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ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
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ВЕСТНИК

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

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NAS RK is pleased to announce that Bulletin of NAS RK scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of Bulletin of NAS RK in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential multidiscipline content to our community.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабаршысы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Web of Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабаршысының Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді мультидисциплинарлы контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Вестник НАН РК» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Вестника НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному мультидисциплинарному контенту для нашего сообщества.

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ISOLATION AND CHARACTERIZATION ISOLATES OF NOSOCOMIAL INFECTIONS

Abstract. The study aimed to identify clinical isolates of nosocomial infections to characterize and create a Bank of strains to study the phenomenon of reversion of antibiotic sensitivity in different types of pathogenic bacteria.

Methods: Clinical isolates from patients suffering from nosocomial infections were plated on respective media to obtain pure cultures, which then were identified and characterized by morpho-cultural, tinctorial, physiological and biochemical traits carried out using standard test systems. The antibiotic sensitivity profile of clinical isolates was studied by the disco-diffusion method. Isolation of DNA from bacteria was carried out using the PureLink Genomic DNA Kit. DNA library for sequencing was obtained by the enzymatic approach using the Ion Xpress Plus Fragment Library Kit (Life Technologies, USA). Barcoding of isolates was carried out using the Xpress Barcode Adapters Kit (Life Technologies, USA). Sequencing of the resulting library was carried out on 318 chips using the Ion PGM Hi-Q View Sequencing Kit. Quality of the reads and trimming of DNA fragments were carried out using the computer programs fastqc and Cassava fastq filter. Genome fragments were assembled using Velvet 1.2.10. Large genome fragments were used to search for the closest reference genomes in the NCBI database. The complete genomes of the isolates were used as reference sequences for an alignment of the generated DNA reads using the program bowtie2. The completeness of the final assemblies was evaluated by the program BUSCO. Genome annotation was carried out using the NCBI annotation pipeline.

The results of the quantitative studies were processed using one-way ANOVA single-factor variance analysis followed by a statistical analysis using the software package GraphPad Prism 6.

In total, 12 clinical isolates were obtained. Complete genome sequence of four isolates was determined - *Enterococcus faecalis* PHRX1, *Pseudomonas aeruginosa* WND1-2019, *P. aeruginosa* WND2-2019, and *P. aeruginosa* WND3-2019. Annotated genomes were deposited at the NCBI database.

Key words: nosocomial infections, isolates, DNA, sequencing, *Enterococcus faecalis*, *Pseudomonas aeruginosa.*

Introduction. Despite the achievements of the modern medicine, the problem of nosocomial infections remains one of the most urgent at present, which leads to over-spending of additional resources and poses a threat to human health and life. Maternity and obstetric facilities (40% of all registered diseases) and surgical hospitals (15%) are recognized as the most vulnerable areas suffering from the spread of infections [1-3].

Pathogens of nosocomial infections are formed in hospitals in close contact between individual patients, as well as between patients and staff, where there is a possibility of circulation of highly virulent pathogens [4]. Against the background of the use of antibiotics, selection of antibiotic-resistant strains that pose the greatest threat to the health of patients occurs. The most commonly isolated nosocomial pathogens are *Escherichia coli* and *Staphylococcus aureus*, and every 10 cases of nosocomial infections caused by *Pseudomonas aeruginosa*. Less common are *Clostridium difficile*, *Enterococcus* and

Enterobacter, *Candida albicans*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus*, in several cases – other types of *Candida*, *Acinetobacter*, *Serratia marcescens*, *Citrobacter*, *Haemophilus influenza*, etc. [5-7].

For reasons of nosocomial infections, the course of treatment of patients is extended by an average of 10 days, the cost of treatment increases 3-4 times and 5-7 times increases the risk of death. Of every 100 hospitalized patients, 7 in developed and 10 in developing countries are infected with nosocomial infections [8]. This problem arose because of the poorly developed system of epidemic surveillance and the lack of effective control methods [9, 10].

Annually 6 to 27% patients in Europe are suffering from hospital infections. These numbers in the USA are about 7-8% and 2-3% in Russian Federation [11] bringing the economic damage to 10-15 billion rubles in Russia; – 7 billion euros in Europe and 6.5 billion dollars per year in the USA [12].

Currently, the number of antibiotics effective against nosocomial infections has sharply decreased [13-15]. The key of prevention of nosocomial infections is timely diagnosis with an emphasis on the use of targeted antimicrobial therapy. Genomic analysis of individual organisms and characterization of population dynamics of microbial communities make it easier to identify new pathogens to better track disease outbreaks and to study the evolution of antibiotic resistance.

The study aimed to identify clinical isolates of nosocomial infections to characterize and create a Bank of strains to study the mechanisms and prospects of clinical use of drugs that cause reversion of pathogens to antibiotic sensitive phenotype.

Methods. Primary identification of clinical isolates, preparation of pure cultures, morpho-cultural, tinctorial, physiological and biochemical trials were carried out following the *«Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World» (*WHO/CDS/CSR/RMD/2003.6), M35-A2 *Abbreviation Identification of Bacteria and Yeasts Approved Guideline-Second Edition*[16, 17], CLSI and Bergey's Manual of Systematic Bacteriology [18]. For typing isolates used well-standardized commercial test system STREPTOtest 16, STAPHYtest 16, EN-COCCUStest and NEFERMtest 24 [19]. Alpha-hemolysis (α-hemolysis) is a partial or "green" hemolysis associated with reduction of red cell hemoglobin. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin. Beta-hemolysis (β-hemolysis) is associated with complete lysis of red cells surrounding the colony.

Antibiotic sensitivity profiling of clinical isolates was performed by the disco-diffusion method [20]. Determination of the biofilm-forming ability of the isolates was carried out as described before [21].

DNA samples were extracted from bacterial cells using PureLink Genomic DNA Kits (Publication Number: MAN0000601, Revision 2.0) following the manufacturer's recommendations. The quality and quantity of the resulting DNA samples were determined using the NanoDrop 2000c spectrophotometer (Thermo Scientific, USA) at optical wavelengths of 260 and 280 nm.

The Ion Torrent PGM sequencer (USA) was used to sequence genomic DNA samples. The library for sequencing was obtained by an enzymatic approach using the Ion Xpress Plus Fragment Library Kit (Life Technologies, USA). The degree and quality of fragmentation of the library were evaluated by capillary electrophoresis Bioanalyzer 2100 (Agilent). DNA read barcoding for multiplex sequencing was carried out using the Xpress Barcode Adapters Kit (Life Technologies, USA). Sequencing of the resulting library was carried out on a 318 chip using Ion PGM Hi-Q View Sequencing Kit. At each of the intermediate stages, the quality and quantity of the DNA library were monitored. All operations were carried out according to the manufacturer's instructions.

The quality control and trimming of DNA reads were carried out using the computer programs *fastqc* and *Cassava fastq filter*. DNA reads were assembled using Velvet 1.2.10. Large genome contigs were used to search for the closest reference genomes in the NCBI database. The complete genomes of the isolates were then assembled by aligning DNA reads against the respective reference genomes using the program Bowtie2 implemented in UGENE 1.32.0 [22].

The completeness of the final assemblies was evaluated by benchmarking universal single-copy orthologous genes (BUSCO) [23]. Automatic search for coding genes was carried by NCBI annotation pipeline.

The results of the quantitative studies were processed using the one-way ANOVA single-factor variance analysis followed by a statistical analysis using the software package GraphPad Prism 6.

Results. During the period from 2018 to 2019, a collection of clinical isolates of nosocomial infections from hospitals of phthisiological profile in Almaty and Almaty region was carried out. In result, a collection of 12 strains of pathogenic microorganisms causing nosocomial infections has been created. The primary identification of the isolates was carried out on selective and differential diagnostic media, according to generally accepted methods. Table 1 presents the data about the source and species affiliation of the obtained isolates.

Table 1 – Clinical isolates, their sources and species identification

#	Isolate	Source	Cell morphology
1	*Staphylococcus spp. 01-18*	Urine	Single, pairs and in the form in grape-like clusters
2	*Staphylococcus spp. 02-18*	Purulent wound	Single, pairs and in the form in grape
3	*Staphylococcus spp. 03-18*	Vagina	Single, pairs and in the form in grape
4	*Staphylococcus spp. 04-18*	Pharynx	Single, pairs and in the form in grape
5	*Staphylococcus spp. 05-18*	Incubation tube of an intensive care unit	Single, pairs and in the form in grape
6	*St.pneumoniae spp. 01-18*	Sputum	Paired cocci, oval and slightly elongated lanceolate cells
7	*P.aeruginosa spp. 01-18*	Purulent wound	Rod-shaped asporogenic cells in pairs or in short chains
8	*P.aeruginosa spp. 02-18*	Purulent wound	Rod-shaped asporogenic cells in pairs or in short chains
9	*E.faecalis spp. 01-18*	Pharynx	Cells in pairs (diplococci) or short chains
10	*P.aeruginosa spp. 01-19*	Purulent wound	Rod-shaped asporogenic cells in pairs or in short chains
11	*P.aeruginosa spp. 02-19*	Purulent wound	Rod-shaped asporogenic cells in pairs or in short chains
12	*P.aeruginosa spp. 03-19*	Purulent wound	Rod-shaped asporogenic cells in pairs or in short chains

Tinctorial properties of all obtained clinical isolates were studied using the standard commercial kits.

A study of morpho-cultural and physiology-biochemical characteristics of the isolates was carried out subsequently. Morphological characteristics of colonies including the characteristic pigmentation of the bacterial growth, ability to grow on diagnostic liquid and solid media were recorded for different isolates.

Table 2 characterize physiological and biochemical features of clinical isolates of *Staphylococcus* spp.

Table 2 – Physiological and biochemical features of clinical isolates of *Staphylococcus* spp.

Name of test	Isolates				
	Stapylococcus spp. 01-18	*Stapylococcus spp. 02-18*	*Stapylococcus spp. 03-18*	*Stapylococcus spp. 04-18*	*Stapylococcus spp. 05-18*
	Reaction				
Hemolysis	+ (α-hemolysis)	+ (α-hemolysis)	+ (β- hemolysis)	+ (β- hemolysis)	+ (α-hemolysis)
DNase	+	–	+	+	+
Plasmocoagulase	+	+	+	+	+
Lecithinase	+	+	–	–	+
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Ornithine decarboxylase	+	–	+	+	+
Arginine dihydrolase	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+
Fermentation of carbohydrates					
Sucrose	+	+	+	+	+
Lactose	–	–	+	+	+
Ramnose	+	+	–	–	–
Dulcitol	–	–	–	–	–
Mannitol	+	+	+	+	+
Biofilm formation index	1,13	1,10	1,21	1,18	1,29
Note: «+» positive test result, «–» negative test result.					

According to the data in table 2, the clinical isolates of *Staphylococcus* spp. showed the hemolytic activity with formation of zones of α - and β-hemolysis; DNase, plasmocoagulase, lecithinase (for some isolates), catalase, oxidase and arginine dihydrolase activities, as well as the ability to decarboxylate ornithine and lysine. Coagulase causes clotting of blood plasma. Staphylococci producing this enzyme are covered with a fibrin capsule that protects them from phagocytosis. Large concentrations of coagulase circulating in the host body lead to a decrease in blood clotting, hemodynamic disorders, progressive oxygen starvation of tissues. In laboratories, an identification of the coagulase activity deems possible pathogenicity of isolated *Staphylococcus* strains. The enzyme lecithinase destroys lecithin in the cell membranes of leukocytes and other cells, which promotes leukopenia. DNase causes depolymerization of DNA and also is considered as a pathogenicity factor characteristic also for infections caused by *S. aureus*. The presence of these exoenzymes, as well as the ability to ferment sucrose, lactose, rhamnose, and mannitol, all indicate a high virulence potential of the isolates.

Besides, active formation of biofilm by these strains was recorded. The indexes of biofilm formation ranged from 1.10 to 1.29, which is regarded as a strong propensity for biofilm formation [21]. Given the above features, these isolates can be attributed to cultures with a high virulent potential.

The results of the biochemical trial of *Streptococcus pneumoniae* isolates are shown in table 3.

Table 3 – Physiological and biochemical traits of the clinical isolate of *Streptococcus pneumoniae*

Name of test	Reaction
Hemolysis	α-hemolysis
Catalase test	–
Motility test	–
Growth in anaerobic conditions	+
Arginine dihydrolase	+
Ornithine decarboxylase	–
Fermentation of carbohydrates:	
Sucrose	+
Lactose	+
Mannitol	+
Note: «+» positive test result, «–» negative test result.	

The clinical isolate of *Streptococcus pneumoniae* grew under anaerobic conditions and fermented sucrose, lactose and mannitol. The presence of the exotoxin, hemolysin, which is responsible for hemolytic and cytotoxicity activities, was recorded by the formation of zones of α-hemolysis. The index of biofilm formation for this strain was around 2.10, which indicates a high propensity to form biofilms. All these features including the arginine dihydrolase activity imply high virulence of these isolates.

Similar biochemical trials were carried out for the isolates affiliated with the genus *Pseudomonas* (table 4).

Isolates of *Pseudomonas aeruginosa* could grow at 42 °Cand produce pigment pyocyanin. Pyocyanin producing isolates are commonly found in the sputum of patients with cystic fibrosis. An important factor of the pathogenicity of *P. aeruginosa* is the production of extracellular mucus formed by surface glycoproteins. This mucus forms a biofilm around the infected tissues leading eventually to chronic infections. Such biofilm can be formed on objects of the hospital environment that contributes to the spread of nosocomial infections. All isolated strains of *P. aeruginosa* actively formed biofilms. The value of the biofilm formation indexes was in the range of 2.56 to 7.30, which is seen as a strong biofilm propensity [21]. Additionally, the isolates produced positive reactions to oxidase and catalase. The complex of the recorded traits allows referring the isolates to high-virulent strains.

E. faecalis isolate from throat was characterized by such traits as α-hemolysis, β-galactosidase, glucuronidase, arginine dihydrolase activity and the ability to decarboxylate lysine and ornithine. The isolate also activity fermented lactose, sucrose, mannitol, trehalose, cellobiose, melibiose, sorbitol, inositol, adonitol and arabitol (table 5). It was concluded that this isolate represents a highly virulent strain of *E. faecalis*.

Table 4 – Physiological and biochemical traits of the clinical isolates of *Pseudomonas aeruginosa*

Name of test	Isolates				
	Pseudomonas aeruginosa spp. 01-18	*Pseudomonas aeruginosa spp. 02-18*	*Pseudomonas aeruginosa spp. 01-2019*	*Pseudomonas aeruginosa spp. 02-2019*	*Pseudomonas aeruginosa spp. 03-2019*
	Reaction				
Growth at 42 ^{0} C	+	+	+	+	+
Pyocyanin production	+	+	+	+	+
Oxidase test	+	+	+	+	+
Catalase test	+	+	+	+	+
Lysine decarboxylase	–	–	–	–	–
Arginine dihydrolase	–	–	+	+	+
Ornithine decarboxylase	+	+	–	–	–
Gelatin hydrolysis test	+	+	+	+	+
Fermentation of carbohydrates					
Glucose	–	–	–	–	–
Maltose	–	–	–	–	+
Mannitol	–	–	–	–	–
Sucrose	–	–	–	–	–
Lactose	–	–	–	–	–
Biofilm formation index	2,92	2,56	7,3	6,2	5,9
Note: «+» positive test result, «–» negative test result.					

Table 5 – Physiological and biochemical traits of the clinical isolate *E. faecalis.*

Name of test	Reaction
Hemolysis	α-гемолиз
Lecithinase test	–
β-galactosidase	+
β-xylosidase	–
β-glucuronidase	+
Ornithine decarboxylase	+
Arginine dihydrolase	+
Lysine decarboxylase	+
Fermentation of carbohydrates	
Lactose	+
Sucrose	+
Raffinose	–
Dulcitol	–
Mannitol	+
Trehalose	+
Cellobiose	+
Mellibiose	+
Sorbitol	+
Salicine	–
Malonate	–
Aesculine	–
Inositol	+
Adonitol	+
Arabitol	+
Simmons citrate	–
H_2S	–
Urease	–
Note: «+» positive test result, «–» negative test result.	

All studied isolates were properly identified to the genus and species level and accession numbers of the JSC "Scientific Centre for Anti-infectious Drugs" - SCAID culture collection were assigned according to international standards and specifications of collecting virulent clinical isolates [17-19].

On the next step of the study, profiles of antibiotic sensitivity were detected for all isolates. The evaluation was performed by the disco-diffusion method using 12 the most common antibiotics (table 6).

Table 6 – Sensitivity of clinical isolates to antibacterial drugs

Isolates	Antibiotics concentration, μg/disc											
	OX 1	CZ 30	AMX 10	GEN 30	MRP 10	IMP 10	AK 10	E 10	AZM 30	CTR 30	TOB 30	CB 10
	Zones of growth inhibition, M±StD, mm (according to CLSI)											
S.aureus SCAID URN1-2018	25 ± 1.15 (S)	40 ± 1.15 (S)	30 ± 1.15 (S)	25 ± 0.58 (S)	33 ± 1.15 (S)	50 ± 1.15 (S)	20 ± 0.58 (S)	10 ± 0.58 (R)	6 ± 0 (R)	30 ± 1.15 (S)	25 ± 1.15 (S)	42 ± 0.58 (S)
S.aureus SCAID WND1-2018	23 ± 1.15 (S)	40 ± 1.15 (S)	45 ± 1.15 (S)	30 ± 0.58 (S)	35 ± 1.15 (S)	56 ± 1.15 (S)	20 ± 0.58 (S)	32 ± 0.58 (S)	28 ± 0.58 (S)	30 ± 1.15 (S)	30 ± 0.58 (S)	25 ± 0.58 (S)
S.aureus SCAID VGN1-2018	18 ± 0.58 (S)	36 ± 1.15 (S)	30 ± 1.15 (S)	25 ± 0.58 (S)	33 ± 1.15 (S)	52 ± 1.15 (S)	25 ± 0.58 (S)	6 ± 0 (R)	6 ± 0 (R)	30 ± 1.15 (S)	23 ± 0.58 (S)	30 ± 0.58 (S)
S.aureus SCAID PHRX1-2018	23 ± 0.58 (S)	40 ± 1.15 (S)	40 ± 1.15 (S)	30 ± 0.58 (S)	40 ± 1.15 (S)	53 ± 1.15 (S)	23 ± 0.58 (S)	27 ± 0.58 (S)	30 ± 1.15 (S)	30 ± 0.58 (S)	30 ± 1.15 (S)	45 ± 1.15 (S)
St.pneumoniae SCAID SPT1-2018	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	12 ± 0.58 (R)	6 ± 0 (R)	10 ± 0.58 (R)	20 ± 0.58 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	25± 0.58 (S)	6 ± 0 (R)
E. faecalis SCAID PHRX1-2018	6 ± 0 (R)	23 ± 1,15 (S)	25 ± 1,15 (S)	21 ± 0,58 (S)	29 ± 1,15 (S)	23 ± 0,58 (S)	15 ± 0,58 (I)	6 ± 0 (R)	14 ± 0,58 (I)	34 ± 1,15 (S)	19 ± 0,58 (S)	25 ± 1,15 (S)
Pseudomonas aeruginosa SCAID WND1-2018	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	30 ± 1,15 (S)	35 ± 1,15 (S)	36 ± 1,15 (S)	25 ± 0,58 (S)	6 ± 0 (R)	30 ± 1,15 (S)	25± 0,58 (S)	35± 1,15 (S)	27± 0,58 (S)
Pseudomonas aeruginosa SCAID WND2-2018	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	10 ± 0,58 (R)	23 ± 1,15 (S)	30 ± 0,58 (S)	15 ± 0,58 (S)	6 ± 0 (R)	22 ± 0,58 (S)	6 ± 0 (R)	18 ± 0,58 (S)	10 ± 0,58 (R)
S.aureus SCAID ITICU1-2018	25± 0,58 (S)	35± 1,15 (S)	35 ± 1,15 (S)	25 ± 0,58 (S)	35 ± 1,15 (S)	50 ± 1,15 (S)	23 ± 0,58 (S)	30 ± 1,15 (S)	30 ± 1,15 (S)	30 ± 1,15 (S)	28 ± 0,58 (S)	35 ± 1,15 (S)
Pseudomonas aeruginosa spp. SCAID WND1-2019	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	23 ± 0 (S)	34 ± 0.58 (S)	27 ± 0,58 (S)	20 ± 0,58 (S)	15 ± 0 (R)	23 ± 0,58 (S)	14 ± 0,58 (I)	6 ± 0 (R)	25 ± 0.58 (S)
Pseudomonas aeruginosa spp. SCAID WND2-2019	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	20 ± 0,58 (S)	20 ± 0,58 (S)	8 ± 0.58 (R)	24 ± 0.58 (S)	6 ± 0 (R)	6 ± 0 (R)	12 ± 0 (R)	25 ± 0,58 (S)	14 ± 0,58 (R)
Pseudomonas aeruginosa spp. SCAID WND3-2019	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	25 ± 0,58 (S)	25 ± 0,58 (S)	20 ± 0,58 (S)	15 ± 0,58 (I)	25 ± 0,58 (S)	6 ± 0 (R)	15 ± 0,58 (S)	20 ± 0,58 (I)

Note: «OX» - Oxacillin, «CZ» - Cefazolin, «AMX» - Amoxycillin, «GEN» - Gentamicin, «MRP» - Meropenem, «IMP» - Imipenem, «AK» - Amikacin, «E» -Erythromycin, «AZM» - Azithromycin, «CTR» - Ceftriaxone, «TOB» - Tobramycin, «CB» - Carbenicillin, «R» - Resistant, «I» - Intermediate, «S» - Sensitive.

The studied clinical isolates were characterized by varying degrees of sensitivity to antibiotics. The isolates *S. aureus* SCAID URN1-2018 and *S. aureus* SCAID VGN1-2018 showed sensitivity to almost all classes of investigated antibiotics. Surprisingly, these strains showed resistance to azithromycin that is considered as a last resort reserved drug against staphylococcal infections. Clinical isolates *S. aureus* SCAID WND1-2018, *S. aureus* SCAID ITICU1-2018 and *S. aureus* SCAID PHRX1-2018 were sensitive to all the antibacterial drugs in the study.

Antibiotic sensitivity profiling of *S. pneumoniae* SCAID SPT1-2018 revealed a polyresistance of this culture. Resistance to oxacillin, cefazolin, amoxicillin, gentamicin, meropenem, imipenem, erythromycin, azithromycin, ceftriaxone and carbenicillin was observed. The strain was sensitive to amikacin and tobramycin, only.

The strain *P. aeruginosa* SCAID WND2-2018 also showed a significant resistance to a wide range of antibiotics including oxacillin, cefazolin, amoxicillin, gentamicin, erythromycin, ceftriaxone and carbenicillin; to carbapenem antibiotics, meropenem and imipenem, and also to to amikacin, azithromycin and tobramycin. Another *P. aeruginosa* isolate, SCAID WND1-2018, was resistant to oxacillin, cefazolin, amoxicillin and erythromycin but sensitive to gentamicin, meropenem, imipenem, amikacin, azithromycin, ceftriaxone, tobramycin and carbenicillin.

The strain *P. aeruginosa* SCAID WND1-2019 was characterized by resistance to oxacillin, cefazolin, amoxicillin, erythromycin and tobramycin. This clinical isolate was sensitive to carbapenems, – meropenem and imipenem, as well as to amikacin, azithromycin, carbenicillin, and gentamicin. An intermediate resistance was observed against ceftriaxone. Of the strain *P. aeruginosa* SCAID WND2-2019 was a multidrug resistant isolated showing sensitivity only to gentamicin, meropenem, amikacin and tobramycin. It was resistant to oxacillin, cefazolin, amoxicillin, imipenem, erythromycin, azithromycin, carbenicillin and ceftriaxone. The last *P. aeruginosa* isolate, SCAID WND3-2019, was characterized by resistance to oxacillin, cefazolin, amoxicillin, gentamicin and ceftriaxone. It was sensitive to carbapenems and to amikacin, azithromycin and tobramycin. An intermediate resistance was observed against erythromycin and carbenicillin.

E. faecalis SCAID PHRX1-2018 showed sensitivity to all the studied antibiotics but to erythromycin and oxacillin with an intermediate sensitivity to azithromycin.

Four isolates were selected for subsequent whole genome sequencing that was carried out on the Ion Torrent PGM platform. Assembly and annotation of complete genome sequences was performed as it was explained in detail in the section Methods.

De novo assembly was carried out by Velvet 1.2.10 assembler. The largest contigs were used to search for homologous sequences through NCBI *nr* database. Identified closed genomes were used for re-sequencing by mapping DNA reads with the program Bowtie2. For the isolate *P. aeruginosa* SCAID WND1-2019 the reference genome was the strain *P. aeruginosa* Pb18 (access number CP015650), for *P. aeruginosa* SCAID WND2-2019 – the strain *P. aeruginosa* AJ D 2 (access number CP038661) and for *P. aeruginosa* SCAID WND3-2019 – *P. aeruginosa* AR442 (access number CP029090).

Analysis of contigs of *E. faecalis* SCAID PHRX1-2018 revealed presence of a plasmid. Therefore, two reference sequences were identified: complete genome sequence of *E. faecalis* KB1 (access number CP022712), and for plasmid sequence of an *E. faecalis* plasmid pPD1 (access number KT290268).

Consensus complete genome sequences of the selected isolates were obtained BAM files of alignments of DNA reads using preserving the length of the reference genome that allowed copying the annotation of the reference genome to the obtained sequences. The gene prediction then was checked by the NCBI annotation pipeline. Unmapped regions and loci of ambiguity were marked by 100 N' trails. The resulted genome sequences were deposited at NCBI as shown in table 7.

Table 7 – Whole genome sequences of SCAID virulent clinical isolates deposited at NCBI

Isolate	GenBank AC	Length including gaps	GC-content	BUSCO genome completeness
P. aeruginosa SCAID WND1-2019	CP041787.1	6,267,146 bp	66.57%	95.1%
P. aeruginosa SCAID WND2-2019	CP041786.1	6,185,611 bp	66.7%	98.2%
P. aeruginosa SCAID WND3-2019	CP041785.1	6,382,154 bp	66.61%	97.8%
E. faecalis SCAID PHRX1-2018 (chromosome)	CP041877.1	2,597,557 bp	37.92%	43.8%
E. faecalis SCAID PHRX1-2018 (plasmid)	CP041878.1	98,263 bp	35.81%	Not applicable

Raw sequencing data for all these sequenced strains also was made available from NCBI at BioProject accession numbers: PRJNA555137 for *E. faecalis* SCAID PHRX1-2018 and PRJNA554979 for all sequenced *P. aeruginosa* clinical isolates.

A search for genetic drug resistance determinants in whole genome sequences was performed by the on-line service CARD RDI (https://card.mcmaster.ca/analyze/rgi) using NCBI accession numbers of the sequenced genomes as an input. A summary of this analysis is shown in table 8.

Table 8 – Identification of drug resistance determinants in whole genome sequences by CARD RDI

Genome	Gene category	Gene number	Possible association with drug resistance
P. aeruginosa CP041787	Antibiotic efflux pumps	43	Acridine; aminocoumarins; aminoglycosides; carbapenems; cephalosporins; fluoroquinolones; macrolides; tetracycline and others
	Lactamases	5	Beta-lactam antibiotics
	Other antibiotic inactivators excluding lactamases	5	Aminoglycosides; carbapenems; cephalosporins
	Antibiotic target alteration enzymes	3	Polypeptide antibiotics
P. aeruginosa CP041786	Antibiotic efflux pumps	43	Acridine; aminocoumarins; aminoglycosides; carbapenems; cephalosporins; fluoroquinolones; macrolides; tetracycline and others
	Lactamases	5	Beta-lactam antibiotics
	Other antibiotic inactivators excluding lactamases	5	Aminoglycosides; carbapenems; cephalosporins
	Antibiotic target alteration enzymes	4	fluoroquinolones; nybomycins and peptide antibiotics
P. aeruginosa CP041785	Antibiotic efflux pumps	42	Acridine; aminocoumarins; aminoglycosides; carbapenems; cephalosporins; fluoroquinolones; macrolides; tetracycline and others
	Lactamases	4	Beta-lactam antibiotics
	Other antibiotic inactivators excluding lactamases	5	Aminoglycosides; carbapenems; cephalosporins
	Antibiotic target alteration enzymes	3	Polypeptide antibiotics
	Antibiotic target replacement proteins	2	Sulfone and sulfonamide antibiotics
E. faecalis CP041877 (chromosome)	Antibiotic efflux pumps	3	Cephalosporins; fluoroquinolones; macrolides; peptide antibiotics; rifamycin and tetracycline
	Lactamases	2	Beta-lactam antibiotics
	Antibiotic target protection proteins	1	Lincosamide; macrolides; oxazolidinone; phenicol; streptogramin; tetracycline
	Antibiotic target replacement proteins	1	Diaminopyrimidine
E. faecalis CP041878 (plasmid)	Antibiotic inactivators	2	Aminoglycoside antibiotics
	Antibiotic target alteration enzyme	1	Streptomycin

Note: Genome accession numbers are the same as in table 7.

P. aeruginosa isolates are naturally resistant to many antibiotics as they contain multiple efflux pumps removing antibiotics from cytoplasm. Majority of drug resistance genes are constitutionally present in all sequenced genomes of *P. aeruginosa*. Our study shows that the sequenced genomes differed by several genes encoding antibiotic target alteration and replacement proteins, which probably were acquired by horizontal gene transfer. Contrary, *E. faecalis* isolates normally are sensitive to all antibiotics. The drug-resistant isolate *E. faecalis* SCAID PHRX1-2018 acquired the antibiotic resistance through horizontal gene transfer including an acquisition of a large virulence plasmid comprising at least 3 antibiotic inactivator genes of APH(3')-IIIa and aad(6) families, and a SAT-4 streptomycin acetyltransferase. The BUSCO analysis of genome completeness (table 7) showed that the genome of *E. faecalis* SCAID PHRX1-2018 is still rather fragmented and many genes may be missed including some additional drug resistance genes.

Conclusion. Collection of clinical isolates of nosocomial infections from hospitals of phthisiological profile in Almaty and Almaty region was carried out. In result, a collection of 12 strains of pathogenic microorganisms causing nosocomial infections has been created. Using classical microbiological approaches of morpho-cultural and physiology-biochemical characteristics of the isolates was carried out subsequently. Morphological characteristics of colonies including the characteristic pigmentation of the bacterial growth, ability to grow on diagnostic liquid and solid media were recorded for different isolates. On the basis of the conducted researches, all investigated isolates of *S. aureus* belong to cultures with high virulent potential, capable to form biofilms. Clinical isolates of *P. aeruginosa* is also characterized as highly virulent strains with a high propensity to form biofilms. Isolates of *Streptococcus pneumoniae* and *E. faecalis* spp. were attributed to virulent strains. All studied isolates were assigned according to international standards and specifications of collecting virulent clinical isolates.

The profiles of antibiotic sensitivity were detected for all isolates. The evaluation was conducted to 12 of the most common antibiotics. The clinical isolates are characterized by varying degrees of sensitivity to antimicrobial drugs. Surprisingly, that some strains were resistant to to azithromycin that is considered as a last resort reserved drug. This fact causes concern about the circulation and distribution in hospitals of microorganisms resistant to antibiotics of recent generation.

Four isolates were selected for subsequent whole genome sequencing. After sequencing and assembling reads the full-genome sequences of strains *E. faecalis* SCAID PHRX1-2018, *P. aeruginosa* SCAID WND1-2019, *P. aeruginosa* SCAID WND2-2019 and *P. aeruginosa* SCAID WND3-2019 were obtained. All sequencing data are deposited at NCBI under access numbers CP041877 for isolate *E. faecalis* SCAID PHRX1-2018 (plasmid DNA access number CP041878), CP041787, CP041786, and CP041785 for isolates *P. aeruginosa* SCAID WND1-2019, *P. aeruginosa* SCAID WND2-2019 and *P. aeruginosa* SCAID WND3-2019 respectively.

A search for genetic drug resistance determinants in whole genome sequences was carried out. Our study shows that the genomes differed by several genes encoding antibiotic target alteration and replacement proteins, which probably were acquired by horizontal gene transfer.

The data on the genetic characteristics of microorganisms that cause nosocomial infections in the future will help to improve the monitoring of nosocomial diseases, and will also contribute to the improvement of the protocols of treatment of the population, to prevent the occurrence and spread of antibiotic resistance.

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АУРУХАНАІШІЛІК ИНФЕКЦИЯЛАР ИЗОЛЯТОРЛАРЫНЫҢ БӨЛІНУІ ЖӘНЕ СИПАТТАМАСЫ

Аннотация. Зерттеудің мақсаты патогенді бактериялардың әртүрлі түрлерінде антибиотиктерге сезімталдық реверсиясының феноменін зерттеу мақсатында штаммдардың банкін құру және сипаттау үшін нозокомиальды инфекциялардың клиникалық изоляторларын анықтау болып табылады.

Әдістері: клиникалық оқшаулағыштарды алғашқы идентификациялау: таза дақылдарды алу, морфокультуралық, тинкториалдық, физиологиялық және биохимиялық қасиеттерін зерттеу коммерциялық тест-жүйелерді қолдану арқылы жүзеге асырылды. Клиникалық изоляторлардың сезімталдық профилі диско-диффузиялық әдіспен зерттелді. Бактериялардан ДНҚ бөлу PureLink Genomic DNA Kit жиынтығымен жүргізілді. Секвенирлеуге арналған кітапхана Ion Xpress Plus Fragment Library kit (Life Technologies, АҚШ) жиынтығымен ферментативті әдіспен алынды. Баркодирование изоляттарды жүзеге асырылды жиынтығы көмегімен

Xpress Barcode Adapters (Life Technologies, USA). Алынған кітапхананы секвенирлеу 318 чипте Ion PGM Hi-Q View Sequencing Kit жиынтығымен жүргізілді. Алынған Риддердің сапасы және ДНҚ фрагменттерінің кесілуі FastQC және cassava fastq filter компьютерлік бағдарламаларының көмегімен жүзеге асырылды. Геном фрагменттері Velvet 1.2.10. Геномның үлкен фрагменттері NCBI деректер базасында ең жақын референттік геномдарды іздеу үшін пайдаланылды. Оқшаулағыштардың толық геномы Bawtie2 бағдарламасын пайдалана отырып референс геномдарымен бастапқы фрагменттерді теңестіру жолымен алынды. Соңғы жинақтардың толықтығы BUSCO бағаланды. Кодтаушы гендерді автоматты түрде іздеу RAST серверінің көмегімен жүзеге асырылды.

Сандық зерттеулердің нәтижелері One-Way ANOVA бір факторлы дисперсиялық талдау әдісін пайдалана отырып, GraphPad Prism 6 бағдарламалар пакетінің көмегімен кейіннен статистикалық талдаумен өңделді.

12 клиникалық изолятор бөлінді. Анықталған полногеномная нуклеотидная реттілігі төрт изоляттарды - Enterococcus faecalis PHRX1, Pseudomonas aeruginosa WND1-2019, Pseudomonas aeruginosa WND2-2019 және Pseudomonas aeruginosa WND3-2019. Аннотацияланған геномдар NCBI деректер базасында сақтауға алынған.

Түйін сөздер: ауруханаішілік инфекциялар, оқшаулау, ДНҚ, секвенирлеу, *Enterococcus faecalis*, *Pseudomonas aeruginosa.*

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ВЫДЕЛЕНИЕ И ХАРАКТЕРИСТИКА ИЗОЛЯТОВ ВНУТРИБОЛЬНИЧНЫХ ИНФЕКЦИЙ

Аннотация. Целью исследования являлось выявление клинических изолятов нозокомиальных инфекций для характеристики и создания банка штаммов с целью изучения феномена реверсии чувствительности к антибиотикам у различных видов патогенных бактерий.

Методы. Первичная идентификация клинических изолятов: получение чистых культур, изучение морфокультурных, тинкториальных, физиологических и биохимических свойств осуществлялась с использованием коммерческих тест-систем. Профиль чувствительности клинических изолятов изучали дисково-диффузионным методом. Выделение ДНК из бактерий производили набором PureLink Genomic DNA Kit. Библиотека для секвенирования была получена ферментативным методом с использованием набора Ion Xpress Plus Fragment Library kit (Life Technologies, США). Баркодирование изолятов осуществлялось с помощью набора Xpress Barcode Adapters (Life Technologies, USA). Секвенирование полученной библиотеки проводили на 318 чипе с использованием набора Ion PGM Hi-Q View Sequencing Kit. Качество полученных ридов и обрезку фрагментов ДНК осуществляли с помощью компьютерных программ FastQC и Cassava fastq filter. Фрагменты генома были собраны с использованием Velvet 1.2.10. Большие фрагменты генома были использованы для поиска ближайших референсных геномов в базе данных NCBI. Полный геном изолятов был получен путем выравнивания исходных фрагментов с референсными геномами с использованием программы Bawtie2. Полнота окончательных сборок была оценена BUSCO. Автоматический поиск кодирующих генов осуществлялся с помощью сервера RAST.

Результаты количественных исследований были обработаны с использованием метода однофакторного дисперсионного анализа One-Way ANOVA с последующим статистическим анализом при помощи пакета программ GraphPad Prism 6.

Было выделено 12 клинических изолятов. Определена полногеномная нуклеотидная последовательность четырех изолятов - *Enterococcus faecalis* PHRX1, *Pseudomonas aeruginosa* WND1-2019, *Pseudomonas aeruginosa* WND2-2019 и *Pseudomonas aeruginosa* WND3-2019. Аннотированные геномы были депонированы в базе данных NCBI.

Ключевые слова: внутрибольничные инфекции, изоляция, ДНК, секвенирование, *Enterococcus faecalis*, *Pseudomonas aeruginosa.*

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