Heliyon 10 (2024) e40821

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Two multidrug-resistant *Proteus mirabilis* clones carrying extended spectrum beta-lactamases revealed in a single hospital department by whole genome sequencing

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ARTICLE INFO

Keywords:
Proteus mirabilis
Antimicrobial resistance
Whole genome sequencing
Bacterial clones
Mobile genetic element
Salmonella genomic island 1
Genomic epidemiology

ABSTRACT

Proteus mirabilis bacteria is a component of normal intestinal microflora of humans and animals, but can also be found in hospital settings causing urinary tract infections and sepsis. The problem of treating such infections is complicated by multidrug-resistant isolates producing extended spectrum beta-lactamases (ESBL), and the number of ESBL-carrying *P. mirabilis* strains has significantly increased recently.

This study presents a detailed analysis of 12 multidrug-resistant *P. mirabilis* isolates obtained from the wounds of different patients in one surgical department of a multidisciplinary hospital in Moscow, Russia, using the short- and long-read whole genome sequencing. The isolates under investigation divided into two clusters (clones) C1 and C2 based on their genomic profiles and carried antimicrobial resistance (AMR) genes corresponding well with phenotypic profiles, which was the first case of reporting two different *P. mirabilis* clones obtained simultaneously from the same specimens at one hospital, to the best of our knowledge. Some genes, including ESBL encoding ones, were specific for either C1 or C2 (*aac(6′)-Ib10, ant(2″)-Ia, qnrA1, bla*$_{VEB-6}$ and *fosA3*, *bla*$_{CTX\ -M-65}$, correspondingly). Additionally, the *Salmonella* genomic islands 1 were found that differed in composition of multiple antibiotic resistance regions between C1 and C2 groups. CRISPR-Cas system type I-E was revealed only in C2 isolates, while the same set of virulence factors was determined for both *P. mirabilis* clones.

Diversity of all genetic factors found in case of simultaneous existence of two clones collected from the same source at one department indicates high pathogenic potential of *P. mirabilis* and poses a requirement of proper spreading monitoring. The data obtained will facilitate the understanding of AMR transfer and dynamics within clinical *P. mirabilis* isolates and contribute to epidemiological surveillance of this pathogen.

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https://doi.org/10.1016/j.heliyon.2024.e40821
Received 23 September 2024; Received in revised form 25 November 2024; Accepted 28 November 2024
Available online 29 November 2024

1. Introduction

Bacterial multidrug resistance (MDR) is a global problem worldwide arose due to the uncontrolled antibiotic usage according to the World Health Organization. This problem is especially challenging for the members of *Enterobacteriaceae* family including *Proteus* genus, whose members are gram-negative, anaerobic, non-sporulated, and motile bacteria. One of the most prevalent species within this genus is *P. mirabilis*, which usually inhabits the intestinal tract of humans and animals as normal flora, and can also be found in soil and wastewater environments [1,2]. Nevertheless, it is considered an opportunistic pathogen causing community-acquired and nosocomial urinary tract infections, as well as foodborne infections [1,3].

Environmental isolates of *P. mirabilis* are usually susceptible to beta-lactam antibiotics, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole, but are naturally resistant to polymyxins, nitrofurantoin, macrolides, lincosamides and tetracycline [4,5]. However, resistance to 3-rd and 4-th generation cephalosporins, aztreonam, trimethoprim-sulfamethoxazole, and fluoroquinolones has been previously reported [1,6–8].

Mobile genetic elements (MGEs), including conjugative plasmids, integrative conjugative elements and integrative mobilizable elements (IMEs) play a key role in acquisition, accumulation and transfer of antimicrobial resistance (AMR) genes [9]. IMEs are integrated into chromosomes and can excise themselves, but are not self-transferrable and need to use the conjugation system of some plasmid. *Salmonella* genomic islands (SGI) 1 represent one of the most studied groups of IMEs in gram-negative bacteria, the elements of which were first revealed in *S. enterica*, but later were found to be rather common in *P. mirabilis*, especially in MDR clones [9,10]. SGI1 elements harbor or can acquire a multiple antibiotic resistance (MAR) region, which contains several AMR genes and thus plays a crucial role in AMR transfer. Whole genome sequencing (WGS) allowed revealing multiple SGI1 and other SGI variants, as well as the structures of various MARs they contained, in different bacterial species including *P. mirabilis*, in particular [10,11]. To summarize, WGS currently represents an essential tool for studying the AMR acquisition and spread via MGEs in pathogenic bacteria.

Despite the obvious public health threats due to infections caused by MDR *P. mirabilis* isolates, the present level of their investigation and WGS is significantly inferior to the ones of ESKAPE pathogens. Currently, only 3863 whole-genome assemblies of *P. mirabilis* are available in Genbank in comparison to, e.g., *Acinetobacter baumannii* (29,228 assemblies) (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=584, accessed on August 09, 2024). Genomic data, including horizontally transferable elements, such as insertion sequences, integrons, and transposons, greatly facilitates the epidemiological surveillance of *P. mirabilis* and developing effective prevention measures against this pathogen in clinical settings.

This study was focused on the clinical *P. mirabilis* isolates obtained from the wounds of the patients from a single surgical department of a multidisciplinary hospital in Moscow, Russia. Wound infections can become chronic, which are difficult to treat due to the formation of biofilms in the wound environment. According to the previous findings, the most frequent agents causing wound infections are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *P. mirabilis* [2,12].

The short- and long-read WGS was applied to characterize 12 MDR *P. mirabilis* isolates representing two clonal groups obtained from the different patients of the same surgical department at a multidisciplinary hospital. The data presented include antimicrobial resistance phenotypes and genotypes, virulence factors, CRISPR-Cas systems and phylogenetic analysis of the isolates studied. WGS also allowed to reveal mobile elements of SGI1-PmSC1111 type harboring multiple resistance genes in all of the isolates studied. To the best of our knowledge, this is the first report describing comprehensive characteristics of multiple *P. mirabilis* clones present simultaneously within a single hospital department and isolated from the same specimen. The data shown will facilitate the understanding of AMR transfer within clinical *P. mirabilis* isolates and provide useful epidemiological insights into its spreading within bacterial populations.

2. Materials and methods

2.1. Bacteria isolation and antibiotic susceptibility determination

The study involved 12 clinical isolates obtained from the wounds of different patients of a surgical department of a Moscow hospital within one week in 2023 (April 17 – April 23). In total, 37 *P. mirabilis* isolates were obtained originally during the period specified, but only the representative set was selected for the current study. The species were identified by time-of-flight mass spectrometry (MALDI-TOF MS) using the VITEK MS system (bioMerieux, Marcy-l'Étoile, France). The antibiotic susceptibility was determined using the disc diffusion method using the Mueller-Hinton medium (bioMerieux, Marcy-l'Étoile, France) and disks with antibiotics (BioRad, Marnes-la-Coquette, France), and also by the boundary concentration method on VITEK2 Compact 30 analyzer (bioMerieux, Marcy-l'Étoile, France). The isolates were tested for susceptibility/resistance to the following drugs: amikacin, gentamicin, ciprofloxacin, levofloxacin, ceftazidime, cefepime, ceftolozane-tazobactam, piperacillin-tazobactam, meropenem, ertapenem, and trimethoprim/sulfamethoxazole. EUCAST clinical breakpoints, version 13 (http://www.eucast.org, accessed on April 15, 2023), were applied to interpret the susceptibility/resistance results obtained.

2.2. DNA isolation, sequencing, and genome assembly

Genomic DNA was isolated with the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), and the quantity was evaluated by fluorimetry using Qubit 4.0 (Thermo Fisher Scientific, USA). The Nextera™ DNA Sample Prep Kit (Illumina, San Diego, USA) was used for paired-end library preparation and WGS of the isolates on Illumina NextSeq 2000 platform (Illumina, San Diego, USA).

Oxford Nanopore MinION sequencing system (Oxford Nanopore Technologies, Oxford, UK) was used for long-read WGS. The

libraries were prepared according to the manufacturer protocols with Rapid Barcoding Sequencing kit SQK-RBK004 (Oxford Nanopore Technologies, Oxford, UK) and were sequenced on FLO-MIN106 R9.4 flow cell with a standard 72 h sequencing protocol using the MinKNOW software version 22.03 (Oxford Nanopore Technologies, Oxford, UK). Long read sequencing was performed for three representative isolates CriePir1631, CriePir1633 and CriePir1652.

Genome assembly was performed by SPAdes version 3.15.4 for short-read assemblies. Base calling of the raw MinION data was made using Guppy Basecaller version 6.4.6 (Oxford Nanopore Technologies, Oxford, UK), and demultiplexing was performed using Guppy Barcoder version 6.4.6 (Oxford Nanopore Technolo-gies, Oxford, UK). Hybrid short- and long-read assemblies were obtained using Unicycler version 0.5.0 (normal mode) [13].

Genome assemblies were uploaded to NCBI Genbank under the project number PRJNA1145476.

2.3. Data processing and annotation

The assembled genomes were processed by the custom annotation pipeline described earlier [14,15]. Briefly, the Resfinder 4.3.0 database (https://cge.cbs.dtu.dk/services/ResFinder/, accessed on June 25, 2024) was used for antimicrobial gene and resistance mutation detection. CRISPR arrays and Cas cassettes were identified in genomic sequences of *P. mirabilis* using CRISPRCasFinder version 4.2.20 [16].

The set of virulence genes was derived from literature data [17,18] and revealed using Prokka annotation of the genomes (prokka 1.14.5 [19]).

The search for *Salmonella* and *Proteus* Genomic Islands (SGI, PGI) was performed by BLASTn search against reference strains described earlier [9,20].

Core genome analysis was performed using chewBBACA version 2.8.5 [21], and minimum spanning tree was build using PHYLOViz online (http://online.phyloviz.net, accessed on June 27, 2024).

3. Results

3.1. Isolate typing and antibiotic resistance

Proteus mirabilis WGS data is significantly inferior to the pathogens belonging, for example, to the ESKAPE group. In addition, there is no standard multilocus sequence typing (MLST) scheme, which is usually based on the fragments of the six to eight housekeeping loci, for *P. mirabilis* typing, and no generally accepted cgMLST scheme was defined either. Thus, in order to obtain information regarding the genomic and phylogenetic relationship of the isolates under investigation, a minimum spanning tree was built based on 2264 ad hoc core genome alleles (Fig. 1).

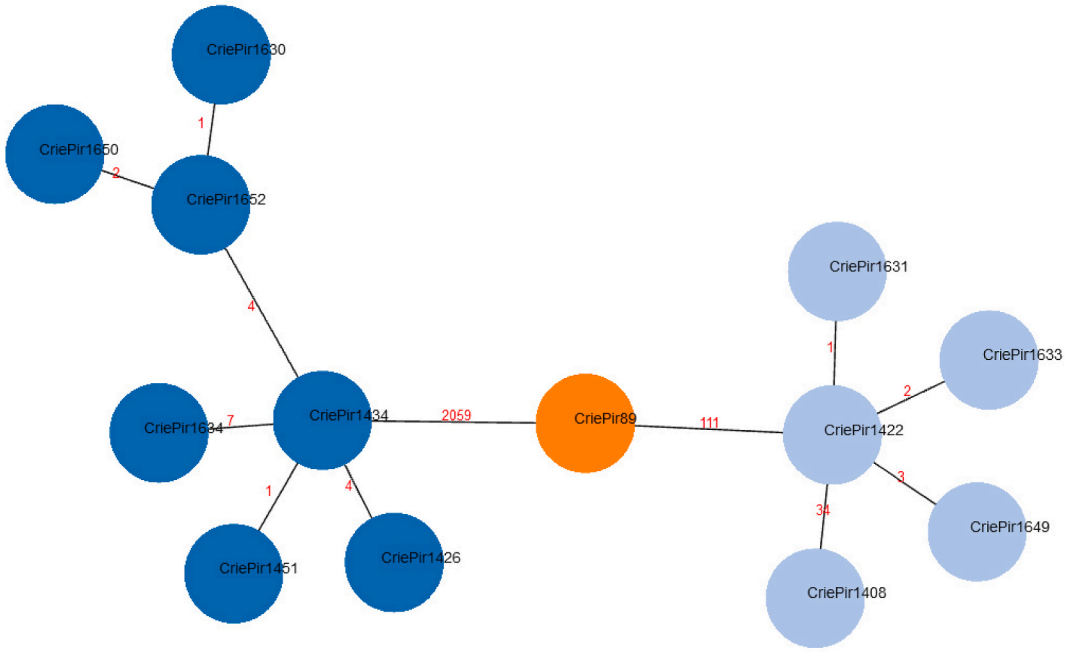

Fig. 1. Minimum spanning core genome tree based on 2264 alleles. Red numbers indicate allele differences. The isolates belonging to clone 1 are shown in blue, while the isolates of clone 2 are shown in cyan. CriePir89 previously revealed in the same hospital was added for comparison purposes.

This dendrogram clearly demonstrates that all isolates were divided into two clusters C1 and C2. C1isolates were characterized by 1–7 allelic mismatches within their group, while C2 contained 1–34 allelic mismatches according to the comparison of common genes. The data obtained indicate that the isolates studied belong to two different clones of *P. mirabilis*.

The isolate CriePir89 obtained from the same hospital earlier [8] was added to the current study for comparison purposes. Although it was rather close to C2, it clearly did not belong to any of the clones.

All 12 *P. mirabilis* isolates were tested for antibiotic susceptibility/resistance both by boundary concentration method using VITEK 2 system and bioinformatics analysis to search for known acquired resistance genes in genomic sequences. The phenotypes and genotypes of the 12 isolates studied are presented in Fig. 2.

According to the antimicrobial susceptibility test (Fig. 2A), all of the wound-isolated samples were resistant to ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole, while most of the isolates studied had additional gentamicin (n = 10), ceftazidime and cefepime (n = 11) resistance. Moreover, five isolates belonged to C1 were resistant to amikacin.

In silico searching for AMR determinants provided the results which corresponded well to the phenotypic profile (Fig. 2B). For example, the presence of $bla_{TEM\text{-}1B}$, $bla_{SAT\text{-}2}$, $bla_{OXA\text{-}1}$ complies with the resistance to cephalosporins, and the combination of *dfrA12/dfrA14* genes with *sul1/sul2* confers the resistance to trimethoprim/sulfamethoxazole. Furthermore, a number of genes were specific to the isolates of a particular genetic line. For example, C1 was characterized by the presence of AMR determinants to aminoglycosides *aac(6′)-Ib10, ant(2″)-Ia*, fluoroquinolones (*qnrA1*), and extended spectrum β-lactamase (ESBL) gene $bla_{VEB\text{-}6}$, while C2-isolates carried ESBL gene $bla_{CTX\text{-}M\text{-}65}$, as well as *fosA3* responsible for the resistance to fosfomycin.

3.2. *Salmonella genomic island (SGI1) variants*

The comparative genomic analysis allowed us to reveal SGI1 in all 12 isolates. In general, CriePir *P. mirabilis* isolates included SGIs of one type, namely, SGI1-PmSC1111 previously revealed in *P. mirabilis* [22]. However, the contents of multiple antibiotic resistance (MAR) regions was different between C1 and C2 groups of the isolates. The current study was focused on CriePir1631, CriePir1633 and CriePir1652 as representative genomes since high-resolution genomic sequences were obtained for them.

According to the comparative analysis and searching for *attL* and *attR* signature sites located at the beginning and the end of SGI1 backbone, respectively, the size of SGI1 in CriePir1631 was estimated to be 52k. It included the MAR regions containing all AMR genes revealed in the isolate except *tet(J)*. CriePir1633 had virtually the same SGI except for several minor inconsistencies likely representing assembly artifacts.

CriePir1652 possessed the SGI1 whose length was estimated to be 54k. Its MAR regions also included most resistance genes except *ant(2″)-Ia* and *tet(J)*. The difference in MAR regions between C1 and C2 also included $bla_{VEB\text{-}6}$ for C1 and $bla_{CTX\text{-}M\text{-}65}$ and $bla_{OXA\text{-}1}$ for C2, which made the latter more similar to the reference SGI from PmSC1111. In general, the SGI1 region from CriePir1652 was more similar to the reference than the one from CriePir1631.

Both variants of SGI1 included IS26-element, which was known to increase the plasticity of the MAR region since it facilitates the insertion of plasmid genes [23]. Since neither of the isolates possessed a plasmid, the previous plasmid-mediated insertion event for AMR gene acquisition can be supposed.

A.

Isolates	amikacin	gentamicin	ciprofloxacin	levofloxacin	ceftazidime	cefepime	ceftolozane-tazobactam	piperacillin-tazobactam	meropenem	ertapenem	trimethoprim/sulfamethoxazole
Crie-Pir1426	R	R	R	R	S	S	S	S	S	S	R
Crie-Pir1434	S	R	R	R	R	R	S	S	S	S	R
Crie-Pir1451	S	R	R	R	R	R	S	S	S	S	R
Crie-Pir1630	R	R	R	R	R	R	S	S	S	S	R
Crie-Pir1634	R	R	R	R	R	R	S	S	S	S	R
Crie-Pir1650	R	R	R	R	R	R	S	S	S	S	R
Crie-Pir1652	R	R	R	R	R	R	S	S	S	S	R
Crie-Pir1422	S	S	R	R	R	R	S	S	S	S	R
Crie-Pir1631	S	R	R	R	R	R	S	S	S	S	R
Crie-Pir1633	S	R	R	R	R	R	S	S	S	S	R
Crie-Pir1649	S	R	R	R	R	R	S	S	S	S	R
Crie-Pir1408	S	S	R	R	R	R	S	S	S	S	R

B.

Isolates	AAC(3)-IId	AAC(6′)-Ib-cr6	AAC(6′)-Ib10	ANT(2″)-Ia	APH(3″)-Ib	aadA5	APH(3″)-Ia	APH(6)-Id	CTX-M-3	CTX-M-65	OXA-1	SAT-2	SHV-12	TEM-1	VEB-6	FosA3	QnrA1	catA1	catA4	catB3	dfrA1	dfrA17	sul1	sul2	qacEdelta1 (antiseptics)	tet(J)
Crie-Pir1426	+	.	+	+	+	+	.	+	+	.	.	+	+	+	+	.	+	+	+	.	+	+	+	+	+	+
Crie-Pir1434	.	.	+	+	+	.	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	.	+	+	+	+
Crie-Pir1451	.	.	+	+	+	.	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	.	+	+	+	+
Crie-Pir1630	.	.	+	+	+	.	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	.	+	+	+	+
Crie-Pir1634	.	.	+	+	+	.	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	.	+	+	+	+
Crie-Pir1650	.	.	+	+	+	.	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	.	+	+	+	+
Crie-Pir1652	.	.	+	+	+	.	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	.	+	+	+	+
Crie-Pir1422	.	+	.	.	+	+	+	+	.	+	+	+	.	+	.	+	.	+	+	+	+	+	+	+	+	+
Crie-Pir1631	+	+	.	.	+	+	+	+	.	+	+	+	.	+	.	+	.	+	+	+	+	+	+	+	+	+
Crie-Pir1633	+	+	.	.	+	+	+	+	.	+	+	+	.	+	.	+	.	+	+	+	+	+	+	+	+	+
Crie-Pir1649	+	+	.	.	+	+	+	+	.	+	+	+	.	+	.	+	.	+	+	+	+	+	+	+	+	+
Crie-Pir1408	.	.	.	.	.	+	.	.	.	+	.	+	.	.	.	+	.	+	+	.	+	+	+	.	+	+

Fig. 2. A. Phenotypic antimicrobial resistance profile of MDR *P. mirabilis* isolates from one surgical department of a Moscow hospital. **B.** Genotypic antimicrobial resistance profile of MDR *P. mirabilis* isolates. Orange group of isolates belonged to C1; blue belonged to C2. Colors indicate different antibiotic classes and corresponding AMR determinants: gray is used for aminoglycosides; green for β-lactams; yellow shows fluoroquinolones; and violet indicates sulfonamides. Cyan highlighted the intrinsic gene *tet(J)*.

3.3. Virulence genes

Virulence genes can be located on plasmids or a chromosome, and are involved in a complex of tasks including bacteria survival, signaling, metabolism and interactions with the host. All the isolates analyzed, regardless of belonging to C1 or C2, were characterized by the same set of virulence factors (Table 1), which included genes responsible for swarming activity (*cheW*, adherence and biofilm formation factor *mrpA*, protease expression regulator *zapA*), interactions between bacteria (quorum sensing, *luxS*), tissue damage (hemolysin *hpmA*), urovirulence and antibiotic efficiency (*rpoA*), adherence and biofilm formation determinants (*ureABCDEFG* and *mrpA*).

3.4. CRISPR-cas systems

C2 isolates possessed CRISPR-Cas system type I-E, while C1 did not include any CRISPR-Cas structures. The type I-E system revealed was located in the chromosome and included endonucleases Cas1, Cas2, Cas3, Cas5, Cas6, Cas7, and also two Cse cassettes were found (Cse1 and Cse2). The scheme is presented below (Fig. 3).

4. Discussion

Proteus mirabilis is a common opportunistic pathogen causing severe illness in humans and animals. Risk factors are associated with the acquisition of resistance to three or more classes of antimicrobial drugs [24]. According to the core genome analysis of the isolates studied, two clones (genetic lines), named C1 and C2, were obtained in a single surgical department of a multidisciplinary hospital in Moscow, Russia. Currently, there is no established criteria for *P. mirabilis* belonging to the same or different epidemic strains due to the lack of generally accepted cgMLST typing scheme for this species. However, in the current case the differences between the isolates within each lineage were insignificant (1–7 alleles of ad hoc core genome) allowing to suggest that they belonged to the same epidemiological clone. To the best of our knowledge, this is the first comprehensive WGS analysis of more than one clone of *P. mirabilis* collected simultaneously from the same type of specimens of isolation in a single hospital department.

Different clonal lineages were obtained from three departments in one hospital in Croatia within 3 years and, moreover, clonality of the isolates was confirmed by pulsed-field gel electrophoresis (PFGE) [25]. Other studies also described few groups of clones obtained from a single hospital, which were confirmed by PFGE analysis, but they were collected from different types of specimens [26,27]. On the other hand, there were several works describing single clones obtained from a single medical department and several specimens of isolation [28,29].

In the current study, the AMR determinants of 12 clinical isolates derived from WGS data corresponded well to the phenotypic profile provided by susceptibility test. Each clonal group studied was characterized by specific AMR genotypic profile, for example, C1 carried ESBL gene $bla_{VEB\text{-}6}$ and fluoroquinolone resistance gene *qnrA1,* while C2 had $bla_{CTX\ \text{-}M\text{-}65}$ ESBL gene and *fosA3* fosfomycin resistance determinant. High prevalence of extended-spectrum cephalosporin resistance could be caused by the presence of $bla_{VEB\text{-}6}$, which was considered to be the source of resistance in extended-spectrum-cephalosporin-resistant clinical *P. mirabilis* isolates [30]. Moreover, a cephalosporin-resistant *P. mirabilis* clinical isolate from France harbored $bla_{VEB\text{-}6}$ and *qnrA1* genes [31] similarly to C1 isolates in the present study, and WGS data for cephalosporin-resistant Italian clinical isolates showed a complex array of resistance genes including *qnrA1* and the $bla_{VEB\text{-}6}$ [32]. $bla_{CTX\ \text{-}M\text{-}65}$ and *fosA3* determinants revealed in C2 were also characterized by chromosomal localization, as well as *fosA3* and $bla_{CTX\text{-}M}$ genes in *P. mirabilis* from Chinese chicken farm [33]. Interestingly, there was a number of studies from China describing the same set of AMR genes for *P. mirabilis* isolated from swine farm [34], retail chicken [35], livestock farms [36], and clinical samples of patients at the Hejiang County People's Hospital [37]. Additionally, the same combination of genes was possessed by two isolates in the autonomous community of Spain [38], which were located in transposon, thus possessing the ability to spread among *Enterobacteriaceae*. Also, $bla_{CTX\ \text{-}M\text{-}65}$ and *fosA3* genes were more prevalent in chicken strains and human urine samples isolated from the same geographical area of Southern Brazil [39].

In order to better understand the mechanisms and pathways of AMR acquisition and spreading, it is essential to investigate the location of AMR genes and possible ways of their transfer. In this study, most genes conferring resistance were located inside the MAR region of SGI1 for three representative isolates sequenced by the third-generation equipment, with the exclusion of *tet(J)* genes. The presence of a rather large number of AMR genes in SGI of *Proteus mirabilis* isolates is a rather common phenomenon, e.g., RGF134-1 obtained from swine fecal samples [40] had the SGI structure very similar to the one of CriePir1631 and CriePir1633, including the

Table 1
Virulence factors of the clinical *P. mirabilis* isolates.

Gene/gene cluster	Function
cheW	swarming ability on solid surfaces
hpmA	tissue damage
luxS	quorum sensing
mrpA	adherence of bacteria to the epithelial tissue and biofilm formation
pmfA	fimbriae formation
rpoA	urovirulence and antibiotic efficiency
ureABCDEFG	urease production, facilitates bacterial adherence and biofilm formation
zapA	protease expression at the differentiation of swimmer cells to swarmer cells

Fig. 3. The scheme of the CRISPR-Cas system type I-E of *P. mirabilis* isolates belonged to the C2 clone.

same AMR genes except for $bla_{TEM\text{-}1B}$. The PmSC1111 isolate [23] possessing an SGI backbone structure selected as a reference for CriePir1631 and CriePir 1633, also included a broad variety of AMR genes, including *aac(6′)-Ib* and $bla_{OXA\text{-}1}$, within its SGI region. Although it is known that SGI1-related elements cannot self-transfer, they harbor AcaCD binding sites and thus are mobilizable by IncC conjugative plasmids [20]. CriePir1631, CriePir1633 and CriePir1652 apparently did not possess any plasmids. However, their SGI1 regions will be able to be mobilized by the corresponding plasmids once they are acquired. Interestingly, similar SGI1-related elements containing AMR genes both in a plasmid and a chromosome were revealed in CriePir89 isolate obtained in 2017 from the department of thoracic and vascular surgery of the same hospital [8], which additionally highlights the need for continuous monitoring of SGI1-related elements in *P. mirabilis* due to their possible role in AMR transfer. It also shows that similar SGI1-elements can be carried by genomically diverged isolates since CriePir89 did not belong to either C1 or C2 clones studied. At the same time, CriePir89 could be a predecessor of C2, to which it was more similar genomically, and the isolates making C2 group in the present study could diverge from this ancestor over time, which was not uncommon for *P. mirabilis* previously shown to exhibit within-host evolution [41].

All isolates analyzed were characterized by the same set of virulence factors, which was identical to the one of CriePir89 *P. mirabilis* isolate [8]. Similar findings regarding the shared set of virulence factors were revealed for a larger sample of 183 *P. mirabilis* isolates from Brazil [18]. In general, key *P. mirabilis* virulence factors include the ones involved in swarming activity, as well as ureases, fimbrial adhesins, toxins and proteases [17]. The members from each of these groups were revealed in the set of the isolates from the current study, which additionally highlights the need for continuous monitoring of *P. mirabilis* in clinical conditions due to its rather high pathogenic potential.

Another interesting feature of two clonal groups detected was the presence of CRISPR-Cas system only in C2. Such systems were revealed in less than a half of the sequenced *P. mirabilis* genomes [42]. In the present study, a type I-E system was detected. CRISPR-Cas systems, which were found in 75 % bacteria and archaea, combine both genomic (CRISPR) and proteomic (Cas) components and mediate an adaptive immune response against invading viruses and other threats [43]. All *Proteus* species described to date harbored exclusively a class I subtype B (type I-E) system [44,45]. CRISPR-Cas systems support an evolutionary connection between CRISPR and pathogenicity in different species. For instance, the presence of CRISPR limits acquisition of virulence genes in *Enterococcus faecalis* [46]; a connection of CRISPR elements with serotypes and virulence potential of *E. coli* strains has been established [47], and *A. baumannii* can more easily adapt to environmental conditions through acquisition of antibiotic resistance genes due to the presence of some CRISPR arrays [48]. These findings suggest that I-E type of *P. mirabilis* CRISPR-Cas system could be related to the expression regulation of virulence genes described for other microorganisms, in which Cas proteins enabled or increased their pathogenicity [47, 49].

All the findings above demonstrate strong dissemination potential of *P. mirabilis* clones and their AMR genes. Furthermore, despite the relatively low number of samples collected, these isolates can be assumed to represent the nosocomial clones based on the original metadata and WGS analysis.

5. Conclusions

In this study, 12 MDR clinical *P. mirabilis* isolates were characterized using the second- and third generation WGS. Genomic and phenotypic analysis of the isolates revealed the following key points:

- Two clones (C1 and C2) of *P. mirabilis* isolated from the same specimen (wounds) simultaneously occurred within the same hospital department
- Both clones included Salmonella genomic island (SGI) 1, but multiple antibiotic resistance (MAR) regions of these islands were different between C1 and C2, with C2 having slightly smaller number of AMR genes
- Neither of the isolates was revealed to possess plasmids; at the same time, the presence of IS26 element within the genomes could indicate the possible previous plasmid-mediated SGI insertion event, by which the AMR genes were acquired
- Only C2 possessed an apparently functional CRISPR-Cas system of I-E type

The simultaneous existence of two clones and the presence of MGEs carrying multiple resistance genes, as well as rather diverse set of virulence factors carried by all 12 isolates studied, highlights their pathogenic potential and poses a requirement of proper spreading monitoring. The data presented will facilitate the understanding of AMR transfer within clinical *P. mirabilis* isolates and will contribute to the epidemiological surveillance of this pathogen.

CRediT authorship contribution statement

Anna Karpenko: Writing – original draft, Resources, Investigation, Visualization. **Andrey Shelenkov:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Lyudmila Petrova: Validation, Resources, Methodology, Investigation. **Vitaly Gusarov:** Supervision, Formal analysis, Conceptualization, Methodology. **Mikhail Zamyatin:** Validation, Resources, Investigation, Formal analysis, Methodology, Project administration. **Yulia Mikhaylova:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Vasiliy Akimkin:** Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization.

Data availability statement

Genome assembly was uploaded to NCBI Genbank under the project number PRJNA1145476.

Ethics and consent

Review and/or approval by an ethics committee was not needed for this study due to the regulations provided by the Order of the Ministry of Health of the Russian Federation dated April 1, 2016 №200n "On approval of the rules of good clinical practice" since the samples were routinely collected from the patients for the purpose of antibiotic therapy prescription, no additional samples and/or interventions were made exclusively for this study, and the bacterial isolates processing was performed retrospectively without referencing the subjects from which they were taken.

All patients provided written informed consent for their anonymized data to be published. The study was conducted in accordance with the Declaration of Helsinki.

Funding statement

This work was supported by a grant from the Ministry of Science and Higher Education of the Russian Federation (agreement No. 075-15-2019-1666).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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