GENETIC CHARACTERIZATION OF *AEROCOCCUS* SP. 1KP-2016 STRAIN ISOLATED FROM A PATIENT WITH BLOODSTREAM INFECTION

Chaplin AV[1,2] ✉, Chagina IA[1], Pimenova AS[1], Gadua NT[1], Kargaltseva NM[1], Borisova OYu[1,2], Donskikh EE[2], Kafarskaya LI[2]

[1] Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russia

[2] Pirogov Russian National Research Medical University, Moscow, Russia

Aerococcus genus bacteria are often associated with human urinary tract and bloodstream infections. The *Aerococcus* sp. 1KP-2016 strain isolated from the buffy coat had the 16S rRNA sequence that was a 98.7% (and less) match with the previously described members of this genus. The purpose of this study was to perform whole genome sequencing of *Aerococcus* 1KP-2016 followed by phylogenetic reconstruction. We have shown that *Aerococcus* 1KP-2016 belongs to the new species of the *Aerococcus* genus that is closest to *Aerococcus* viridans and *Aerococcus urinaeequi.* The genomic sequence, which consists of 2.042 million bps with GC content at 38.5%, was deposited in the DBJ/EMBL/GenBank under identifier NEEY00000000.

Keywords: *Aerococcus*, bloodstream infection, buffy coat (blood leukocyte layer), whole genome sequencing, phylogenetic analysis

Author contribution: AV Chaplin — phylogenetic analysis, data analysis, manuscript authoring; IA Chagina, AS Pimenova, NT Gadua — microbiological studies, manuscript authoring; NM Kargaltseva, LI Kafarskaya — literature analysis, manuscript authoring; OYu Borisova — molecular genetic studies, data analysis, literature analysis, manuscript authoring; EE Donskikh — data analysis, literature analysis, manuscript authoring.

Compliance with ethical standards: the study was approved by the Ethics Committee of the Gabrichevsky Research Institute for Epidemiology and Microbiology (Minutes #28 of November 18, 2014); the patient signed a voluntary consent to participation in the study.

✉ **Correspondence should be addressed:** Andrey Viktorovich Chaplin
10 Admiral Makarov str., Moscow, 125212, Russia; okolomedik@gmail.co

Received: 22.03.2023 **Accepted:** 16.04.2023 **Published online:** 25.04.2023

DOI: 10.24075/brsmu.2023.012

ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ШТАММА *AEROCOCCUS* SP. 1KP-2016, ВЫДЕЛЕННОГО ОТ ПАЦИЕНТА С ИНФЕКЦИЕЙ КРОВОТОКА

А. В. Чаплин[1,2] ✉, И. А. Чагина[1], А. С. Пименова[1], Н. Т. Гадуа[1], Н. М. Каргальцева[1], О. Ю. Борисова[1,2], Е. Е. Донских[2], Л. И. Кафарская[2]

[1] Московский научно-исследовательский институт эпидемиологии и микробиологии имени Г. Н. Габричевского, Москва, Россия

[2] Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва, Россия

Бактерии рода *Aerococcus* часто ассоциированы с инфекциями мочевыводящих путей и кровотока у человека. Штамм *Aerococcus* sp. 1KP-2016, выделенный из лейкоцитарного слоя крови, обладал последовательностью 16S рРНК, совпадающей на 98,7% и менее с ранее описанными представителями данного рода. Целью работы было провести полногеномное секвенирование *Aerococcus* 1KP-2016 с последующей филогенетической реконструкцией. Показано, что *Aerococcus* 1KP-2016 является представителем нового вида рода *Aerococcus*, наиболее близкого к *Aerococcus viridans* и *Aerococcus urinaeequi*. Геномная последовательность, имеющая длину 2,042 млн п.н. и GC-состав 38,5%, депонирована в DBJ/EMBL/GenBank под идентификатором NEEY00000000.

Ключевые слова: *Aerococcus*, инфекция кровотока, лейкоцитарный слой крови, полногеномное секвенирование, филогенетический анализ

Вклад авторов: А. В. Чаплин — филогенетический анализ, анализ данных, подготовка рукописи; И. А. Чагина, А. С. Пименова, Н. Т. Гадуа — микробиологические исследования, подготовка рукописи; Н. М. Каргальцева, Л. И. Кафарская — анализ литературы, подготовка рукописи; О. Ю. Борисова — молекулярно-генетические исследования, анализ данных, анализ литературы, подготовка рукописи; Е. Е. Донских — анализ данных, анализ литературы, подготовка рукописи.

Соблюдение этических стандартов: исследование одобрено этическим комитетом ФБУН МНИИЭМ им. Г. Н. Габричевского Роспотребнадзора (протокол № 28 от 18 ноября 2014 г.); от пациента получено добровольное письменное согласие на участие в исследовании.

✉ **Для корреспонденции:** Андрей Викторович Чаплин
ул. Адмирала Макарова, д. 10, г. Москва, 125212, Россия; okolomedik@gmail.com

Статья получена: 22.03.2023 **Статья принята к печати:** 16.04.2023 **Опубликована онлайн:** 25.04.2023

DOI: 10.24075/vrgmu.2023.012

The *Aerococcus* genus was first described in 1953, with the first studied representative thereof being *Aerococcus* viridans isolated from the air and street dust [1]. Currently, we know of eight species comprising the *Aerococcus* genus: *A. viridians*, *A. urinae*, *A. sanguinicola*, *A. christensenii*, *A. urinaehominis*, *A. urinaeequi*, *A. suis*, *A. vaginalis* [2].

Aerococcus bacteria are commonly associated with the urinary tract infection and urosepsis [3]. At the same time, within the last 10 years there were many reports of complications caused by the representatives of this genus, such complications manifesting as bloodstream infection and infective endocarditis, the etiology of which is most often linked to *A. urinae* and *A. sanguinicola* [2, 3]. Moreover, it was established that aerococci can cause invasive infections, such as osteomyelitis, meningitis, septic arthritis, peritonitis, and soft tissue infections, with isolated *Aerococcus*-like microorganisms and *A. urinae* often assumed to be behind the etiology thereof [2]. Since 1987, 17 cases of bacteremia/septicemia caused by *Aerococcus*-like microorganisms (pure culture isolated from the blood) have been reported in Denmark, with 6 of them being endocarditis cases and 11 — septicemia cases; despite adequate antimicrobial therapy, 7 patients died [4]. Other authors, considering diseases of the urinary system, took isolation of *Aerococcus* in blood culture as an etiological factor of bacteremia [5].

The virulence of *Aerococcus* species is associated with their ability to build biofilms (in particular, on heart valves *in vivo*), form aggregation of platelets, and adhere to surfaces using capsular polysaccharide the presence of which was confirmed by comparative genomic analysis that revealed a wide intraspecific diversity of loci synthesizing it [2, 6, 7].

This study aimed to genetically characterize the *Aerococcus* sp. 1KP-2016 strain isolated from the blood of a patient with a bloodstream infection.

METHODS

We cultivated the *Aerococcus* sp. 1KP-2016 strain under microaerophilic conditions for 24–48 h at 37±1°C on Columbia agar base (Conda; Spain) with 10% of sheep blood. The cultural and morphological properties of the resulting colonies were uncovered using a SteREO Discovery V12 stereoscopic microscope (Carl Zeiss; Germany) with a PlanApo S 1.0 × FWD 60 mm lens objective and a Pl 10 × 23 Br foc eyepiece. Gram staining (ZAO ECOlab; Russia) allowed establishing stain-related properties. The stained smears were examined through an Axio Scope A1 light microscope with EC Plan-NEOFLUAR 100 × 1.3 lens and Pl 10 × 23 Br foc eyepiece (Carl Zeiss; Germany). The biochemical properties of the bacteria were studied with the help of Micro-LA-Test STREPTOtest 16 (Lakhema, Czech Republic), a commercially available biochemical test system, and a catalase test prepared in the laboratory.

As per the guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), we studied the colonies' susceptibility to antibiotics with the disk diffusion test using the commercial standardized paper disks (HiMedia Laboratories Pvt Limited; India).

The cells were boiled to release the chromosomal DNA. The 16S rRNA gene fragment was amplified as per the generally accepted protocol [8]. The PCR reaction mixture contained 1.5 mM of $MgCl_2$, 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 0.1 μM of primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-ACGGYTACCTTGTTACGACTT -3'), 200 mM of each nucleoside triphosphate and 1 unit of Taq DNA polymerase (Thermo Fisher Scientific; Lithuania). The amplification was done with 1 μl of the DNA preparation in the total volume of the reaction mixture (25 μl) using the Tertsik amplifier (DNK-Technologiya; Russia). The PCR products were purified and sequenced at ZAO Evrogen (Moscow, Russia) (http://evrogen.ru/). To process the results of sequencing, we used the ChromasLite software (Technelysium Pty Ltd, Australia) (for the chromatogram format); the sequences were collated with the EMBL/NCBI (http://www.ncbi.nlm.nih.gov/nuccore) international online database using the megablast algorithm.

The genome of the *Aerococcus* sp. 1KP-2016 strain was sequenced using the Ion Proton system (Thermo Fisher Scientific; USA) at Gabrichevsky Research Institute for Epidemiology and Microbiology. The genome was assembled de novo with the help of the SPAdes software [9]. Contest16S [10] and CheckM [11] enabled checking of the assembly for contamination. In the search for CRISPR-Cas systems we relied on CRISPRCasFinder [12], for integrated phage genomes — on PHASTER [13], for antibiotic resistance genes — ResFinder [14].

We used all publicly available genomes of the Aerococcus genus from the Refseq database (as of October 4, 2021) for comparative analysis, and Abiotrophia defectiva ATCC 49176T as an outgroup. As per the annotations given in the databases, protein-coding regions of the genomes were clustered into groups of orthologs with the help of the ProteinOrtho software (standard settings) [15]. Ultimately, we obtained a conservative part of the proteome consisting of 543 such groups, each containing a single-copy protein-coding gene from each genome. The amino acid sequences of proteins in these groups of orthologs were aligned using MUSCLE [16] and concatenated. RapidNJ algorithm [17] guided the phylogenetic reconstruction based on the resulting concatenate. To calculate the average nucleotide identity (ANI) between genomes, we applied the ANIb approach and used the JSpeciesWS online service [18]. The ANI data are represented by two values divided by a slash in order to show the differences between mapping of fragments of the first genome onto the second one and second onto the first, respectively. As of the time of writing of this article, the release of the GTDB database, which contains an alternative taxonomy based on a purely phylogenetic approach, was 202 [19].

RESULTS

The *Aerococcus* sp. 1KP-2016 strain was isolated from the buffy coat of blood collected from a patient with a bloodstream infection (36 years old, city of Stavropol) in August 2016; the patient had subfebrile temperature for a long period of time (over a year).

Individual small smooth colonies less than 1 mm in size with uneven edges, a raised center, translucent grayish-white in color, with a small hemolysis zone formed on Columbia agar in 24–48 hours (Fig. 1). Gram-stained smear contained Gram-positive cocci forming tetrads (Fig. 2). Biochemical tests of the isolated culture returned positive for galactosidase, esculin, lactose, trehalose, and negative for catalase, hippurate, phosphatase, leucine, alpha-arginine, urease, mannitol, sorbitol, raffinose, inulin, melibiose, ribose. The culture proved to be resistant to ciprofloxacin, ofloxacin, penicillin, erythromycin, doxycycline, showed intermediate resistance to clindamycin and susceptibility only to imipenem.

The *Aerococcus* sp. 1KP-2016 strain genome assembled to the level of contigs was deposited in the DBJ/EMBL/GenBank under identifier NEEY00000000; subsequently,

Fig. 1. *Aerococcus* sp. 1KP-2016 colonies on Columbia agar (Stereo Discovery V12 stereomicroscope (Carl Zeiss; Germany))

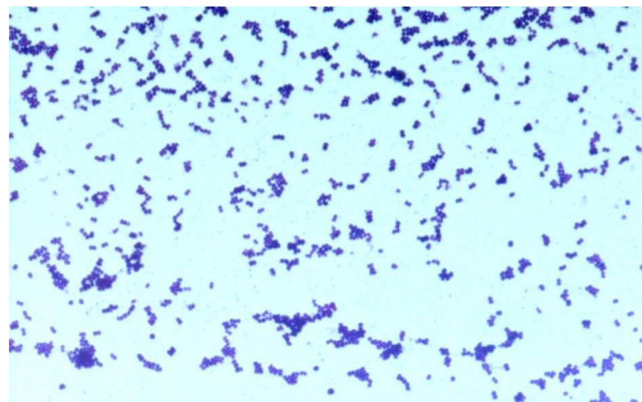

Fig. 2. Micrograph of a Gram-stained smear of *Aerococcus* sp. 1KP-2016 (Axio Scope A1 light microscope, EC Plan-NEOFLUAR 100 — 1.3 lens, Pl 10 × 23 Br foc eyepiece (Carl Zeiss; Germany))

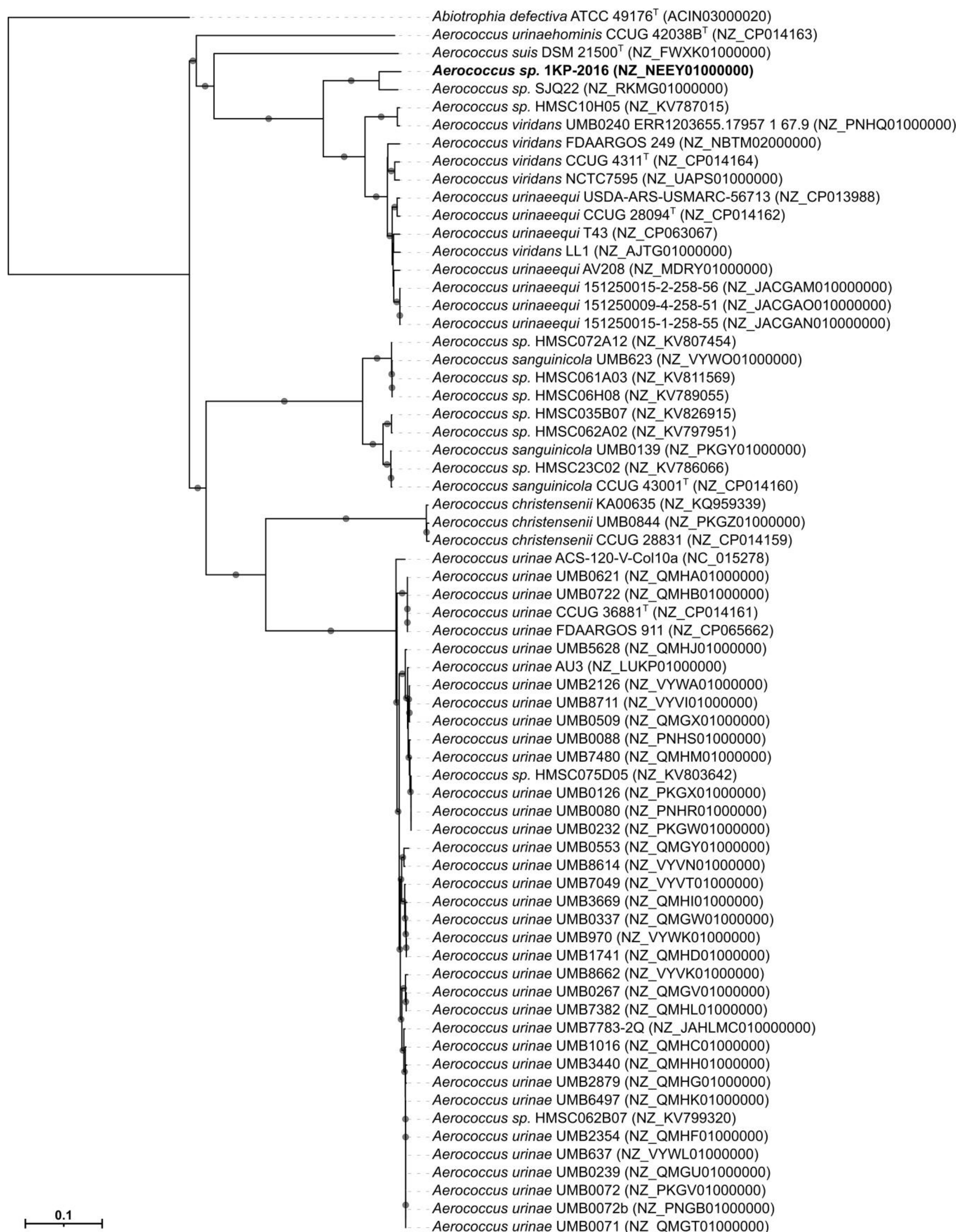

Fig. 3. Phylogenetic tree reconstructed from the sequences of 543 conserved single-copy proteins. The strain described in this work is highlighted in bold. Type strains are marked with a superscript "T". Species names are given as per the Refseq database. Clades with bootstrap level >90 are marked with circles

it was included in the NCBI Refseq as NZ_NEEY00000000. The assembly yielded 119 contigs with the mean coverage of 78x; the resulting genomic sequence consisted of 2.042 million bps, with the GC content at 38.5%. The Contest16S algorithm did not reveal any differing fragments of the 16S rRNA gene, which would have signaled of contamination. Verification of the set of conserved genes with CheckM has shown that the genome was 98.9% complete with contamination at 1.1%, which confirms high quality of the assembly. There were no CRISPR loci found in the genome. According to the PHASTER data, the genome contains two putative prophages, one of which includes intact genes of the major capsid protein (B9P78_00230), tail protein (B9P78_00200), phage terminase (B9P78_00240), and primase (B9P78_00325), which indicates its intactness. Despite the low susceptibility of the strain to antibacterial drugs, the search for antibiotic resistance genes yielded only the gene encoding chloramphenicol-O-acetyltransferase (B9P78_09255). A region at the beginning of the NEEY01000023 contig encodes a number of enzymes participating in biosynthesis of polysaccharides (B9P78_05530-B9P78_05565) that form part of the cell wall or capsule.

The sequence of the *Aerococcus* sp. 1KP-2016 strain's 16S rRNA was 98.7% and 98.6% identical to the sequences of the type *A. viridans* and *A. urinaeequi* strains, respectively. Currently, the bacterial species differentiation threshold is accepted at 98.7% [20], which disallows relying on the 16S rRNA's similarity to make unambiguous conclusions about the taxonomic position. At the same time, the ANI between the sequenced genomic sequence and the genomes of the type strains *A. viridans* ATCC 11563 and *A. urinaeequi* DSM 20341

strains is 77.38/77.39% and 76.49/76.26%, respectively. This is significantly below the generally accepted threshold of 95–96% that allows assigning bacteria to a species [20, 21], which indicates the need to introduce a new species. The result is additionally confirmed by the GTDB, an alternative taxonomy database based on the comparison of genomes, in which the 1KP-2016 strain belongs to the separate *Aerococcus* sp002252085 species.

Reconstruction of the phylogeny (Fig. 3) of *Aerococcus* sp. 1KP-2016 strain shows that its closest relative is the *Aerococcus* sp. SJQ22 (RKMG01000000) strain, which was isolated from the soil and is currently not assigned to any validated species of the *Aerococcus* genus. However, the ANI between *Aerococcus* sp. 1KP-2016 and this strain was only 87.87/88.05%, which is below the generally accepted level of similarity within the species.

DISCUSSION

Aerococcus cause sporadic urinary tract disease, endocarditis, CSF and bloodstream infections. The most commonly isolated strains are *A. urinae* (55–60%) and *A. sanguinicola* (26–46%); *A. viridians* is isolated less frequently. The prevalent strain in Europe and the US is *A. sanguinicola*, *A. viridians* is found less often. *Aerococcus* bacteria are considered as part of the normal microbiota of the urogenital tract. They are isolated from the urine in the absence of clinical symptoms of the disease. In 2010-2015, a retrospective cohort study showed the etiological role played by the *Aerococcus* bacteria in urinary tract infections and asymptomatic bacteriuria (mainly in elderly women), with a noteworthy presence of other microorganisms in 35% of the cases [5, 22]. According to a retrospective cohort study of 2005–2020, 22.4% of the involved patients had a proven clinical picture of the aerococci blood infection, and their mortality depended on the duration of the disease: at 30 days, it was 17%, and at three months — 24% [2]. For microbiologists, it is difficult to properly identify the *Aerococcus* bacteria when they are isolated from urine or blood samples: the morphology often leads to their confusion with staphylococci, and hemolysis — with α-hemolytic streptococci. Given their low biochemical activity, biochemical tests do not yield reliable results. In this regard, it is possible to identify the species using mass spectrometry, but this method allows identification of only five species of the aerococci: *A. christensenii*, *A. sanguinicola*, *A. urinae*, *A. urinaehominis*, *A. viridians*. Therefore, sequencing of the 16S rRNA gene is used to identify strains of aerococci isolated from blood [5, 22].

This study is the first successful attempt at identification of the *Aerococcus* sp. 1KP-2016 strain isolated from the buffy coat that has a 16S rRNA sequence that matches the previously described representatives of this genus 98.7% or less. The whole genome sequencing and the subsequent phylogenetic reconstruction we undertook have shown that this strain is a representative of a new species of the *Aerococcus* genus, one closest to *A. viridans* and *A. urinaeequi*.

Consequently, aerococci are still the microorganisms the epidemiological and clinical characteristics of which have not been studied in full. There are many open fundamental questions about the pathogenetic role played by them in the invasive infectious pathologies. The complexity of identification of species within the *Aerococcus* genus requires use of the modern molecular genetic technologies that enable identification of the new types of microorganisms and expansion of the etiological spectrum of pathogens of severe invasive diseases.

CONCLUSIONS

The genotypic features of the studied strain allow attributing the 1KP-2016 strain isolated from the blood as a new species of the *Aerococcus* genus that is evolutionarily close to *A.viridans* and *A. urinaeequi* yet different from them. It is necessary to further study its phenotypic and chemotaxonomic properties while applying the polyphasic approach to bacterial taxonomy.

References

1. Williams RE, Hirch A, Cowan ST. Aerococcus, a new bacterial genus. J Gen Microbiol. 1953; 8: 475–80.
2. Tai DBG, Go JR, Fida M, Saleh JA. Management and treatment of Aerococcus bacteremia and endocarditis. International J Infect Dis. 2021; 102: 584–9.
3. Rasmussen M. Aerococcus: an increasingly acknowledged human pathogen. Clin Microbiol Infect. 2016; 22: 22–7.
4. Christensen JJ, Jensen IP, Faerk J, Kristensen B, Skov R, Korner B. The Danish ALO Study Group. Bacteremia / Septicemia Due to Aerococcus-Like Organisms: Report of Seventeen Cases. Clin Infect Dis. 1995; 21 (4): 943–94.
5. Rasmussen, M. Aerococci and aerococcal infections. J Infection. 2013; 66: 467–74.
6. Yaban B, Kikhney J, Musci M, Petrich A, Schmidt J, Hajduczenia M et al. Aerococcus urinae – A potent biofilm builder in endocarditis. PLoS One. 2020; 15: e0231827.
7. Carkaci D, Højholt K, Nielsen XC, Dargis R, Rasmussen S, Skovgaard, O. et al. Genomic characterization, phylogenetic analysis, and identification of virulence factors in Aerococcus sanguinicola and Aerococcus urinae strains isolated from infection episodes. Microb Pathog. 2017; 112: 327–40.
8. Mathieu A, Delmont TO, Vogel TM, Robe P, Nalin R, Simonet P. Life on human surfaces: skin metagenomics. PLoS One. 2013; 8 (6): e65288.
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS. et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012; 19: 455–77.
10. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H, Chun J. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. Int J Syst Evol Microbiol. 2017; 67: 2053–57.
11. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015; 25: 1043–55.
12. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res. 2018; 46: W246–51.
13. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y. et al. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res. 2016; 44: W16.
14. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012; 67: 2640–4.
15. Lechner M, Findeiß S, Steiner L, Marz M, Stadler PF, Prohaska SJ. Proteinortho: Detection of (Co-)orthologs in large-scale analysis. BMC Bioinformatics. 2011; 12.
16. Edgar RC MUSCLE: multiple sequence alignment with high

accuracy and high throughput. Nucleic Acids Res. 2004; 32: 1792–7.
17. Simonsen M, Mailund T, Pedersen CNS. Rapid Neighbour-Joining. In Algorithms in Bioinformatics; Springer Berlin Heidelberg: Berlin, Heidelberg, 2008; 113–22.
18. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics. 2016; 32: 929–31.
19. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol. 2018; 36: 996.
20. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol. 2018; 68: 461–6.
21. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA. 2009; 106: 19126–31.
22. Narayanasamy S, King K, Dennison A, Spelman DW, Aung AK. Clinical characteristics and laboratory identification of Aerococcus infections: an Australian tertiary centre perspective. Hindawi International J Microbiology. 2017; 5684614.

Литература

1. Williams RE, Hirch A, Cowan ST. Aerococcus, a new bacterial genus. J Gen Microbiol. 1953; 8: 475–80.
2. Tai DBG, Go JR, Fida M, Saleh JA. Management and treatment of Aerococcus bacteremia and endocarditis. International J Infect Dis. 2021; 102: 584–9.
3. Rasmussen M. Aerococcus: an increasingly acknowledged human pathogen. Clin Microbiol Infect. 2016; 22: 22–7.
4. Christensen JJ, Jensen IP, Faerk J, Kristensen B, Skov R, Korner B. The Danish ALO Study Group. Bacteremia / Septicemia Due to Aerococcus-Like Organisms: Report of Seventeen Cases. Clin Infect Dis. 1995; 21 (4): 943–94.
5. Rasmussen, M. Aerococci and aerococcal infections. J Infection. 2013; 66: 467–74.
6. Yaban B, Kikhney J, Musci M, Petrich A, Schmidt J, Hajduczenia M et al. Aerococcus urinae – A potent biofilm builder in endocarditis. PLoS One. 2020; 15: e0231827.
7. Carkaci D, Højholt K, Nielsen XC, Dargis R, Rasmussen S, Skovgaard, O. et al. Genomic characterization, phylogenetic analysis, and identification of virulence factors in Aerococcus sanguinicola and Aerococcus urinae strains isolated from infection episodes. Microb Pathog. 2017; 112: 327–40.
8. Mathieu A, Delmont TO, Vogel TM, Robe P, Nalin R, Simonet P. Life on human surfaces: skin metagenomics. PLoS One. 2013; 8 (6): e65288.
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS. et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012; 19: 455–77.
10. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H, Chun J. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. Int J Syst Evol Microbiol. 2017; 67: 2053–57.
11. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015; 25: 1043–55.
12. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res. 2018; 46: W246–51.
13. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y. et al. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res. 2016; 44: W16.
14. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012; 67: 2640–4.
15. Lechner M, Findeiß S, Steiner L, Marz M, Stadler PF, Prohaska SJ. Proteinortho: Detection of (Co-)orthologs in large-scale analysis. BMC Bioinformatics. 2011; 12.
16. Edgar RC MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32: 1792–7.
17. Simonsen M, Mailund T, Pedersen CNS. Rapid Neighbour-Joining. In Algorithms in Bioinformatics; Springer Berlin Heidelberg: Berlin, Heidelberg, 2008; 113–22.
18. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics. 2016; 32: 929–31.
19. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol. 2018; 36: 996.
20. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol. 2018; 68: 461–6.
21. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA. 2009; 106: 19126–31.
22. Narayanasamy S, King K, Dennison A, Spelman DW, Aung AK. Clinical characteristics and laboratory identification of Aerococcus infections: an Australian tertiary centre perspective. Hindawi International J Microbiology. 2017; 5684614.