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Research article

Multidrug-resistant *Serratia marcescens* isolated from indoor air in a childcare facility: genomic insights into antibiotic resistance, virulence, and biofilm formation

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Abstract: Genomic data on multidrug (MDR) airborne bacteria from nonclinical settings in Tunisia, particularly childcare facilities, remain scarce. We aimed to characterize the genome of an MDR *Serratia marcescens* (*S. marcescens*) isolate recovered from indoor air and assess its relevance for public health surveillance.

Phenotypic characterization included antibiotic susceptibility testing (disc diffusion and broth microdilution) and quantification of biofilm formation. The underlying genetic basis was investigated via whole-genome sequencing (WGS), followed by bioinformatic analysis to characterize the resistome, virulome, and plasmid content. The *S. marcescens* strain EA1 exhibited an MDR phenotype,

with resistance to 10 of the 23 tested antibiotics, including amoxicillin-clavulanic acid. Phenotypically, it displayed a strong biofilm-forming capacity and α-hemolytic activity. *In silico* resistome analysis supported the phenotypic profile by revealing antibiotic resistance determinants such as the AmpC β-lactamase gene (*bla*SRT-2) and efflux systems belonging to all four major families (ABC, MFS, RND, and SMR). The detection of genes associated with disinfectant tolerance (e.g., *qacG*) indicates the presence of genetic determinants that, if expressed, could contribute to persistence under hygiene-related pressures; however, functional studies are needed to confirm this phenotype. Additionally, genomic islands characterized by insertion sequences and transposons were detected, suggesting a high potential for horizontal gene transfer. These elements were also present on the single pSM22 plasmid, highlighting a highly mobile and adaptable genome.

In this study, we report the first characterization of an MDR airborne *S. marcescens* strain (EA1) within the framework of environmental surveillance of indoor air quality in a childcare facility in Tunisia. These findings highlight the presence of genetic determinants associated with antimicrobial resistance, virulence, and disinfectant tolerance. Based on a single sentinel isolate, larger-scale sampling is required to assess generalizability.

Keywords: *Serratia marcescens*; MDR; airborne pathogens; childcare facility; WGS

1. Introduction

The genus *Serratia*, a group of gram-negative bacteria within the *Enterobacteriaceae* family, represents a significant microbial constituent of diverse ecosystems. While *Serratia marcescens* is frequently documented in clinical infections [1], its presence is increasingly recognized in the broader environmental context. As an opportunistic pathogen, it is a known agent of healthcare-associated infections (HAIs), particularly among neonates and patients in intensive care units (ICUs) [2,3]. However, the organism's clinical impact is underpinned by its ecological resilience across niches, including water, soil, air, and biological hosts [1]. This environmental versatility, coupled with expanding resistance profiles, positions *S. marcescens* as a significant subject of public health surveillance beyond the hospital setting [4].

Antibiotic resistance genes (ARGs) are fundamental components of the *Serratia* genome. Environmental strains represent a frequently overlooked pool of resistance determinants [5]. Interestingly, even though clinical isolates constitute more than three-quarters (76.5%) of the sequenced *Serratia* genomes, the total number of ARGs is remarkably similar between clinical and environmental strains [5].

S. marcescens has three major resistance mechanisms: acquired, adaptive, and intrinsic [1]. Acquired resistance typically occurs via horizontal gene transfer (HGT). Adaptive resistance develops under environmental pressures, leading to modified cell permeability and increased biofilm production [1,5,6]. Intrinsic resistance is mediated by chromosomal determinants, including efflux pumps, β-lactamases, and LPS modifications. The synergy between intrinsic and acquired resistance frequently results in MDR phenotypes, limiting therapeutic options and facilitating ARG dissemination via HGT [1,4,7]. Genomic investigations have clarified many of the genetic drivers of

this resistance [4,5,7], and the full functional range of efflux systems in *S. marcescens* is being established. Six of eight putative RND-type pumps have been associated with MDR phenotypes [8]. These proteins enable the extrusion of various antimicrobial agents from the cell [8]. Furthermore, resistance to β-lactams is driven primarily by enzymes such as AmpC-type cephalosporinases, extended-spectrum β-lactamases (ESBLs), and carbapenemases [1,7]. In environmental and clinical strains, the formation of biofilms serves as a physical defense that further limits antibiotic efficacy [6].

National studies have largely prioritized isolates from hospital environments [9,10]. Consequently, the role of nonclinical reservoirs, such as high-contact childcare settings, as potential facilitators of the persistence of antibiotic resistance remains underresearched [9,10]. WGS has been underutilized in these settings, despite its ability to characterize environmental isolates accurately [3–5].

In the context of indoor air quality surveillance, WGS was used to characterize the resistome and virulome of an MDR *S. marcescens* isolate from a Tunisian childcare facility, with genomic findings integrated with phenotypic antimicrobial susceptibility and quantitative biofilm formation assays.

2. Materials and methods

2.1. Description of the site, physicochemical analyses, and isolation steps

This study was conducted in a school daycare facility in Korba, Tunisia during the summer period, with all sampling performed during active hours while children were present (occupied state) to capture the microbial profile under typical functional conditions. Environmental parameters, including temperature, relative humidity, and carbon dioxide (CO_2) concentrations, were monitored via a Q-TRAK 7575 analyzer, whereas formaldehyde (CH_2O) and particulate matter (PM_{10}) levels were recorded via a HAL-HFX205 meter and a DUSTTRAK II monitor, respectively. For microbiological and genomic analyses, a total of eight air samples (four indoor and four outdoor) were collected via a BK-BAS biocollector on tryptic soy agar (TSA) plates. To ensure a representative assessment of the breathing zone, the biocollector was positioned in the center of the rooms at a height of 1.5 meters above the floor, maintaining a minimum distance of 2 meters from doors and mechanical ventilation points to avoid the influence of localized drafts. Each sample consisted of 1000 liters of air collected over a 10-minute duration. Following collection, the TSA plates were incubated at 30°C for 3 days; all resulting bacterial isolates were then harvested and preserved in 30% (v/v) glycerol for long-term storage. The preserved isolates were subsequently cultured in TSA supplemented with augmentin and amphotericin B and incubated at 30°C for 24 hours to selectively enrich for β-lactamase-producing bacteria while preventing fungal contamination.

2.2. Antimicrobial susceptibility testing and screening for ESBL production

2.1.1. Disc diffusion method

The antimicrobial susceptibility of the *S. marcescens* EA1 isolate was determined via the disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute [11]. The following antibiotics (Oxoid) were used (μg/disk): nalidixic acid (30 μg), ciprofloxacin (5 μg),

ofloxacin (5 μg), chloramphenicol (30 μg), cefoxitin (30 μg), ertapenem (10 μg), imipenem (10 μg), meropenem (10 μg), gentamycin (10 μg), amikacin (30 μg), vancomycin (30 μg), teicoplanin (30 μg), tetracyclines (30 μg), streptomycin (10 μg), erythromycin (E, 15 μg), penicillin (10 μg), cephalothin (30 μg), fosfomycin (200 μg), and colistin (30 μg). The isolate was defined as MDR if it exhibited resistance to at least one agent belonging to three or more antimicrobial classes [12]. A double-disk synergy test (DDST) with cefotaxime (30 μg), ceftazidime (30 μg), aztreonam (30 μg), and cefepime (30 μg) in proximity to amoxicillin-clavulanic acid (20/10 μg) was used for screening ESBL production [11].

2.1.2. Minimal inhibitory concentration

The minimum inhibitory concentrations (MICs) of the selected antibiotics against *S. marcescens* EA1 were determined via the broth microdilution method in accordance with CLSI guidelines [11]. Antibiotic stock solutions of cefotaxime, colistin, tetracycline, ampicillin, amoxicillin, penicillin G, streptomycin, trimethoprim, and sulfamethoxazole (Sigma–Aldrich, St. Louis, MO, USA) were prepared in distilled water at a concentration of 1024 μg/mL. Serial twofold working dilutions were subsequently performed in cation-adjusted Mueller–Hinton broth (CAMHB; Merck, Darmstadt, Germany) in sterile 96-well microtiter plates (Sigma–Aldrich, St. Louis, MO, USA) to yield final antibiotic concentrations ranging from 0.125 to 512 μg/mL.

A single colony of *S. marcescens* EA1 grown overnight on TSA was inoculated into CAMHB and incubated at 35 ± 2°C until it reached a turbidity equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The suspension was subsequently diluted 1:150 in CAMHB to obtain a working inoculum of approximately 1×10^6 CFU/mL. Aliquots of 100 μL of this suspension were then added to each well containing 100 μL of antibiotic dilution, resulting in a final inoculum density of approximately 5×10^5 CFU/mL per well and a total well volume of 200 μL, which is consistent with CLSI specifications. The accuracy of the inoculum was verified by counting viable plates on a subset of wells before incubation.

Growth controls (CAMHB inoculated with bacteria but without antibiotics) and sterility controls (uninoculated CAMHB only) were included on each plate to confirm normal bacterial growth and the absence of contamination, respectively. The plates were sealed and incubated at 35 ± 2°C for 16–20 h under ambient atmospheric conditions without agitation.

The MIC was defined as the lowest antibiotic concentration that completely inhibited visible bacterial growth, as determined by the absence of turbidity upon visual inspection. Breakpoints for susceptibility interpretation were applied in accordance with CLSI M100 performance standards. All the assays were performed in triplicate across three independent experiments conducted on separate days. The MIC was reported as the mean of the three determinations.

2.3. Hemolytic assay

The hemolytic assay was performed by spotting an overnight culture adjusted to 0.5 McFarland standard over the surface of sheep blood agar plates as described in the study by Hebert G et al. [13]. *S. aureus* ATCC 25923 was used as a positive control.

2.4. Biofilm assay

2.4.1 Congo red agar

Slime production ability was assessed on Congo red agar (CRA) according to the method of Freeman et al. 1989 [14]. The CRA medium was composed of brain heart infusion (BHI) agar (47 g/L), sucrose (50 g/L), and Congo red dye (0.8 g/L). Congo red solution was autoclaved separately at 121°C for 15 min and added to the melted agar before it was cooled to 45–50°C. The EA1 strain was inoculated into CRA media, and the plates (triplicates) were placed in a 37°C incubator for 2 days. After incubation, the slime-positive strains appeared as black colonies with dry crystalline consistency, and the slime-negative strains remained red. *Staphylococcus epidermidis* RP62A was used as a positive control.

2.4.2. Crystal violet

The isolates were tested for biofilm formation in 96-well microtiter plates (Bio-Rad), as described in the study by Stepanović S et al. [15]. An O/N culture was diluted (1:100) in tryptic soy broth (TSB) containing 1% glucose and inoculated onto microtiter plates at 37°C for 18 h without aeration. The free-floating planktonic bacteria were removed, washed, dried for 60 min at 60°C, and stained with 0.06% crystal violet.

Biofilms were quantified after the addition of 30% acetic acid via a microtiter plate reader (OD 570 nm) (Bio-Rad). Each isolate was tested in triplicate, and each assay was performed in duplicate. *S. epidermidis* RP62A and ATCC12228 were used as positive and negative controls, respectively. The isolates were classified as strong biofilm producers: $4 \times ODc < OD$; moderate biofilm producers: $2 \times ODc < OD \leq 4 \times ODc$; weak biofilm producers: $ODc < OD < 2 \times ODc$; and no biofilm producers: $OD \leq ODc$. The cutoff value (ODc) is defined as three standard deviations (SDs) above the mean OD of the negative control (TSB plus 1% glucose without bacterial cells) [15].

2.5. DNA extraction, library preparation, genome sequencing, assembly, and annotation

Genomic DNA was extracted via the GeneJET Genomic DNA Purification Kit (Thermo Scientific), and the quality and quantity of the extracted DNA were verified via the Qubit dsDNA HS Assay Kit (Invitrogen) and the NanoDrop 2000c UV–Vis Spectrophotometer (Thermo Scientific, USA). Library preparation was conducted via the Illumina DNA Flex Prep Kit following the manufacturer's protocols. The final library was denatured with NaOH and sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) utilizing 2 × 250 paired-end reads. Sequence reads were assembled through the Galaxy platform via Shovill version 1.1.0, incorporating an optional adaptor-trimming step *via* Trimmomatic version 0.39 and leveraging the SPAdes genome assembler within an optimized pipeline for smaller genomes (https://galaxyproject.org). Genome annotation was performed via the RAST server [16] and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [17]. The BioProject accession number is PRJNA1059522 (BioSample SAMN39208621), and the Whole Genome Shotgun project is deposited at DDBJ/ENA/GenBank under the accession number JAYJMW000000000.

2.6. In silico analysis

For whole-genome taxonomic analysis, genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS) [18]. A genomic map was generated. Antimicrobial resistance determinants were identified via the CARD Resistance Gene Identifier tool *via* Proksee [19] and ResFinder v.3.2 (https://cge.cbs.dtu.dk/services/ResFinder/) from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). PathogenFinder (https://cge.food.dtu.dk/services/PathogenFinder/) was used to predict the pathogenicity of the EA1 strain. The plasmid content was analyzed via PlasmidFinder v.2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/). Mobile genetic elements and restriction-modification sites were predicted *via* MobileElementFinder (https://cge.food.dtu.dk/services/MobileElementFinder/) and Restriction-Modification Finder (https://crisprcas.i2bc.paris-saclay.fr/). Genome annotation was performed via the Rapid Annotations using the Subsystems Technology (RAST) server [20]. The pSM22 genomic region, including the *rep*A and *tra* genes (e.g., *tra*C, *tra*D, *tra*G, *tra*N, *tra*I), was annotated via RAST and then aligned and compared with the *S. marcescens* strain 14VA4 plasmid p14VA4_1 (AP028481.1) and *S. marcescens* strain ET_W2 plasmid pET_W2 (AP028600.1) via MAUVE. The plasmid region alignments were generated with Easyfig v2.2.5 [21] at an 80% minimum sequence identity.

Furthermore, the genome of the *S. marcescens* strain EA1 was uploaded to IslandViewer4 [22], with the complete genome of

S. marcescens FGI94 used as a reference. IslandViewer4 uses four genomic island prediction methods (IslandPick, IslandPath-DIMOB, SIGI-HMM, and Islander) to identify genomic islands [22].

3. Results

3.1. Physicochemical parameters

The environmental conditions in the studied room included a mean temperature of 27.3°C, a relative humidity of 79.8%, and a carbon dioxide concentration of 533.1 ppm. Airborne formaldehyde and PM_{10} levels were measured at 88.9 μg/m³ and 99.78 μg/m³, respectively.

3.2. Bacterial isolation and identification

A total of 176 bacterial isolates were initially recovered on tryptic soy agar (TSA). Among them, a single pigmented isolate was selected on TSA supplemented with amoxicillin-clavulanic acid. Amoxicillin–clavulanic acid (augmentin) remains a primary pediatric therapeutic in Tunisia; resistance to this combination in environmental isolates from childcare settings is poorly understood. Because of its resistance to augmentin, the EA1 isolate was designated a sentinel strain for indoor air resistance determinants. Consequently, this isolate was prioritized for comprehensive phenotypic and genomic characterization to elucidate its resistome and virulome. Species identification via the TYGS platform confirmed that strain EA1 belongs to the species *S. marcescens*. Phylogenomic analysis revealed that EA1 clusters with *Serratia nematodiphila* DSM 21420 [23] and *S. marcescens* subsp. *sakuensis* KCTC 42172 (strain KRED^T) [24], which is consistent with previous reports [25].

3.3. Genomic characterization

The genome of strain EA1 is 5,564,637 bp in size, with a GC content of 59.1%. The assembled genome comprises 107 contigs, with an N50 value of 295520 bp. Moreover, the genome of strain EA1 contains 5611 coding sequences (CDSs), 318 subsystems, and 90 RNAs.

3.4. The S. marcescens EA1 strain is multidrug-resistant

The *S. marcescens* EA1 strain displayed an MDR profile, exhibiting resistance to 10 of the 23 antibiotics tested. This profile included resistance to amoxicillin-clavulanic acid, cephalothin, colistin, erythromycin, fosfomycin, penicillin, streptomycin, teicoplanin, tetracycline, and vancomycin (Figure 1).

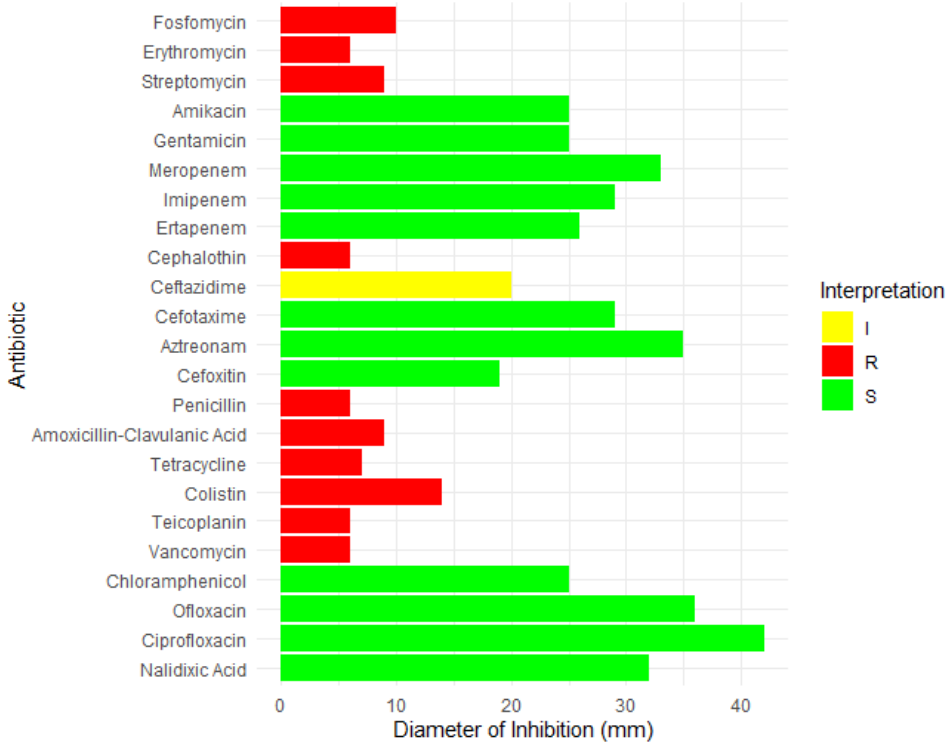

Figure 1. Antibiotic susceptibility testing and inhibition zone diameters of strain EA1. S: Susceptible. I: Intermediate. R: Resistant.

A negative double-disk synergy test (DDST) excluded classic ESBL-mediated cephalosporin resistance. Broth microdilution assays confirmed that EA1 was resistant to amoxicillin (MIC=128 μg/mL), penicillin G (MIC=256 μg/mL), colistin (MIC=512 μg/mL), tetracycline (MIC=32 μg/mL), streptomycin (MIC=128 μg/mL), and sulfamethoxazole (MIC=512 μg/mL), whereas the isolate remained susceptible to cefotaxime (MIC=1 μg/mL) and trimethoprim (MIC=4 μg/mL) (Table 1).

Table 1. Antimicrobial susceptibility of the *S. marcescens* EA1 isolate.

Antimicrobial Class	Antimicrobial Agents	Mean of MIC (μg/mL)	Interpretation
β-lactams	Cefotaxime	1	S
	Amoxicillin	128	R
	Penicillin G	512	R
Polymyxins	Colistin	512	R
Tetracyclines	Tetracycline	32	R
Aminoglycosides	Streptomycin	128	R
Diaminopyrimidines	Trimethoprim	4	S
Sulfamides	Sulfamethoxazole	512	R

In silico analysis revealed genes conferring resistance to various antibiotic classes (Figure 2. Table 2), including aminoglycosides (*AAC(6')-Ic*, *kpnH*), beta-lactams (*PBP3*), cephalosporins (*SRT-2*, *kpnH*), diaminopyrimidines and phenicols (*rsm*A), fluoroquinolones (*CRP, emrR, adeF, glpT, rsmA*), fosfomycin (*glp*T), glycopeptides (*van*G), macrolides (*crp*, *kpnH*), penam (*crp*), peptides (*kpnH*), rifamycin (*kpnH*), and tetracyclines (*tet(41), adeF, kpnH*). ResFinder confirmed the presence of $bla_{SST\text{-}1/SRT\text{-}2}$, *tet*(41), and *AAC(6')-Ic* genes, corresponding to cephalosporin, tetracycline, and aminoglycoside resistance, respectively.

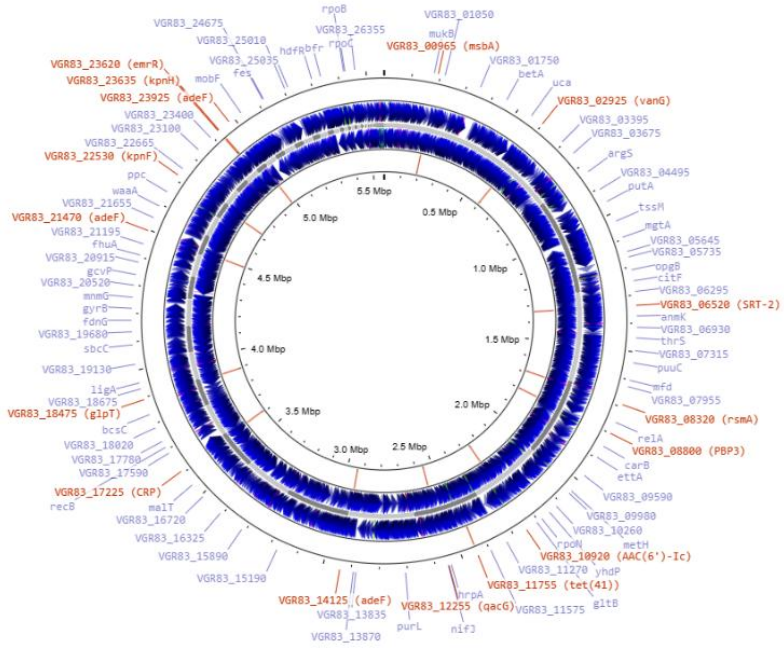

Figure 2. Whole-genome map of strain EA1. Comprehensive Antibiotic Resistance Database (CARD) resistance gene identifier results are indicated in red.

Table 2. Comprehensive antibiotic resistance database (CARD) resistance gene identifier results.

Locus tag	Best Hit ARO	Drug Class	Resistance Mechanism	AMR Gene Family
VGR83_00965	MsbA	Nitroimidazole antibiotic	Antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump
VGR83_02925	VanG	Glycopeptide antibiotic	Antibiotic target alteration	Glycopeptide resistance gene cluster; Van ligase
VGR83_06520	SRT-2	Cephalosporin	Antibiotic inactivation	SRT beta-lactamase
VGR83_08320	RsmA	Fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
VGR83_08800	*Haemophilus influenzae* PBP3 conferring resistance to beta-lactam antibiotics.	Cephalosporin; cephamycin; penam	Antibiotic target alteration	Penicillin-binding protein mutations conferring resistance to beta-lactam antibiotics
VGR83_10920	AAC(6')-Ic	Aminoglycoside antibiotic	Antibiotic inactivation	AAC(6')
VGR83_11755	Tet(41)	Tetracycline antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
VGR83_12255	QacG	Disinfecting agents and antiseptics	Antibiotic efflux	Small multidrug resistance (SMR) antibiotic efflux pump
VGR83_14125 VGR83_21470 VGR83_23925	AdeF	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
VGR83_17225	CRP	Macrolide antibiotic; fluoroquinolone antibiotic; penam	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
Locus tag	Best Hit ARO	Drug Class	Resistance Mechanism	AMR Gene Family
VGR83_18475	*Escherichia coli* GlpT with a mutation conferring resistance to fosfomycin	Phosphonic acid antibiotic	Antibiotic target alteration	Antibiotic-resistant GlpT
VGR83_21470	AdeF	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
VGR83_22530	*Klebsiella pneumoniae* KpnF	Macrolide antibiotic; aminoglycoside antibiotic; cephalosporin; tetracycline antibiotic; peptide antibiotic; rifamycin antibiotic; disinfecting agents and antiseptics	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
VGR83_23620	EmrR	Fluoroquinolone antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
VGR83_23635	*Klebsiella pneumoniae* KpnH	Macrolide antibiotic; fluoroquinolone antibiotic; aminoglycoside antibiotic; carbapenem; cephalosporin; penam; peptide antibiotic; penem	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
VGR83_23925	AdeF	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump

RAST-based subsystem analysis assigned 73% of the EA1 coding sequences (CDSs) to known functional subsystems, whereas 27% remained unclassified, reflecting the organism's genomic complexity and the presence of novel or poorly characterized genes not yet represented in the RAST subsystem database (Figure 3). RAST annotation identified the *ept*A (Lipid A phosphoethanolamine transferase) and *pmr*K (undecaprenyl phosphate-alpha-4-amino-4-deoxy-L-arabinose arabinosyl transferase) genes. Additionally, the *msbA, qacG*, and *kpnH* genes related to resistance to nitroimidazoles, disinfectants, and antiseptics were detected.

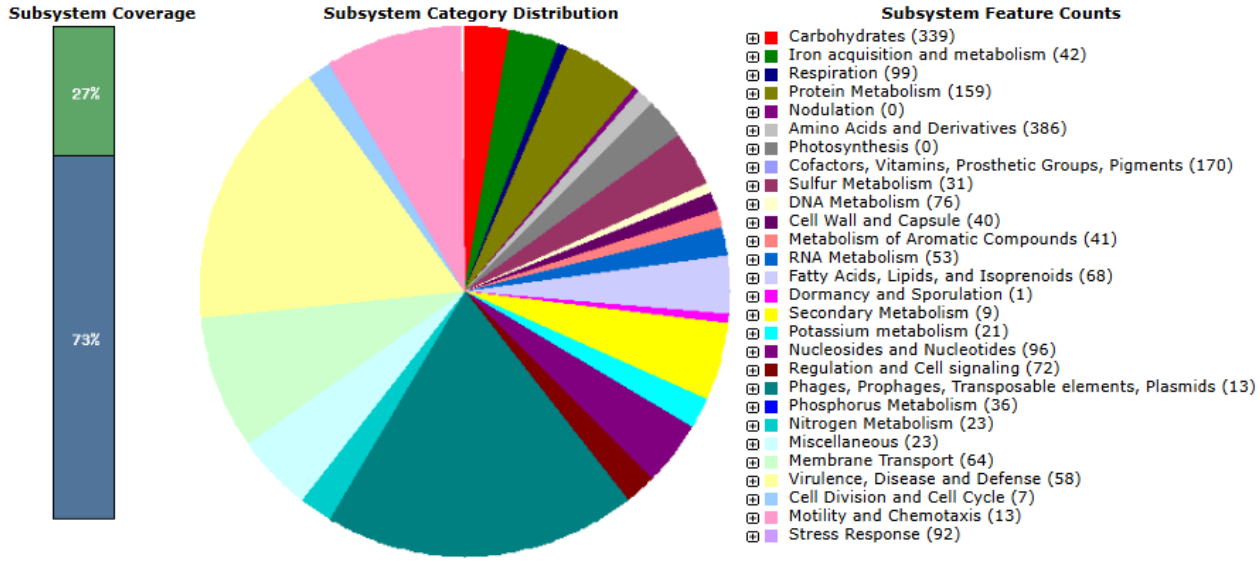

Figure 3. Subsystem statistical information on the genome of the *S. marcescens* strain EA1 obtained via RAST annotation. The subsystem categories and corresponding counts are presented in the legend.

3.5. The S. marcescens EA1 strain carries efflux systems

In addition to ARGs, the genome of the *S. marcescens* EA1 strain contains genes encoding potential efflux systems from four of the six major families, ABC, MFS, RND, and SMR, as identified via CARD (Table 2).

3.6. The S. marcescens EA1 strain has pathogenic potential

The EA1 strain exhibited α-hemolysis on sheep blood agar and strong slime and biofilm production (Congo Red Agar, microtiter plate). RAST identified cellular adhesion genes, including *fimH*, *fimD*, *fimC*, *fimI*, and *fimF*, as well as the adhesin-encoding gene *PGA*. Pathogenic genes such as *rsm*A (repressor of secondary metabolites) and *CRP* were present. PathogenFinder predicted that the EA1 strain is a potential human pathogen with a 73.7% probability. Restriction-ModificationFinder-1.1 detected two Type II methyltransferase genes (M1. Cko10770II, M2. Cko10770II) but no genes for Type I, III, or IV restriction enzymes were identified.

3.7. The S. marcescens EA1 strain carries the pSM22 plasmid

The PlasmidFinder-2.0 tool (https://cge.food.dtu.dk/services/PlasmidFinder-2.0/) identified a single plasmid, pSM22, within contig 25 of the *S. marcescens* EA1 strain, with 95.36% identity. However, the *bla*$_{SRT2}$ gene encoding AmpC β-lactamases was found in contig 03, suggesting that the AmpC enzyme is chromosomally encoded in our strain. The sequence of the 51,431 bp pSM22 *Inc*F plasmid found in the *S. marcescens* EA1 strain included genes encoding conjugative transfer pilus assembly proteins such as *TraC*, *TraD*, *TraG*, *TraH*, *TraI*, *TraK*, and *TraN*, which are essential for plasmid transfer. These genes are located alongside the replication initiator gene *repA*, as well as the plasmid partitioning *par*A gene, an insertion sequence (*IS91*), and a gene encoding a DNA methyltransferase protein (Figure 4). The plasmid has a GC content of 56.4% and harbors 61 protein-coding genes. Sequence analysis via the National Center for Biotechnology Information database revealed that the pSM22 plasmid found in the *S. marcescens* EA1 strain presented the highest similarity with the p14VA4_1 plasmid of the *S. marcescens* strain 14VA4 (97.21% nucleotide identity and 80% query coverage, accession number: AP028481.1) and the pET_W2 plasmid of *S. marcescens* strain ET_W2 (95.70% nucleotide identity and 70% query coverage, accession number: AP028600.1), which were isolated from humans and soil, respectively. Additionally, mobile genetic elements, including IS91 and the transposase STM474_p1058, were identified in the plasmid of the *S. marcescens* EA1 strain (Figure 4).

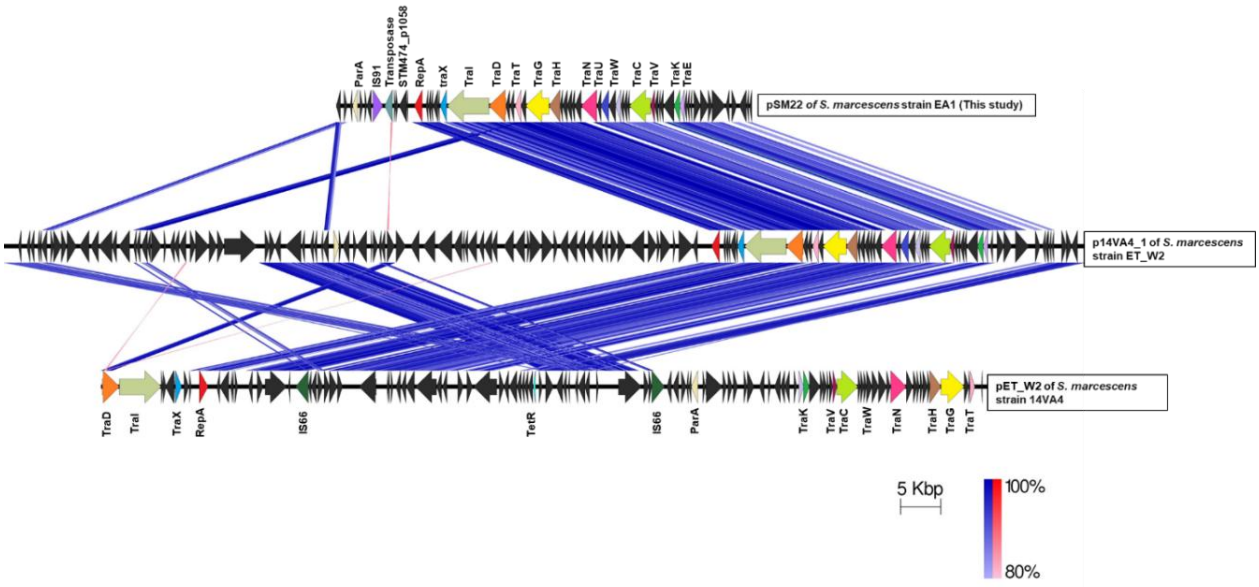

Figure 4. Linear schematic comparison of the pSM22 plasmid with the p14VA4_1 plasmid of the S. marcescens strain 14VA4 (accession number: AP028481.1) and the pET_W2 plasmid of S. marcescens strain ET_W2 (accession number: AP028600.1). The selected key genes are highlighted and labeled. The areas in blue represent regions conserved between sequences, and homologous coding DNA sequences are marked in the same color, with the percentage identity key shown on the bottom right. Areas in red denote inverted sequence alignment.

3.8. The S. marcescens strain EA1 harbors genomic islands

Using the IslandViewer4 tool, a comparison between the genome of *S. marcescens* strain EA1 and the complete reference genome of *S. marcescens* FGI94 led to the identification of 52 genomic islands (GIs) (Figure 5). Within these GIs, a variety of genes encoding mobile genetic elements were found. These included genes for multiple families of transposases, such as the *IS21* family transposase, *IS3* family transposase, and *IS91* family transposase. The islands also carried elements associated with transposition, including the *IS21*-like element helper ATPase IstB, the *IS66*-like element accessory protein TnpA, and the *IS66* family insertion sequence element accessory protein TnpB and its transposase. This analysis also revealed genes encoding the Rpn family recombination-promoting nuclease/putative transposase, an integrase arm-type DNA-binding domain-containing protein, and a DDE-type integrase/transposase/recombinase. These islands also harbor genes encoding TetR/AcrR family transcriptional regulators, alongside other functionally relevant genes.

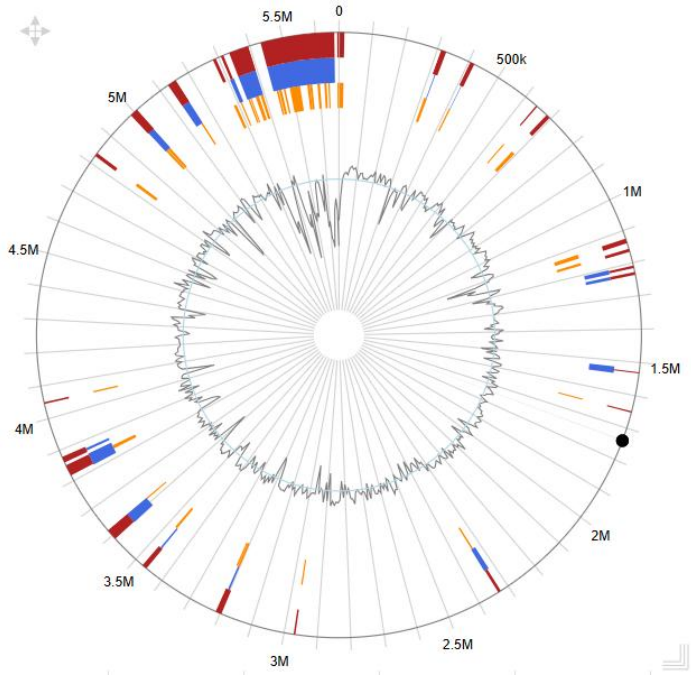

Figure 5. Circular plot of the genomic islands identified from the S. marcescens strain EA1 genome aligned against the reference genome Serratia marcescens FGI94 via IslandViewer 4. The black outer circle represents the scale line of the genome in Mbps, and the identified genomic islands are represented in three colors: red (integrated detection), blue (IslandPath-DIMOB), and orange (SIGI-H). The center circle represents the G+C content (%).

4. Discussion

The physicochemical characteristics of the childcare facility's indoor air, including elevated CO_2 and PM_{10} concentrations, provide an environmental context in which *S. marcescens* EA1 was isolated. The measured CO_2 concentration (533.1 ppm) was close to ambient levels. Whether such modest

elevations influence HGT under real-world indoor conditions requires direct experimental testing, which was beyond our scope for this study.

The close phylogenetic relationship between *S. marcescens* EA1 and the insect-associated *S. nematodiphila* supports an environmental origin. Its detection in daycare air samples reflects its environmental presence rather than confirmed airborne transmission or respiratory exposure. Possible introduction pathways described for *Serratia* species, such as wind-borne soil dust or passive transfer via insects, were not investigated in this study. Moreover, air sampling reveals the presence of viable bacteria in the indoor environment but does not provide information on exposure dose, colonization, or infection risk. Therefore, these findings indicate a widespread environmental presence rather than effective airborne dissemination.

Once indoors, airborne bacteria are associated with settled dust and surfaces, as reported in other indoor microbiome studies [26,27]. These findings underscore the dynamic interface between environmental and indoor microbiomes, highlighting the potential for environmental pathogens to colonize indoor environments.

Phenotypic characterization via disc diffusion revealed resistance to 10 antimicrobial agents across six classes, whereas broth microdilution confirmed resistance to six of the eight antibiotics tested. This intermethod is a recognized phenomenon in antimicrobial susceptibility testing and reflects inherent differences in the physicochemical conditions of each assay format, including antibiotic diffusion properties in agar, inoculum density effects, and breakpoint-specific thresholds. Notably, resistance to colistin and tetracycline was confirmed by both methods, which is consistent with the well-documented intrinsic and acquired resistance mechanisms of *S. marcescens* to these drug classes [1,4,28]. For example, in a prior study, the strain KREDT was resistant to benzylpenicillin, erythromycin, lincomycin, polymyxin, and tetracycline but was susceptible to ampicillin, carbenicillin, chloramphenicol, gentamicin, kanamycin, and streptomycin [24].

This report represents the first identification of an MDR *S. marcescens* isolate from air in Tunisia, a significant finding given the WHO's emphasis on cephalosporin-resistant *Enterobacteriaceae* [29]. These results align with other reports of *bla*SRT-2-carrying *S. marcescens* strains, including one from Chile [7] and a clinical isolate from China coproducing *bla*SRT-2 and *bla*IMP-4 [30]. Furthermore, our results reflect those of Gaultier et al. [31], who isolated *S. marcescens* SGAir0764 from air samples in Singapore and identified 112 genes associated with virulence, disease, and defense, 87 of which were potentially linked to resistance to antibiotics and toxic compounds.

The genome of the *S. marcescens* strain EA1 harbors the *eptA* and *pmrK* genes, which are involved in lipid A modification pathways associated with polymyxin resistance. These genes encode enzymes responsible for the addition of phosphoethanolamine (*pEtN*) and 4-deoxyaminoarabinose (L-Ara4N) to lipopolysaccharide (LPS), resulting in reduced binding affinity of polymyxins to the bacterial outer membrane [32]. Importantly, resistance to polymyxin B (including colistin) is a well-documented intrinsic characteristic of *S. marcescens* and the *Serratia* genus and therefore does not represent an acquired or emergent resistance trait in this species [1]. Consequently, the presence of these genes reflects the expected species-specific resistance rather than a novel or critical resistance threshold.

The MDR pattern of EA1 is supported by genomic predictions identifying genes encoding resistance proteins, including MdtG, MdtH, ErmA, Bcr/CflA, and SMR, with direct public health

relevance given their activity against antibiotics typically effective against *Serratia*. However, their contribution to resistance in EA1 is inferred from genomic predictions, as gene expression levels and functional activity were not experimentally assessed in this study.

Efflux pump genes from four major families (ABC, MFS, RND, and SMR) were identified in strain EA1, highlighting its genetic adaptability. This finding aligns with those of other studies emphasizing the genetic plasticity of *S. marcescens* [4,5,33]. The ABC superfamily is known to protect *S. marcescens* from polymyxins and aminoglycosides, while the SMR-type pump SsmE and the MFS-type pump SmfY are associated with fluoroquinolone protection [1].

The RAST service identified several genes associated with cellular adhesion, including fimH, which governs type 1 fimbriae length and mannose-binding adhesion. Other related genes, such as fimD, fimC, fimI, fimF, and the Type-1 fimbrial protein A chain, collectively regulate fimbrial adhesion and length [34]. Genes encoding adhesins, such as PGA (poly-β-1,6-N-acetyl-D-glucosamine), were also detected. PGA is critical for biofilm structural integrity, facilitating the transition from temporary polar to permanent lateral attachment during early biofilm formation. It also influences host–microbe interactions, enhancing colonization, virulence, and immune evasion [35]. Our findings align with reports demonstrating the robust ability of *S. marcescens* to adhere to various surfaces, such as contact lenses and epithelial cells [36], a process facilitated by type 1 fimbriae, which are key virulence determinants [37].

Several genes in the EA1 strain are associated with pathogenicity and adaptive potential. The rsmA gene (a repressor of secondary metabolites) regulates swarming motility [38], whereas the CRP gene controls multiple virulence-related functions [3]. In addition, EA1 harbors *msbA*, *qacG*, and *kpnH* genes, which have been linked to tolerance or reduced susceptibility to nitroimidazoles, disinfectants, and antiseptics. Disinfectant susceptibility testing was not performed in this study; therefore, the phenotypic impact of *qacG* and related genes remains unknown.

Our strain also harbors a type II restriction-modification (R-M) system, which consists of a restriction enzyme and a methyltransferase. These systems provide a bacterial defense mechanism against bacteriophage infections [39] and regulate the cell cycle, gene expression, and virulence in response to the environment [40]. The *S. marcescens* EA1 strain exhibited α-hemolytic activity on sheep blood agar, indicating its ability to partially lyse red blood cells. While generally considered less virulent than β-hemolysis, it still reflects the strain's potential to cause tissue damage and evade host immune defenses. This hemolytic activity, combined with the strain's strong biofilm-forming capacity and multidrug resistance profile, underscores its pathogenic potential and ability to persist in challenging environments such as childcare facilities.

Researchers have identified the *bla*SRT gene, encoding AmpC β-lactamases, in various *S. marcescens* plasmid types, including *Inc*X3 and *Inc*L/M incompatibility groups, indicating acquisition *via* conjugation [7,41]. In contrast, the *bla*SRT2 gene in *S. marcescens* EA1 is not on the pSM22 plasmid, and no additional resistance genes or plasmids were identified in the genome. This genomic organization supports the chromosomal localization of the AmpC β-lactamases encoding gene in strain EA1. Alternatively, the gene may have been acquired via natural transformation or phage-mediated transduction, although this cannot be confirmed from genomic data alone. Likewise, the resistance determinants identified *in silico* provide a genetic basis that is consistent with the observed phenotype. The presence of insertion sequences such as *IS401* and elements from the *IS3/IS911* family, previously

implicated in plasmid rearrangements in *Pseudomonas cepacian* [42], underscores the genetic plasticity of this isolate and its potential to acquire and disseminate antibiotic resistance *via* HGT [30].

MDR bacteria frequently harbor resistance islands as a key strategy to increase their survival under antimicrobial pressure [43]. The remarkable flexibility of the *Serratia* genome underscores its ability to acquire mobile genetic elements (MGEs), which contribute to the reduced antibiotic susceptibility commonly observed in this genus [3]. Comparable observations have been noted in *Serratia* sp. HRI, where the genome has accumulated a diverse set of fitness-enhancing determinants over time, bolstering its resilience against a broad spectrum of antimicrobial agents [44].

S. marcescens EA1 contains numerous GIs and an array of mobile elements, including transposases and integrases, highlighting its potential for HGT. GIs are recognized as primary vectors for HGT [45,46]. Integrases, which act as site-specific recombinases that catalyze the integration and excision of GIs, provide a mechanism for rapid genomic plasticity [47,48]. Additionally, the IS21 family of insertion sequences was identified. This class of transposable elements is frequently associated with the spread of resistance and virulence traits, as observed in MDR *Escherichia coli* and *Staphylococcus aureus*, and contributes to the evolution of pathogens such as *Yersinia pestis* [49,50]. Furthermore, the discovery of a gene encoding a TetR/AcrR family transcriptional regulator within GIs has critical implications for strain survival and pathogenicity. TetR/AcrR family proteins are global regulators involved in controlling a wide spectrum of cellular activities, including the stress response, antibiotic biosynthesis, and the expression of virulence genes [51]. However, their most prevalent role is the regulation of efflux pumps and other transporters, which confer antibiotic resistance and tolerance to various toxic chemical compounds [52–55].

Given that the original TetR protein in *E. coli* controls the expression of a tetracycline efflux pump [51], the presence of this regulator family suggests an increased capacity for multidrug resistance in *S. marcescens* EA1. These TetR-like regulators, defined by a conserved helix-turn-helix DNA-binding domain, are increasingly recognized as antimicrobial drug targets [51].

The detection of an MDR *S. marcescens* isolate in Tunisian daycare is consistent with growing evidence that high-occupancy indoor environments can serve as reservoirs of ARGs. Multiomics studies have shown that indoor microbial communities are shaped by human activity, surface materials, and ventilation, resulting in distinct resistome and virulome profiles across settings. In particular, Fu et al. [56] demonstrated that aircraft cabins and school classrooms harbor comparable levels of ARGs, whereas the abundance of virulence factors varies according to surface composition, with higher loads associated with textile materials. These findings underscore the importance of the environmental context, rather than clinical exposure alone, in structuring indoor microbial assemblages.

In this study, strain EA1 was recovered from indoor air, indicating the presence of viable opportunistic bacteria in the airborne compartment of a childcare facility. Moreover, while studies have emphasized surfaces as key reservoirs of virulence- and resistance-associated traits in high-contact environments, our findings show that airborne niches also harbor such bacteria. These observations support the inclusion of daycare environments in surveillance efforts targeting airborne and surface-associated antimicrobial resistance.

A primary limitation of this study is the characterization of a single isolate from a unique sampling site. Furthermore, the CO_2 concentration recorded in this study (533.1 ppm) was only marginally above outdoor ambient levels and should not be interpreted as evidence of enhanced HGT activity;

experimental validation of conjugative transfer rates under these conditions was beyond our scope of this study. Further studies with larger datasets are needed to evaluate associations between environmental factors and antimicrobial resistance patterns, as well as to determine whether the identified genetic determinants translate into reduced disinfectant efficacy under real-world conditions. In addition, the presence of undetected plasmids or resistance genes cannot be excluded, warranting further investigation.

5. Conclusions

In this study, we report the first genomic characterization of an MDR *S. marcescens* strain isolated from the indoor air of a childcare facility in Tunisia as part of an environmental surveillance effort. The strain EA1 exhibited resistance to ten antibiotics, and genomic analysis revealed a repertoire of ARGs, efflux systems, GIs, and virulence-associated determinants, reflecting a high degree of genetic plasticity and adaptability. While analyzing a single isolate is a significant limitation that precludes generalizable conclusions, this strain serves as a critical sentinel indicator. This highlights the resistance genes and mobile genetic elements (MGEs) capable of persisting in bioaerosols, suggesting that sporadic indoor air isolates can serve as sentinel indicators. However, broader conclusions about the prevalence or distribution of MDR *S. marcescens* in childcare facilities await multi-sample investigations. These results support the need for expanded environmental monitoring and systematic epidemiological investigations to characterize the distribution, evolution, and potential environmental burden of MDR *S. marcescens* and related airborne bacteria in childcare facilities in Tunisia and comparable indoor settings.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Tavares-Carreon F, De Anda-Mora K, Rojas-Barrera IC, et al. (2023) *Serratia marcescens* antibiotic resistance mechanisms of an opportunistic pathogen: a literature review. *PeerJ* 11: e14399. https://doi.org/10.7717/peerj.14399
2. Cristina ML, Sartini M, Spagnolo AM (2019) *Serratia marcescens* infections in neonatal intensive care units (NICUs). *Int J Environ Res public health* 16: 610. https://doi.org/10.3390/ijerph16040610
3. Piccirilli A, Cherubini S, Brisdelli F, et al. (2022) Molecular characterization by whole-genome sequencing clinical and environmental *Serratia marcescens* strains isolated during an outbreak in a neonatal intensive care unit (NICU). *Diagnostics* 12: 2180. https://doi.org/10.3390/diagnostics12092180
4. Iguchi A, Nagaya Y, Pradel E, et al. (2014) Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen. *Genome Biol Evol* 6: 2096–2110. https://doi.org/10.1093/gbe/evu160
5. Sandner-Miranda L, Vinuesa P, Cravioto A, et al. (2018) The genomic basis of intrinsic and acquired antibiotic resistance in the genus *Serratia. Front Microbiol* 9 :828. https://doi.org/10.3389/fmicb.2018.00828
6. Ray C, Shenoy AT, Orihuela CJ, et al. (2017) Killing of *Serratia marcescens* biofilms with chloramphenicol. *Ann Clin Microbiol Antimicrob* 16: 1–6. https://doi.org/10.1186/s12941-017-0192-2.
7. Quezada-Aguiluz M, Lincopan N, Cerdeira L, et al. (2020) Draft genome sequence of a multidrug-resistant KPC-2 and SRT-2 co-producing *Serratia marcescens* strain isolated from a hospitalised patient in Chile. *J Glob Antimicrob Resist* 21: 1–2. https://doi.org/10.1016/j.jgar.2020.02.004.
8. Du D, Wang-Kan X, Neuberger A, et al. (2018) Multidrug efflux pumps: structure, function and regulation. *Nat Rev Microbiol* 16: 523–539. https://doi.org/10.1038/s41579-018-0048-6
9. Messaoudi A, Mansour W, Tilouche L, et al. (2021) First report of carbapenemase OXA-181-producing *Serratia marcescens. J Glob Antimicrob Resist* 26: 205–206. https://doi.org/10.1016/j.jgar.2021.06.004
10. Ktari S, Mahjoubi F, Mnif B, et al. (2010) Investigation de trois épidémies d'infections nosocomialesa *Serratia marcescens* survenues dans le service de réanimation-CHU de Sfax Tunisie. *La Tunisie Médicale* 88: 501–506
11. CLSI (2020) Performance Standards for Anti-Microbial Susceptibility Testing. Clinical and Laboratory Standard Institute (CLSI).
12. Magiorakos A-P, Srinivasan A, Carey RB, et al. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection* 18: 268–281
13. Hebert G, Hancock GA (1985) Synergistic hemolysis exhibited by species of staphylococci. *J Clin Microbiol* 22: 409–415. https://doi.org/10.1128/jcm.22.3.409-415.1985
14. Freeman D, Falkiner F, Keane C (1989) New method for detecting slime production by coagulase-negative staphylococci. *J Clin Pathol* 42: 872–874. https://doi.org/10.1136/jcp.42.8.872

15. Stepanović S, Vuković D, Hola V, et al. (2007) Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 115: 891–899. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x
16. Overbeek R, Olson R, Pusch GD, et al. (2014) The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42: D206–D214. https://doi.org/10.1093/nar/gkt1226
17. Tatusova T, DiCuccio M, Badretdin A, et al. (2016) NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 44: 6614–6624. https://doi.org/10.1093/nar/gkw569
18. Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 10: 2182. https://doi.org/10.1038/s41467-019-10210-3
19. Grant JR, Enns E, Marinier E, et al. (2023) Proksee: in-depth characterization and visualization of bacterial genomes. *Nucleic Acids Res* 51: W484–W492. https://doi.org/10.1093/nar/gkad326
20. Aziz RK, Bartels D, Best AA, et al. (2008) The RAST Server: rapid annotations using subsystems technology. *BMC genomics* 9: 75. https://doi.org/10.1186/1471-2164-9-75
21. Sullivan MJ, Petty NK, Beatson SA, et al. (2011) Easyfig: a genome com-parison visualizer. *Bioinformatics* 27: 1009–1010. https://doi.org/10.1093/bioinformatics/btr039
22. Bertelli C, Laird MR, Williams KP, et al. (2017) IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Res* 45: W30–W35. https://doi.org/10.1093/nar/gkx343
23. Zhang C-X, Yang S-Y, Xu M-X, et al. (2009) *Serratia emetophiles* sp. nov., associated symbiotically with the entomopathogenic nematode *Heterorhabditidoides chongmingensis* (Rhabditida: *Rhabditidae*). *Int J Syst Evol Microbiol* 59: 1603–1608. https://doi.org/10.1099/ijs.0.003871-0
24. Ajithkumar B, Ajithkumar VP, Iriye R, et al. (2003) Spore-forming *Serratia marcescens subsp. sakuensis subsp. nov*., isolated from a domestic wastewater treatment tank. *Int J Syst Evol Microbiol* 53: 253–258. https://doi.org/10.1099/ijs.0.02158-0
25. Marques-Pereira C, Proença DN, Morais PV (2020) Genome sequences of *Serratia* strains revealed common genes in both serratomolides gene clusters. *Biology* 9: 482. https://doi.org/10.3390/biology9120482
26. Zhu S, Yang B, Wang Z, et al. (2023) Augmented dissemination of antibiotic resistance elicited by non-antibiotic factors. *Ecotoxicol Environ Saf* 262: 115124. https://doi.org/10.1016/j.ecoenv.2023.115124
27. Liao J, Huang H, Chen Y (2019) CO2 promotes the conjugative transfer of multiresistance genes by facilitating cellular contact and plasmid transfer. *Environ Int* 129: 333–342. https://doi.org/10.1016/j.envint.2019.05.060
28. Moradigaravand D, Boinett CJ, Martin V, et al. (2016) Recent independent emergence of multiple multidrug-resistant *Serratia marcescens* clones within the United Kingdom and Ireland. *Genome Res* 26: 1101–1109. http://genome.cshlp.org/content/26/8/1101.full.html#ref-list-1

29. Tacconelli E, Carrara E, Savoldi A, et al. (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infect Dis* 18: 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3
30. Huang X, Shen S, Shi Q, et al. (2021) First Report of bla IMP–4 and bla SRT–2 Coproducing *Serratia marcescens* Clinical Isolate in China. *Front Microbiol* 12: 743312. https://doi.org/10.3389/fmicb.2021.743312
31. Gaultier NE, Junqueira ACM, Uchida A, et al. (2018) Complete genome sequence of the bacterium *Serratia marcescens* SGAir0764, isolated from Singapore air. *Genome Announc* 6: 10.1128/genomea. 00637–00618. https://doi.org/10.1128/genomea.00637-18
32. Samantha A, Vrielink A. Lipid A (2020) phosphoethanolamine transferase: regulation, structure and immune response. *J Mol Biol* 432: 5184–5196. https://doi.org/10.1016/j.jmb.2020.04.022
33. Srinivasan VB, Rajamohan G (2019) Genome analysis of urease positive *Serratia marcescens*, co-producing SRT-2 and AAC (6′)-Ic with multidrug efflux pumps for antimicrobial resistance. *Genomics* 111: 653–660. https://doi.org/10.1016/j.ygeno.2018.04.001
34. Zeiner SA, Dwyer BE, Clegg S (2012) FimA, FimF, and FimH are necessary for assembly of type 1 fimbriae on *Salmonella enterica* serovar *Typhimurium. Infection and immunity* 80: 3289–3296. https://doi.org/10.1128/iai.00331-12
35. Itoh Y, Rice JD, Goller C, et al. (2008) Roles of pgaABCD genes in synthesis, modification, and export of the *Escherichia coli* biofilm adhesin poly-β-1, 6-N-acetyl-D-glucosamine. *J Bacteriol* 190: 3670–3680. https://doi.org/10.1128/jb.01920-07
36. Hume EB, Stapleton F, Willcox MD (2003) Evasion of cellular ocular defenses by contact lens isolates of *Serratia marcescens. Eye & contact lens* 29: 108–112. https://doi.org/10.1097/01.ICL.0000062461.24391.7F
37. Jonson A, Normark S, Rhen M (2005) Fimbriae, pili, flagella and bacterial virulence. *Contrib Microbiol* 12: 67–89
38. Ang S, Horng Y-T, Shu J-C, et al. (2001) The role of *Rsm*A in the regulation of swarming motility in *Serratia marcescens. J Biomed Sci* 8: 160–169. https://doi.org/10.1159/000054028
39. Morozova N, Sabantsev A, Bogdanova E, et al. (2016) Temporal dynamics of methyltransferase and restriction endonuclease accumulation in individual cells after introducing a restriction-modification system. *Nucleic Acids Res* 44: 790–800. https://doi.org/10.1093/nar/gkv1490
40. Seong HJ, Han S-W, Sul WJ (2021) Prokaryotic DNA methylation and its functional roles. *Journal of Microbiology* 59: 242–248. https://doi.org/10.1007/s12275-021-0674-y
41. Shayer KSA, Shuvo SR, Jabeen I, et al. (2024) Draft genome sequence data of *Serratia marcescens* strain harboring blaNDM-7 from Dhaka, Bangladesh. *Data in Brief* 57: 111133. https://doi.org/10.1016/j.dib.2024.111133
42. Byrne AM, Lessie TG (1994) Characteristics of IS401, a new member of the IS3 family implicated in plasmid rearrangements in *Pseudomonas cepacia*. *Plasmid* 31: 138–147. https://doi.org/10.1006/plas.1994.1015
43. Jiang X, Ren S, Geng Y, et al. (2020) The sug operon involves in resistance to quaternary ammonium compounds in *Listeria monocytogenes* EGD-e. *Appl Microbiol Biotechnol* 104: 7093–7104. https://doi.org/10.1007/s00253-020-10741-6

44. McCarlie SJ, Boucher CE, Bragg RR, et al (2023) Genomic islands identified in highly resistant *Serratia sp*. HRI: a pathway to discover new disinfectant resistance elements. *Microorganisms* 11: 515. https://doi.org/10.3390/microorganisms11020515
45. Juhas M, Van Der Meer JR, Gaillard M, et al. (2009) Genomic islands: tools of bacterial horizontal gene transfer and evolution. *FEMS Microbiol Rev* 33: 376–393. https://doi.org/10.1111/j.1574-6976.2008.00136.x
46. Burrus V, Pavlovic G, Decaris B, et al. (2022) Conjugative transposons: the tip of the iceberg. *Mol Microbiol* 2002;46: 601–610. https://doi.org/10.1046/j.1365-2958.2002.03191.x
47. Wang J, Liu Y, Liu Y, et al. (2018) A novel family of tyrosine integrases encoded by the temperate pleolipovirus SNJ2. *Nucleic Acids Res* 46: 2521–2536. https://doi.org/10.1093/nar/gky005
48. Landy A (2015) The λ integrase site-specific recombination pathway. *Mobile DNA III.* 2015: 91–118. https://doi.org/10.1128/9781555819217.ch4
49. Carvalho R, Aburjaile F, Canario M, et al. (2021) Genomic characterization of multidrug-resistant *Escherichia coli* BH100 sub-strains. *Front Microbiol* 11: 549254. https://doi.org/10.3389/fmicb.2020.549254
50. Filippov A, Oleinikov P, Motin V, et al. (1995). Sequencing of two *Yersinia pestis* IS elements, IS285 and IS100. *Contrib Microbiol Immunol* 13: 306–309
51. Ramos JL, Martínez-Bueno M, Molina-Henares AJ, et al. (2005) The TetR family of transcriptional repressors. *Microbiol Mol Biol Rev* 69: 326–356. https://doi.org/10.1128/MMBR.69.2.326-356.2005
52. Akiba M, Lin J, Barton Y-W, et al. (2006) Interaction of CmeABC and CmeDEF in conferring antimicrobial resistance and maintaining cell viability in *Campylobacter jejuni*. *J Antimicrob Chemother* 57: 52–60. https://doi.org/10.1093/jac/dki419
53. Cagliero C, Mouline C, Payot S, (2005) Cloeckaert A. Involvement of the CmeABC efflux pump in the macrolide resistance of *Campylobacter coli*. *J Antimicrob Chemother* 56: 948–950. https://doi.org/10.1093/jac/dki292
54. Guazzaroni Ma-E, Krell T, Felipe A, et al. (2005) The multidrug efflux regulator TtgV recognizes a wide range of structurally different effectors in solution and complexed with target DNA: evidence from isothermal titration calorimetry. *J Biol Chem* 280: 20887–20893. https://doi.org/10.1074/jbc.M500783200
55. Lin J, Akiba M, Sahin O, et al. (2005) Zhang Q. CmeR functions as a transcriptional repressor for the multidrug efflux pump CmeABC in *Campylobacter jejuni*. *Antimicrob Agents chemother* 49: 1067–1075. 10.1128/AAC.49.3.1067-1075.2005
56. Fu X, Zhang M, Yuan Y, et al. (2023) Microbial Virulence Factors, Antimicrobial Resistance Genes, Metabolites, and Synthetic Chemicals in Cabins of Commercial Aircraft. *Metabolites* 2023; 13: 343. https://doi.org/10.3390/metabo13030343

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