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Original Article

Whole-genome sequencing analysis of multidrug-resistant *Serratia marcescens* isolates in an intensive care unit in Brazil

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Abstract

Introduction: *Serratia marcescens* is an opportunistic pathogen found ubiquitously in the environment and associated with a wide range of nosocomial infections. This multidrug-resistant bacterium has been a cause of concern for hospitals and healthcare facilities due to its ability to spread rapidly and cause outbreaks. Next generation sequencing genotyping of bacterial isolates has proven to be a valuable tool for tracking the spread and transmission of nosocomial infections. This has allowed for the identification of outbreaks and transmission chains, as well as determining whether cases are due to endogenous or exogenous sources. Evidence of nosocomial transmission has been gathered through genotyping methods. The aim of this study was to investigate the genetic diversity of carbapenemase-producing *S. marcescens* in an outbreak at a public hospital in Cuiaba, MT, Brazil.

Methodology: Ten isolates of *S. marcenses* were sequenced and antibiotic resistance profiles analyzed over 12 days.

Results: The isolates were clonal and multidrug resistant. Gentamycin and tigecycline had sensitivity in 90% and 80% isolates, respectively. Genomic analysis identified several genes that encode β-lactamases, aminoglycoside-modifying enzymes, efflux pumps, and other virulence factors.

Conclusions: Systematic surveillance is crucial in monitoring the evolution of *S. marcescens* genotypes, as it can lead to early detection and prevention of outbreaks.

Key words: *Serratia marcescens*; outbreak; antimicrobial resistance; genomic sequencing.

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Introduction

Serratia marcescens belongs to the Enterobacteriaceae family and causes infections and outbreaks in hospital settings that usually involve multidrug-resistant strains [1]. Considerable mortality rates, ranging from 14% to 60%, result from outbreaks of *S. marcescens* infections in intensive care units (ICUs) [2,3].

The main mechanism of carbapenem resistance among Enterobacteriaceae is the production of carbapenemases [4]. Carbapenems are the most effective drugs in the treatment of infections caused by multidrug resistant Gram-negative bacteria, but the recent increase in antibiotic resistance rates in this group has reduced therapeutic options, including in the case of *S. marcescens*, which has intrinsic resistance to several antimicrobial agents, such as polymyxins [5-8].

In addition, it has the ability to acquire multiple mechanisms of virulence and resistance to drugs used during antimicrobial treatment [9,10].

S. marcescens strains with resistance to carbapenems have been reported in Brazil and are associated exclusively with the production of *Klebsiella pneumoniae* carbapenemase (KPC) [2,11-13]. Genotyping of *S. marcescens* has proven to be a valuable tool in tracking the spread and transmission of nosocomial infections [14].

Various molecular typing methods have been employed to identify genotypic differences and genetic relatedness among other nosocomial bacteria isolates [15]. Genomic sequencing is a powerful tool used in investigation of outbreaks to characterize and determine whether the individual microbial strains associated with the infections are identical or closely related to each other [16].

Furthermore, genotyping of antimicrobial resistance genes and virulence factors, such as fimbriae, flagella, and biofilms associated with bacterial movement, fixation, and colonization, indicates increased bacterial persistence and pathogenicity in individuals and environmental reservoirs [17].

This study aimed to characterize the genome of an isolate of *S. marcescens* that caused an outbreak in the ICU of the Tertiary Public Hospital in the Cuiaba, Mato Grosso state of Brazil. This study has great local relevance as it is the first investigated and confirmed outbreak of *S. marcescens* in the state, that led to potential insights that require investigation.

Methodology

Patients and samples

S. marcescens was isolated from ten patients (six males and four females), who had serious complications including underlying diseases, demonstrating the nature of an opportunistic infection by the pathogen. The mean age of the patients was 58.8 (± 18.56) years. Admissions to the Tertiary Public Hospital in the city of Cuiabá, state of Mato Grosso, Brazil, occurred between 8 and 19 November 2018, as described by Cruz *et al.* [7].

Antibiotic resistance to piperacillin + Tazobactam, cefepime, imipenem, meropenem, ertapenem, ciprofloxacin, polymixin B, amikacin, gentamycin, and tigecycline was analyzed using a VITEK 2 system (BioMérieux, Marcy-I'Etoile, France). The VITEK-2 system is used to detect and identify isolated microorganisms, as well as perform automated microorganism sensitivity tests, providing precision and speed in results, thus ensuring agility for faster therapeutic decisions. The average time between collection of the sample for pathogen diagnosis and the subsequent detection of drug sensitivity to the antimicrobial agent was approximately five days.

Epidemiological data

An outbreak occurs when there is a greater than expected increase in the number of infections or colonization by multidrug resistant bacteria in hospitalized patients. This outbreak emerged suddenly, affecting patients admitted to the adult ICU and the internal medicine ward (where ICU patients are transferred after discharge due to clinical improvement).

The measures implemented to contain and control the outbreak were as follows. Firstly, incidence of the outbreak was communicated to all sections of the hospital, including the management. Subsequently, new admissions to the hospital's ICU and medical clinic ward were suspended. Contact precautions were implemented and reinforced. Ongoing education on the topic (outbreak and prevention of infections related to health services) were optimized. Surveillance of all nearby patients was performed using cultures to track and identify infection with the bacteria responsible for the outbreak. Phenotypic characterization of the microorganism was carried out in the microbiology laboratory of the university hospital.

Molecular characterization of the isolates was carried out in the Microbiology Laboratory of the Veterinary Hospital of the Federal University of Mato Grosso (UFMT), after registration in the National System for Ethical Assessment of Research Projects with Human Beings and approval by the Hospital Ethics Committee. Júlio Muller University.

Whole genome sequencing

The bacterial isolates were cultured in brain heart infusion broth at 37 °C overnight. DNA was extracted using proteinase K digestion followed by phenol-chloroform extraction [18]. Genomic libraries were prepared using an Illumina HiSeq 2500 system (Illumina Inc., California, USA) with coverage greater than 1000×. We based the 1000x coverage parameter on genomic analysis of whole genome sequence (WGS) of other strains with a coverage of 662x [19]. The raw data obtained were reassembled using the SPAdes v.3.10.0 algorithm in Pathosystems Resource Integration Centre assembly (PATRIC) server (https://www.patricbrc.org/) [36], and annotations were made using Rapid Annotation using Subsystem Server Technology (RAST) [37]. The assembled genomes

were reconstructed into scaffolds using the multi-CAR tool [38].

Bioinformatic analysis

Genomes assembly and annotation

Unicyler assembler v 0.5.0 (hybrid assembly pipeline for bacterial isolate genomes) was used for the de-novo assembly of Illumina Seq adapter free and error correction of data. Unicycler utilizes SPAdes to build a De Bruijn graph assembly utilizing an extensive variety of k-mer sizes.

Prokka 1.13.4 was used for gene prediction [21] and functional analysis to detect antimicrobial resistance genes (ARGs) were performed using ABRicate [22] with Megares [23], Ecoli_VF [24], and Virulence Factor Database (VFDB) [25].

Comparative genome analysis

BLAST Ring Image Generator (BRIG) was used to generate a circular comparative map (Figure 1) of all the bacterial isolates [26]. In addition to displaying similarity between a reference genome in the center and other query sequences as a set of concentric rings colored according to BLAST identity, BRIG can generate circular comparison images for prokaryote genomes, display multiple genome comparisons in a single image, and generate circular comparison images for other types of genomes as well.

Ethics approval

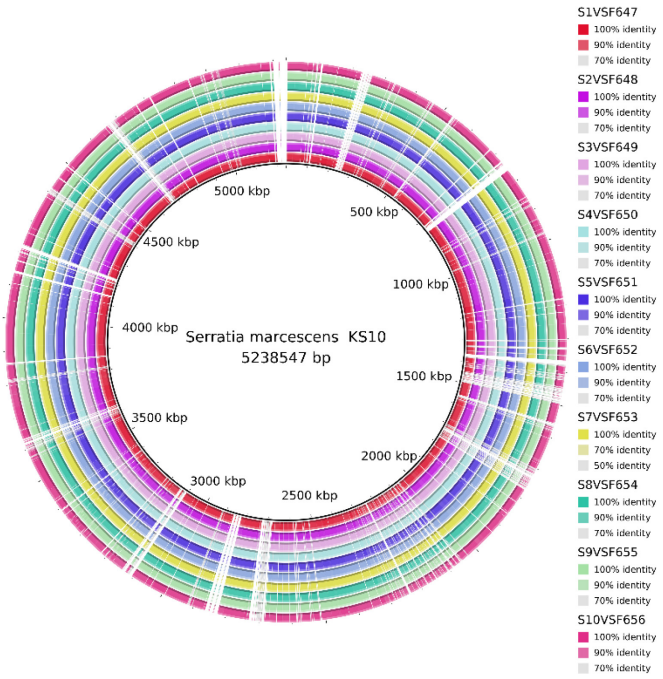
The research project was approved by the Research Ethics Committee of the Júlio Muller University Hospital and registered with the National System of Ethical Evaluation of Human Research Projects (CAAE 17331119.2.0000.5541).

Results

All the patients, except those undergoing cancer treatment, were admitted to the ICU. The isolates collected from the patients were sequenced and the genomic features showed clonality based on identical coding sequences (CDS) (Table 1) probably associated with the short time period of outbreak (12 days). Occurrence of polyclonal outbreaks have been described in hospitals in India and Spain, but over a long period from five-month to two years of investigation [27,28].

We observed the occurrence of a broad group of virulence genes, totaling nine, which were present in all the isolates. Their functions were associated with motility (*cadA*, *fliM*, *cheY*, *fliG*, and *gndA*), stress response (*gndA* and *rpoS*), pathway regulation (*rcsB*), iron uptake (*entF*), and hemolysin production (*shlB*) based on the data from other confirmed bacteria and animal species [29]. These factors give bacteria the

Figure 1. Comparison of sequences of multidrug-resistant *Serratia marcescens* isolates from hospitalized patients to the reference strain.



A BLAST was performed to compare the coding sequence (CDS) regions in the reference genome (*S. marcescens* KS10) and the uploaded query sequences. The inner-most slot (brown) shows CDS regions on the reference, and the outer-most slot (red) represents the core genome. The core genome slot shows regions where a BLAST hit was present between the reference and all of the sequences in the query file.

Table 1. Genomic features of clonal multidrug-resistant *Serratia marcescens* from the outbreak among patients admitted to the intensive care unit, Cuiabá-MT, Brazil.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Contigs	1	1	1	1	1	1	1	1	1	1
Bases	5017862	5022821	5022695	5022821	5022818	5022919	5022802	5022373	5022802	5022725
CDS*	4598	4602	4603	4602	4603	4603	4603	4603	4603	4602
Gene	4677	4684	4685	4684	4685	4686	4685	4685	4685	4681
rRNA	1	1	1	1	1	1	1	1	1	1
tRNA	77	80	80	80	80	81	80	80	80	77
tmRNA	1	1	1	1	1	1	1	1	1	1

CDS: coding DNA sequence.

Table 2. Virulent and resistant genes in *Serratia marcescens.*

S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
ARG									
AAC6	*AAC6*	*AAC6*	*AAC6*	*AAC6*	*AAC6*	*AAC6*	*AAC6*	*AAC6*	*AAC6*
HNS	*HNS*	*HNS*	*HNS*	*HNS*	*HNS*	*HNS*	*HNS*	*HNS*	*HNS*
SRT	*SRT*	*SRT*	*SRT*	*SRT*	*SRT*	*SRT*	*SRT*	*SRT*	*SRT*
SDEB	*SDEB*	*SDEB*	*SDEB*	*SDEB*	*SDEB*	*SDEB*	*SDEB*	*SDEB*	*SDEB*
MEXI	*MEXI*	*MEXI*	*MEXI*	*MEXI*	*MEXI*	*MEXI*	*MEXI*	*MEXI*	*MEXI*
	TEM	*TEM*	*TEM*	*TEM*	*TEM*	*TEM*	*TEM*	*TEM*	*TEM*
SDEX	*SDEX*	*SDEX*	*SDEX*	*SDEX*	*SDEX*	*SDEX*	*SDEX*	*SDEX*	*SDEX*
SDEY	*SDEY*	*SDEY*	*SDEY*	*SDEY*	*SDEY*	*SDEY*	*SDEY*	*SDEY*	*SDEY*
CPXAR	*CPXAR*	*CPXAR*	*CPXAR*	*CPXAR*	*CPXAR*	*CPXAR*	*CPXAR*	*CPXAR*	*CPXAR*
CRP	*CRP*	*CRP*	*CRP*	*CRP*	*CRP*	*CRP*	*CRP*	*CRP*	*CRP*
VIRULENCE									
rcsB	*rcsB*	*rcsB*	*rcsB*	*rcsB*	*rcsB*	*rcsB*	*rcsB*	*rcsB*	*rcsB*
cheY	*cheY*	*cheY*	*cheY*	*cheY*	*cheY*	*cheY*	*cheY*	*cheY*	*cheY*
fliM	*fliM*	*fliM*	*fliM*	*fliM*	*fliM*	*fliM*	*fliM*	*fliM*	*fliM*
fliG	*fliG*	*fliG*	*fliG*	*fliG*	*fliG*	*fliG*	*fliG*	*fliG*	*fliG*
gndA	*gndA*	*gndA*	*gndA*	*gndA*	*gndA*	*gndA*	*gndA*	*gndA*	*gndA*
acrB	*acrB*	*acrB*	*acrB*	*acrB*	*acrB*	*acrB*	*acrB*	*acrB*	*acrB*
rpoS	*rpoS*	*rpoS*	*rpoS*	*rpoS*	*rpoS*	*rpoS*	*rpoS*	*rpoS*	*rpoS*
cadA	*cadA*	*cadA*	*cadA*	*cadA*	*cadA*	*cadA*	*cadA*	*cadA*	*cadA*
ECS88	ECS88	ECS88	ECS88	ECS88	ECS88	ECS88	ECS88	ECS88	ECS88
_3547	_3547	_3547	_3547	_3547	_3547	_3547	_3547	_3547	_3547

Source: Megares, Ecoli_vf and Vfdb. ARGs: antibiotic resistance genes; *AAC6*: Aminoglycoside N(6')-acetyltransferase enzyme; *SRT*: *Serratia marcescens* β-lactamase; *TEM*: β-lactamase class A; *HNS, MEXI, SDEB, SDEX, SDEY, CPXAR, CRP, acrB*: multidrug efflux pump resistance-nodulation-division (RND); *rcsB*: prodigiosin synthesis, biofilm formation and acid resistance; *cheY, fliG, fliM*: modulate the direction of flagellar rotation; *rpoS*: general response to oxidative stress; *gndA*: defense protein thermal shock; *cadA*: metal resistance protein; ECS88_3547: lipoprotein from the *NLPI* gene that provides mechanical strength and resistance to osmotic stress.

ability to colonize, infect and invade the host's immune system [30], thus contributing to an outbreak. These mechanisms are widely known in some bacterial species [29]; however, few studies address their pathogenic mechanisms in *Serratia* spp. Virulence and antibiotic resistance genes (ARG) in *S. marcescens*, from a different database (Megares, Ecoli_vf and Vfdb) are listed in Table 2.

The ARG genes were similar in all samples, except for sample1 (S1) which had no β-lactamase gene (*TEM*), as shown in the phenotypic resistance profile in Table 3. The *blaTEM* gene was previously reported in 54 *S. marcescens* isolates collected from neonatal intensive care units (NICUs) and ICUs in Brazil. Other genes identified in our research, such as *SdeB*, *SdeY*, *aac(6')*, have also been reported previously [31].

Divergent phenotypic antibiotic susceptibility was observed in nine out of ten gentamycin-sensitive isolates that also carried the *aac(6')-Ic* gene, which confers resistance to aminoglycosides [32]. However, in other studies, low resistance was observed in all isolates harboring the acyltransferase gene *aac(6')-Ic* [26,31]. The presence of genes such as phosphotransferase *aph(3')* or adenylyltransferase *aadb*

Table 3. Patient data and the antibiotic resistance profile.

Nº	Patient age/ gender	Sample	Sensitivity	Resistance	Underlying disease	Outcome
S1	74, M	Aspirated tracheal	TG GEN	TZP, CPM, POL, IMP, ETP, MER, CIP, AMI	Neurocryptococcosis	Death
S2	84, F,	Aspirated tracheal	TG GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI	Acute myocardial infarction	Death
S3	60, M,	Tip of catheter	GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI, TG	Adenocarcinoma of prostate	Cured
S4	63, F	Rectal swab	GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI, TG	Leprosy	Cured
S5	57, M	Pleural liquid	TG GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI	Pulmonary aspergillosis	Cured
S6	71, M	Aspirated tracheal	TG	TZP, CPM, POL, IPM, ETP, MER, CPI, AMI, GEN	HIV and Pneumocystosis	Death
S7	40, F	Rectal swab	TG GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI	Leprosy and cholecystitis	Cured
S8	56, M	Abdominal secretion	TG GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI	Diaphragmatic hernia and emergency laparotomy	Cured
S9	18, F	Synovial liquid	TG GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI	Hemolytic anemia and disseminated histoplasmosis	Cured
S10	65, M	Blood	TG GEN	TZP, CPM, POL, IPM, ETP, MER, CIP, AMI	Pemphigus foliaceus and renal failure	Death

TZP: piperaciclin + tazobactam; CPM: cefepime; POL: polymixin B, IPM: imipenem; ETP: ertapenem; MER: meropenem; CIP: ciprofloxacin; AMI: amikacin; TG: tigecycline; GEN: gentamycin; M: male; F: female.

in *S. marcescens* has been previously associated with resistance [33], but they were not present in our isolate.

Low resistance to tigecycline has been described in many studies with inconsistent results [1,8]. In the present study, an 80% susceptibility to tigecycline was observed; however, in other studies this is disrupted by the presence of RND-type drug efflux pumps (*sdeB*, *sdeX*, *sdeY*, and *hasF*) [31,34,35].

Conclusions

S. marcescens is notorious for its increasing antimicrobial resistance and potential to cause outbreaks in hospital settings. Isolates of *S. marcescens* isolated from a tertiary hospital in Cuiabá, showed a multidrug-resistant phenotype that was sensitive to only two of the ten antimicrobial classes tested. *S. marcescens* genotyping proved to be a valuable tool to identify genotypic differences and genetic relatedness among the people attended, in addition to demonstrating clonality by whole genome sequencing, confirming the occurrence of an outbreak in the Tertiary Public Hospital in Cuiabá, Mato Grosso, Brazil.

Our results indicated a diversity of virulence and resistance factors, demonstrating a variety of genetic contexts in which these genes can be inserted. Complete genome sequencing was used as a tool to characterize and confirm an outbreak of multidrug-resistant *S. marcescens* isolates, helping, among other aspects, in the typing and identification of resistance determinants.

This analysis of data generated through bioinformatic tools specialized in the identification of acquired genes provides very useful information to identify important aspects of an isolate, as it brings together information about genomic characteristics and antimicrobial susceptibility in clinical isolates of *S. marcescens* and is also useful for future studies.

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