girdlestone procedure / 310	No		Relative recovery
8	Spacer implantation / 419	Yes	Spacer re-implantation / 419	No	Revision THA			Recovery
9	DAIR / 3716	Yes	Spacer implantation / 3716	Yes	Girdlestone procedure / 3716	No		Relative recovery
10	DAIR / 326	Yes	DAIR / 348	No				Recovery
11	DAIR / 226	No						Recovery
13	Spacer implantation / 399	Yes	Spacer re-implantation / 399	Yes	Girdlestone procedure / 3692			Relative recovery
14	One-stage prosthesis replacement / 217	No						Recovery
17	Spacer implantation / 3825	No	Revision arthroplasty					Recovery
18	One-stage prosthesis replacement / 3808	No						Recovery

Phylogenetically related isolates are highlighted in the same color. DAIR (Debridement, Antibiotics and Implant Retention) — a surgical procedure for PJI with prosthesis retention; Girdlestone procedure — prosthesis removal without spacer implantation; debridement – option for a secondary surgical wound care; chronic osteomyelitis — PJI recurrence; relative recovery — PJI resolution without further revision THA, the supporting limb is shortened; recovery — 12 months after revision hip arthroplasty recurrence is not observed; rec. — recurrence.

Gene composition in genomes of isolates from recurrent infections

During antibiotic therapy, all isolates from patients with recurrent PJI were subjected to prolonged exposure to antibiotics. Therefore, changes in the genomes of isolates from one patient were analyzed. In two patients with significantly different remission periods (4 years and 10 months for patient No 10 and less than a week for patient No 2), the gene composition remained unchanged, despite the presence of 39 and 35 single nucleotide mutations, respectively, differentiating the genomes of the isolates.

For patient No 6, who recovered after treatment, the opposite trend was observed. The number of single nucleotide mutations was 4 and 1, whereas the number of new genes between isolates 359-318 and 318-412 was 2 and 78, respectively. Among these 78 genes, those encoding capsule polysaccharide synthesis enzymes, adhesins, phage proteins, and aminoglycoside-N(6')-acetyltransferase (which confers resistance to aminoglycosides) were identified. Additionally, one of the new genes in isolate 318 (encoding a surface-anchoring protein) was also found in isolate 412, confirming their phylogenetic relationship. However, the source of the new genes in isolate 412 remains an open question for further study, as most of them, according to BLAST NCBI analysis, are present in the genomes of other *S. aureus* strains, suggesting horizontal gene transfer from other bacteria of the same species that must have been in contact with the infecting isolate.

Isolate 212 from patient No 5 contained seven additional genes absent in the genome of isolate 379, including genes encoding mannitol-1-phosphate 5-dehydrogenase, D-mannitol permease of the phosphotransferase system, fibronectin-binding protein, and nickase. In the same patient, the genome of isolate 264 was sequenced at a depth sufficient for phylogenetic analysis but insufficient for *de novo* gene annotation. The acquisition of new genes increasing bacterial pathogenicity likely contributed to the recurrent course of PJI, ultimately leading to chronic osteomyelitis.

DISCUSSION

Using whole-genome sequencing, we analyzed 13 cases of PJI caused by different *S. aureus* strains, five of which involved recurrent PJI (relapse). These recurrent infections were the main topic of the study, as their investigation improves our understanding of how bacteria adapt to new conditions after treatment [32]. Among all antibiotics, cefazolin was the most frequently used (in 73% of admissions), often in combination with other antibiotics. Literature describes the cases of the so-called inoculum effect, where the minimum inhibitory concentration increases fourfold or more at high pathogen concentrations in the presence of the β-lactamase gene (*blaZ*), which hydrolyzes cefazolin molecules with low efficiency [33, 34]. The *blaZ* gene was detected in most isolates (85%), consistent with the high prevalence of *S. aureus* resistance to penicillins.

We identified several discrepancies between phenotypic antibiotic resistance and the presence or absence of genetic determinants. Three isolates resistant to gentamicin and tobramycin but carrying the *aac(6')-aph(2'')* gene remained susceptible to amikacin, a phenomenon frequently described in the literature [35]. Therefore, antibiotic resistance determination should be approached with caution when relying solely on molecular genetic testing, such as whole-genome sequencing or PCR.

Among the 20 collected *S. aureus* isolates, clonal complex 97 (CC97) strains were the most prevalent. Many bacteria of this complex are frequently found in livestock animals [28, 29, 30], and some have been shown to transfer from animals to humans [36, 37, 38]. The possibility that these strains became widespread as nosocomial pathogens after zoonotic transmission cannot be ruled out, especially since half of the patients who underwent operation for hip osteoarthritis were from rural areas.

A potential nosocomial transmission chain of the CC97 strain between multiple patients from 2011 to 2019 was identified. However, confirming this requires a significantly larger number of isolates. Therefore, the study was focused on recurrent infections in the same patients.

WGS data revealed that in four out of five cases, the infection was caused by phylogenetically related isolates, with genomic changes confirming their relatedness. The acquisition of new aminoglycoside resistance genes was observed in only one of six cases, likely due to the use of multiple antimicrobial agents, which reduced the probability of simultaneous uptake of multiple resistance genes.

Thus, whole-genome sequencing demonstrated the phylogenetic relatedness of isolates obtained over long-time intervals from PJI patients, including those with recurrent infections. The findings on genomic changes in *S. aureus* strains in both primary and recurrent nosocomial infections suggest that detailed molecular genetic testing can aid in selecting therapeutic strategies based on bacterial genomic adaptations to antibiotic therapy.

Beyond the tactics of antibiotic therapy, these results may also influence surgical management strategies. If bacteria causing PJI contain antibiotic resistance genes acquired via horizontal transfer, a more radical treatment approach should be considered. When antibiotic resistance develops, requiring increased antibiotic doses that inevitably induce new mutations, continuing a two-stage treatment course with repeated spacer implantation not only loses its effectiveness but also risks the emergence of multidrug-resistant bacteria. In such cases, retaining an infected prosthesis is not advisable. Instead, it should be removed (Girdlestone procedure), the infected site should be thoroughly debrided, and spacer implantation should be avoided. Omitting spacer re-implantation reduces the cumulative doses of local and systemic antibiotics, thereby lowering the mutation pressure on PJI pathogens. Removing the prosthesis with subsequent surgical pause (lasting 6-12 months) shortens the duration of antibiotic therapy, decreases the risk of new mutations and, consequently, the development of antibiotic resistance, and enhances treatment efficacy. Revision arthroplasty after a surgical pause appears to be a more promising approach in such cases.

CONCLUSIONS

Whole-genome sequencing data allow for the identification of phylogenetically related isolates, with genomic changes confirming their relatedness. The detection of related isolates in previously treated periprosthetic joint infection and its recurrence suggests re-infection by persistent isolates rather than exogenous contamination during revision arthroplasty, ruling out surgical site infection as an epidemiological event. Conversely, the presence of related isolates in two or more patients indicates direct nosocomial transmission. Under high-dose antibiotic therapy, both in primary nosocomial periprosthetic joint infection and in recurrent cases, genomic changes accumulate in *S. aureus* strains. Detailed molecular genetic testing, identifying mutations and newly acquired virulence and resistance genes, can help to justify a radical treatment strategy for periprosthetic joint infection that is prosthesis removal.

DISCLAIMERS

Author contribution

Kechin A.A. — data analysis and interpretation, drafting and editing the manuscript.
Borobova V.S. — data analysis and interpretation, drafting the manuscript
Sheraliev T.U. — data acquisition, editing the manuscript.
Chretien S.O. — data acquisition, editing the manuscript.
Tromenshleger I.N. — data acquisition, editing the manuscript.
Pavlov V.V. — study concept, editing the manuscript.
Filipenko M.L. — study design, drafting the manuscript.
All authors have read and approved the final version of the manuscript of the article. All authors agree to bear responsibility for all aspects of the study to ensure proper consideration and resolution of all possible issues related to the correctness and reliability of any part of the work.

Funding source. This work was supported by the Russian state-funded project for ICBFM SB RAS (grant number 125012300671-8).

Disclosure competing interests. The authors declare that they have no competing interests.

Ethics approval. Ethics approval. The study was approved by the local ethics committee of Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics, protocol No 011/19, 20.09.2019.

Consent for publication. The authors obtained written consent from patients to participate in the study and publish the results.

REFERENCES

1. Bozhkova S.A., Preobrazhensky P.M., Kochish A.A., Tikhilov R.M., Artyukh V.A., Klitsenko O.A. Periprosthetic Knee and Hip Infection — Is It Possible to Compare Treatment Outcomes? *Traumatology and Orthopedics of Russia.* 2023;29(4):5-13. (In Russian). doi: 10.17816/2311-2905-15526.
2. Malyuchenko L.I., Nikolaev N.S., Yakovlev V.V., Preobrazhenskaya E.V. Treating Periprosthetic Joint Infection With Silver-Impregnated Carbon-Coated Spacers: Mid-Term Outcomes. *Traumatology and Orthopedics of Russia.* 2023;29(4):14-23. (In Russian). doi: 10.17816/2311-2905-7997.
3. Kasimova A.R., Tufanova O.S., Gordina E.M., Gvozdetsky A.N., Radaeva K.S., Rukina A.N. et al. Twelve-Year Dynamics of Leading Pathogens Spectrum Causing Orthopedic Infection: A Retrospective Study. *Traumatology and Orthopedics of Russia.* 2024;30(1): 66-75. (In Russian). doi: 10.17816/2311-2905-16720.
4. Auñón Á., Tovar-Bazaga M., Blanco-García A., García-Cañete J., Parrón R., Esteban J. Does a New Antibiotic Scheme Improve the Outcome of *Staphylococcus aureus* – Caused Acute Prosthetic Joint Infections (PJI) Treated with Debridement, Antibiotics and Implant Retention (DAIR)? *Antibiotics (Basel).* 2022;11(7):922. doi: 10.3390/antibiotics11070922.
5. Wildeman P., Tevell S., Eriksson C., Lagos A.C., Söderquist B., Stenmark B. Genomic characterization and outcome of prosthetic joint infections caused by *Staphylococcus aureus*. *Sci Rep.* 2020;10(1):5938. doi: 10.1038/s41598-020-62751-z.
6. Renz N., Trampuz A., Perka C., Rakow A. Outcome and Failure Analysis of 132 Episodes of Hematogenous Periprosthetic Joint Infections – A Cohort Study. *Open Forum Infect Dis*. 2022;9(4):ofac094. doi: 10.1093/ofid/ofac094.
7. Hartman C.W., Daubach E.C., Richard B.T., Lyden E.R., Haider H., Kildow B.J. et al. Predictors of Reinfection in Prosthetic Joint Infections Following Two-Stage Reimplantation. *J Arthroplasty.* 2022;37(7S):S674-S677. doi: 10.1016/j.arth.2022.03.017.
8. Bongers J., Jacobs A.M.E., Smulders K., van Hellemondt G.G., Goosen J.H.M. Reinfection and re-revision rates of 113 two-stage revisions in infected TKA. *J Bone Jt Infect*. 2020;5(3):137-144. doi: 10.7150/jbji.43705.
9. Honkanen M., Jämsen E., Karppelin M., Huttunen R., Eskelinen A., Syrjänen J. Periprosthetic Joint Infections as a Consequence of Bacteremia. *Open Forum Infect Dis*. 2019;6(6):ofz218. doi: 10.1093/ofid/ofz218.
10. Chaplin A.V., Korzhanova M., Korostin D.O. Identification of bacterial antibiotic resistance genes in next-generation sequencing data (review of literature). *Klinicheskaya Laboratornaya Diagnostika* [Russian Clinical Laboratory Diagnostics]. 2021;66(11):684-688. (In Russian). doi: 10.51620/0869-2084-2021-66-11-684-688.
11. Kubanov A.A., Runina A.V., Chestkov A.V., Kudryavtseva A.V., Pekov Y.A., Korvigo I.O. et al. Whole-Genome Sequencing of Russian *Neisseria Gonorrhoeae* Isolates Related to ST 1407 Genogroup. *Acta Naturae.* 2018;10(3):68-76. (In Russian). doi: 10.32607/20758251-2018-10-3-68-76.
12. Fedorov E.A., Kretien S.O., Samokhin A.G., Tikunova N.V., Korytkin A.A., Pavlov V.V. Short-term results of treatment of staphylococcal periprosthetic hip joint infection with combined antibiotics and bacteriophages treatment. *Acta Biomedica Scientifica.* 2021;6(4):50-63. (In Russian). doi: 10.29413/ABS.2021-6.4.5.
13. Nabal Díaz S.G., Algara Robles O., García-Lechuz Moya J.M. New definitions of susceptibility categories EUCAST 2019: clinic application. *Rev Esp Quimioter.* 2022;35 (Suppl 3):84-88. doi: 10.37201/req/s03.18.2022.
14. Kechin A., Boldyreva D., Borobova V., Boyarskikh U., Scherbak S., Apalko S. et al. An inexpensive, simple and effective method of genome DNA fragmentation for NGS libraries. *J Biochem*. 2021;170(5):675-681. doi: 10.1093/jb/mvab089.
15. Bolger A.M., Lohse M., Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114-2120. doi: 10.1093/bioinformatics/btu170.
16. Prjibelski A., Antipov D., Meleshko D., Lapidus A., Korobeynikov A. Using SPAdes De Novo Assembler. *Curr Protoc Bioinformatics.* 2020;70(1):e102. doi: 10.1002/cpbi.102.
17. Bortolaia V., Kaas R.S., Ruppe E., Roberts M.C., Schwarz S., Cattoir V. et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother.* 2020;75(12):3491-3500. doi: 10.1093/jac/dkaa345.
18. Clausen P.T.L.C., Aarestrup F.M., Lund O. Rapid and precise alignment of raw reads against redundant databases with KMA. *BMC Bioinformatics*. 2018;19(1): 307. doi: 10.1186/s12859-018-2336-6.
19. Larsen M.V., Cosentino S., Rasmussen S., Friis C., Hasman H., Marvig R.L. et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol.* 2012;50(4):1355-1361. doi: 10.1128/JCM.06094-11.
20. Gostev V.V., Punchenko O.E., Sidorenko S.V. Current concepts of *Staphylococcus aureus* resistance to beta-lactam antibiotics. *Clinical Microbiology and Antimicrobial Chemotherapy*. 2021;23(4):375-387. (In Russian). doi: 10.36488/cmac.2021.4.375-387.
21. Price M.N., Dehal P.S., Arkin A.P. FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One.* 2010;5(3):e9490. doi: 10.1371/journal.pone.0009490.
22. Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.* 2013;30(12):2725-2729. doi: 10.1093/molbev/mst197.
23. Overbeek R., Olson R., Pusch G.D., Olsen G.J., Davis J.J., Disz T. et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.* 2014;42(Database issue):D206-214. doi: 10.1093/nar/gkt1226.
24. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother.* 2009;53(12):4961-4967. doi: 10.1128/AAC.00579-09. doi: 10.1128/AAC.00579-09.

25. Argudín M.A., Dodémont M., Taguemount M., Roisin S., de Mendonça R., Deplano A. et al. *In vitro* activity of ceftaroline against clinical *Staphylococcus aureus* isolates collected during a national survey conducted in Belgian hospitals. *J Antimicrob Chemother*. 2017;72(1):56-59. doi: 10.1093/jac/dkw380.
26. Hamilton S.M., Alexander J.A.N., Choo E.J., Basuino L., da Costa T.M., Severin A. et al. High-Level Resistance of *Staphylococcus aureus* to β-Lactam Antibiotics Mediated by Penicillin-Binding Protein 4 (PBP4). *Antimicrob Agents Chemother*. 2017;61(6):e02727-16. doi: 10.1128/AAC.02727-16.
27. Henze U.U., Roos M., Berger-Bächi B. Effects of penicillin-binding protein 4 overproduction in *Staphylococcus aureus*. *Microb Drug Resist*. 1996;2(2):193-199. doi: 10.1089/mdr.1996.2.193.
28. Gómez-Sanz E., Torres C., Lozano C., Fernández-Pérez R., Aspiroz C., Ruiz-Larrea F. et al. Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups. *Foodborne Pathog Dis*. 2010;7(10):1269-1277. doi: 10.1089/fpd.2010.0610.
29. Feltrin F., Alba P., Kraushaar B., Ianzano A., Argudín M.A., Di Matteo P. et al. A Livestock-Associated, Multidrug-Resistant, Methicillin-Resistant *Staphylococcus aureus* Clonal Complex 97 Lineage Spreading in Dairy Cattle and Pigs in Italy. *Appl Environ Microbiol*. 2015;82(3): 816-821. doi: 10.1128/AEM.02854-15.
30. Hoekstra J., Zomer A.L., Rutten V.P.M.G., Benedictus L., Stegeman A., Spaninks M.P. et al. Genomic analysis of European bovine *Staphylococcus aureus* from clinical versus subclinical mastitis. *Sci Rep*. 2020;10(1):18172. doi: 10.1038/s41598-020-75179-2.
31. Abaev I.V., Skryabin Yu.P., Kislichkina A.A., Korobova O.V., Mitsevich I.P., Mukhina T.N. et al. Genomic analysis of *Staphylococcus aureus* strains of clonal lineage 30 — causative agents of foodborne infection in the Russian Federation. *Bulletin of the Russian Academy of Sciences*. 2017;72(5):346-354. (In Russian). doi: 10.15690/vramn889.
32. Davidovich N.V., Kukalevskaya N.N., Bashilova E.N., Bazhukova T.A. General principles of antibiotic resistance evolution in bacteria (review of literature). *Russian Clinical Laboratory Diagnostics*. 2020;65(6):387-393. (In Russian). doi: 10.18821/0869-2084-2020-65-6-387-393.
33. Vaganova A.N., Borisenko S.V., Nesterova E.V., Trofimova N.N., Litvinenko I.V., Petunova Ya.G. et al. Inoculum effect to cefazolin among methicillin-susceptible *Staphylococcus aureus* isolates from patients with skin diseases. *Clinical Microbiology and Antimicrobial Chemotherapy*. 2021;23(2):205-211. (In Russian). doi: 10.36488/cmac.2021.2.205-211.
34. Mossman A.K., Svishchuk J., Waddell B.J.M., Izydorczyk C.S., Buckley P.T., Hilliard J.J. et al. *Staphylococcus aureus* in Non-Cystic Fibrosis Bronchiectasis: Prevalence and Genomic Basis of High Inoculum β-Lactam Resistance. *Ann Am Thorac Soc*. 2022;19(8):1285-1293. doi: 10.1513/AnnalsATS.202108-965OC.
35. Fathi J., Hashemizadeh Z., Dehkordi R.S., Bazargani A., Javadi K., Hosseini-Nave H. et al. Evaluation of aminoglycoside modifying enzymes, SCC*mec*, *coagulase* gene and PCR-RFLP *coagulase* gene typing of *Staphylococcus aureus* isolates from hospitals in Shiraz, southwest of Iran. *Heliyon*. 2022;8(8):e10230. doi: 10.1016/j.heliyon.2022.e10230.
36. Spoor L.E., McAdam P.R., Weinert L.A., Rambaut A., Hasman H., Aarestrup F.M. et al. Livestock origin for a human pandemic clone of community-associated methicillin-resistant *Staphylococcus aureus*. *mBio*. 2013;4(4):e00356-13. doi: 10.1128/mBio.00356-13.
37. Boswihi S.S., Udo E.E, Mathew B., Noronha B., Verghese T., Tappa S.B. Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Patients Admitted to Kuwait Hospitals in 2016-2017. *Front Microbiol*. 2020;10:2912. doi: 10.3389/fmicb.2019.02912.
38. Lozano C., Gómez-Sanz E., Benito D., Aspiroz C., Zarazaga M., Torres C. *Staphylococcus aureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains. *Int J Med Microbiol*. 2011;301(6):500-505. doi: 10.1016/j.ijmm.2011.02.004.

Authors' information

✉ *Andrey A. Kechin* — Cand. Sci. (Biol.)
Address: 8, Akademika Lavrentieva ave., Novosibirsk, 630090, Russia
https://orcid.org/0000-0002-4822-0251
e-mail: aa_kechin@niboch.nsc.ru

Victoria S. Borobova
https://orcid.org/0000-0002-4443-3997
e-mail: v.borobova@mail.ru

Taalaibek U. Sheraliev
https://orcid.org/0000-0002-7261-2730
e-mail: sheraliev.taalai@mail.ru

Svetlana O. Chretien
https://orcid.org/0000-0003-0074-8062
e-mail: ssonovo64@inbox.ru
Irina N. Tromenshleger
https://orcid.org/0009-0006-9543-3547
e-mail: irinatrom@mail.ru
Vitaliy V. Pavlov — Dr. Sci. (Med.), Associate Professor
https://orcid.org/0000-0002-8997-7330
e-mail: pavlovdoc@mail.ru
Maxim L. Filipenko — Dr. Sci. (Med.)
https://orcid.org/0000-0002-8950-5368
e-mail: max@niboch.nsc.ru