The genomic characterization of carbapenem-resistant *Serratia marcescens* at a tertiary hospital in South Africa

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Running Title

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Thesis Declaration

I, Amanda Julia Overmeyer, hereby declare that the work on which this dissertation is based is my own original work.

Signed by candidate

13.02.2023

Signature date

Abstract

Background

Serratia marcescens is an opportunistic nosocomial pathogen, and recent reports have highlighted the rapid increase in multidrug resistance in this organism. There is a paucity in genomic data for carbapenem resistant *S. marcescens* (CRSM).

Methods

A retrospective cohort study describing laboratory confirmed CRSM from a tertiary academic hospital in Cape Town, South Africa, for the period 2015-2020, was performed. Stored CRSM and control isolates were submitted for whole-genome sequencing using Illumina MiSeq, with the Nextera DNA Flex Library Preparation Kit. Sequence data was analysed in-house using srst2 and Tychus, and CRSM and control isolates were compared.

Results

Twenty-one CRSM and four control isolates were sequenced and analysed. Twenty-four different resistance genes were identified, with all isolates having at least two resistance genes, and seventeen isolates harbouring three or more genes. This correlated well with phenotypic results. The $bla_{OXA\text{-}48\text{-}like}$ carbapenemase was the most common carbapenemase identified in 86% (18/21) of CRSM. A core SNP difference tree indicated that the CRSM could be grouped into three clusters. A minority of isolates had shared plasmids. Several genes and single nucleotide polymorphisms (SNPs) were identified in the CRSM which may putatively augment virulence, but this requires further functional characterisation.

Conclusion

A diverse resistome was observed in CRSM, which was also reflected phenotypically, with bla*OXA-48-like* the most common carbapenemase. Though distinct clusters were observed, no clonality was noted, and a limited number of isolates shared plasmids. This study provides genomic data for emerging CRSM and highlights the importance of ongoing genomic surveillance to inform infection prevention control and antimicrobial stewardship initiatives.

Introduction

Serratia marcescens is a non-spore-forming, gram-negative bacillus. The genus *Serratia* consists of more than 20 species, of which *S. marcescens* is the primary human pathogen. It is a ubiquitous saprophyte that is present in water, soil, plants, animals, and insects[1]. However, it was not until 1951, when Wheat published a report on cases of nosocomial *S. marcescens* infection, that it was considered pathogenic[2]. It has subsequently been recognised as an important cause of opportunistic infections, especially in hospital settings.

The success of *S. marcescens* as a pathogen is in part due to its genetic diversity and genome plasticity. Several intrinsic virulence factors have been encountered, contributing to biofilm formation, chromosomally encoded porins, and efflux pumps. Other virulence factors include haemolysins, proteases, siderophores, and lipopolysaccharide which form part of the gram-negative cell wall[3].

Antibiotic resistance is mediated by both intrinsic and acquired mechanisms. The most common mechanism of resistance is β-lactamase production. S. *marcescens* may harbour chromosomal AmpC β-lactamase genes[4], conferring resistance to penicillins, first generation cephalosporins, cefamycins, and monobactams. This β-lactamase may be hyper expressed due to induction following exposure to β-lactam antibiotics, or through the selection of derepressed mutants. This results in resistance to all β-lactam antibiotics except fourth generation cephalosporins and carbapenems[5].

Acquired resistance to extended spectrum β-lactams in *S. marcescens* is plasmid encoded, and confers resistance to third generation cephalosporins[6] for example *bla*$_{CTX-M}$. Other antibiotics affected by acquired resistance determinants include aminoglycosides, fluoroquinolones, macrolides, and cotrimoxazole. β-lactamases, in combination with decreased cell membrane permeability, can lead to carbapenem resistance[7]. Notably, *S. marcescens* is intrinsically resistant to colistin by modification of lipopolysaccharide, but the exact mechanism is yet to be elucidated[8].

The first carbapenem-resistant *S. marcescens* (CRSM) were described in 1982 and were designated S6, S7 and S8[9]. In 1994, Naas *et al.,* sequenced the first carbapenemase gene from *S. marcescens* (*bla*$_{sme-1}$)[10]. Subsequently, many other carbapenemases have been identified in this organism.

Carbapenem resistant Enterobacterales (CREs) have been reported from every major centre in South Africa[11,12]. The most common organisms causing infections are *Klebsiella pneumoniae, Enterobacter* species*, S. marcescens, Escherichia coli, Citrobacter* spp.*, Providencia* spp., and *Morganella* spp. The most common carbapenemase identified to-date are *bla*$_{NDM}$ and/or *bla*$_{OXA-48}$, with *bla*$_{VIM}$, *bla*$_{GES}$, and *bla*$_{KPC}$ in the minority of cases[11,12].

While molecular studies have focused primarily on *K. pneumoniae*, genomic data for CRSM is limited; however it is needed to understand of the transmission patterns and prevailing resistance determinants. This data could be used to inform data-driven local patient management, antimicrobial stewardship (AMS), and infection prevention control (IPC) strategies.

Methods

Ethics

Ethics approval was obtained from the University of Cape Town Human Research Ethics Committee (Ref: 468/2020) and hospital approval obtained from Groote Schuur Hospital for access to patient information and stored samples.

Isolate selection

All CRSM isolates cultured from in-patients at Groote Schuur Hospital, Cape Town, and stored at the National Health Laboratory Service (NHLS) laboratory for the study period September 2015 to June 2020. All were considered for the study, irrespective of clinical source. Only the first isolate per patient was included and where the first isolate was unavailable for testing, a different isolate from the same patient was used. Isolates were excluded if they were obtained from rectal swabs for CRE screening as this may have represented colonization instead of disease. Carbapenem susceptible control strains were selected from samples collected during the study period.

All samples were cultured onto standard microbiological media, organisms identified, and antimicrobial susceptibility testing performed using the Vitek II automated system (BioMerieux, France) using standard laboratory protocols. The laboratory information system (Trakcare) was accessed to identify intermediate susceptibility or resistance to carbapenems. Presumptive CRSM were confirmed using the modified Hodge's test[13], modified carbapenemase inactivation method (mCIM)[14], or carbapenemase lateral flow assay (LFA) (Corbisbio, USA). Minimum inhibitory concentrations (MIC) were determined using E-test

(BioMerieux, France), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[15].

Clinical data was extracted from the laboratory information system and included the date of birth, sex, sample type, ward, and associated microbiological test results. The raw data was refined using Stata (Statacorp, USA).

DNA extraction and whole genome sequencing

Total genomic DNA was extracted using the Zymogen Bacterial/Fungal DNA extraction miniprep kit (Zymo Research, USA). The resulting quality and yield confirmed using the Nanodrop (Thermo Fisher, USA), as per manufacturer's instructions.

Libraries were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina, USA), according to the manufacturer's instructions. Whole genome sequencing was performed on the MiSeq Platform (Illumina) using the v2 300-cycle reagent kit and flow cell, generating 2 x 150 bp, paired-end reads. The raw reads were inspected and reads of poor quality removed using FastQC[16] and Trimmomatic[17]. Reads were subsequently mapped to a reference genome (*Serratia marcescens* ASM351616v1) to produce draft genomes.

There are currently no *S. marcescens* specific virulence databases for *in silico* analysis. To identify genetic elements which may have potentially contributed to increased pathogenesis or virulence of the CRSM isolates, a statistical comparison was conducted comparing genes in the CRSM to the controls, but excluding the ESBL isolate. The srst2[18] tool was used to identify resistance elements with ≥ 80% homology (ARGannot[19]) and plasmid types with 50% coverage

and 90% homology (PlasmidFinder[20]) based on the short reads[18]. A core genome single nucleotide polymorphism (SNP) dendrogram indicating genetic relatedness was constructed using Tychus[21]. Fisher's exact tests with multiple testing correction was used to identify virulence genes that were differentially present/absent in the CRSM isolates compared to the wild-type controls (excluding the ESBL isolate), and adjusted P values ≤0.05 deemed significant.

Results

Carbapenem-resistant *Serratia marcescens*

During the study period (September 2015 to June 2020), a total of 128 246 bacterial isolates were collected and analysed by the NHLS laboratory using the Vitek II system. Of those, a total of 1396 (1.09%) were identified as *S. marcescens.* Phenotypic reduced susceptibility to carbapenems was noted in 169/1396 (12.11%) of the isolated *S. marcescens*, but only 39/169 (23.1%) were non-repeat specimens, with 21/39 (53.85%) available for sequencing and inclusion in the study. The number of CRSM isolates per month remained relatively stable over the study period, except for a noticeable increase in July 2018, peaking during January to April 2019, and again in January to March 2020 (Figure 1).

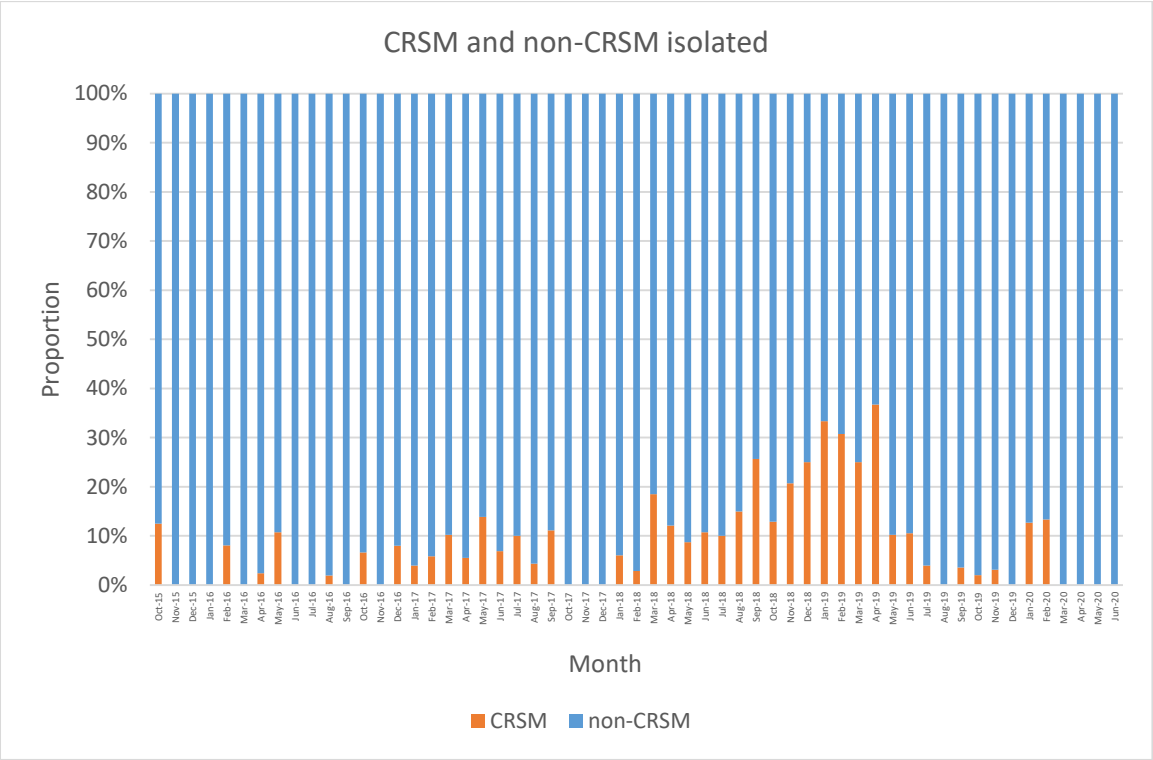

Figure 1. Proportions of CRSM and non-CRSM isolated over the study period.

Twenty out of twenty-one CRSM isolates selected were confirmed to be carbapenemase-positive using either the modified carbapenemase inactivation method (mCIM) (17/21) or the carbapenemase lateral flow assay (LFA) (Corbisbio, USA) (3/21), with two isolates also confirmed by PCR to harbour *bla*$_{\text{OXA-48}}$, at the National Reference Laboratory, Johannesburg. The methods of confirmation were different because of the advancement in and implementation of different methodology over time.

Of the 21 CRSM isolates sequenced, 14/21 (66.7%) were from pure cultures and 7/21 (33.3%) were part of mixed cultures (data not shown). Based on specimen type, the majority [13/21 (61.9%)] were cultured from blood, 3/21 (14.29%) from urine, 2/21 (9.52%) from intra-abdominal fluids, 2/21 (9.52%) from CVP tips, and 1/21 (4.76%) from a pus aspirate. Most of

the patients were male (17/21, 80.95%), with a median age of 42 years (range 14-64 years). The median duration of admission before isolation of a CRSM was 18 days with a range of 1-110 days. Most isolates were obtained from samples submitted while the patient was in ICU (17/21, 80.95%). Of the 21 CRSM infected patients included, 7/21 (33.3%) had another CRE cultured. These included 4 *Klebsiella pneumoniae,* 2 *Enterobacter cloacae,* and 1 *Citrobacter freundii*. Other multi-drug resistant organisms were previously cultured in 15/21 (71.43%) of patients.

All 21 CRSM isolates demonstrated phenotypic resistance to penicillins, second, third, and fourth generation cephalosporins, and the β-lactam-β-lactamase inhibitor combinations amoxicillin-clavulanate and piperacillin-tazobactam, with variable resistance to carbapenems (Supplemental Table S2). CRSM isolates exhibited varying degrees of resistance to the non-β-lactam antibiotics, but no single agent displayed less than 50% resistance, where resistance to ciprofloxacin, gentamycin, tigecycline, amikacin and cotrimoxazole were 90.5%, 85.7%, 66.7%, 61.9% and 57.1%, respectively (data not shown).

Antibiotic prescription data was available for 20/21 (95.24%) of the CRSM infected patients. Nineteen patients were on antibiotics prior to the isolation of CRSM. The most common antibiotics prescribed were β-lactams, specifically piperacillin-tazobactam. The other most frequently prescribed antibiotics were cephalosporins, aminoglycosides, and carbapenems in 9/19 (47%) of cases. Colistin was administered in four cases prior to isolation of CRSM. Most patients received a median of two (range 1–15) antibiotics prior to culturing a CRSM.

The control isolates included 2 phenotypically wild type, 1 non-wild type, and an extended spectrum β-lactamase (ESBL)-producing isolate.

Whole genome sequencing

There were approximately 0.8–2.2 million reads per sample, and the mean quality score was Phread > 28.[22]

Genetic relatedness

A core SNP difference dendrogram indicated that the isolates could be grouped into three clusters (Figure 2). Cluster 1 (isolates 4, 5, 7-11, 13-15, 18, 19) was the largest and comprised 12/21 (57%) of CRSM isolates. Cluster 2 (isolates 12, 17, 25) consisted of 3/21 (14%) of CRSM isolates and was closely related to Cluster 1. The third cluster (isolates 1-3, 6, 16, 23) comprised 6 isolates (29%) which harboured the *bla*$_{\text{OXA-1}}$ gene, the OXA-48 plasmid, and a unique set of resistance genes.

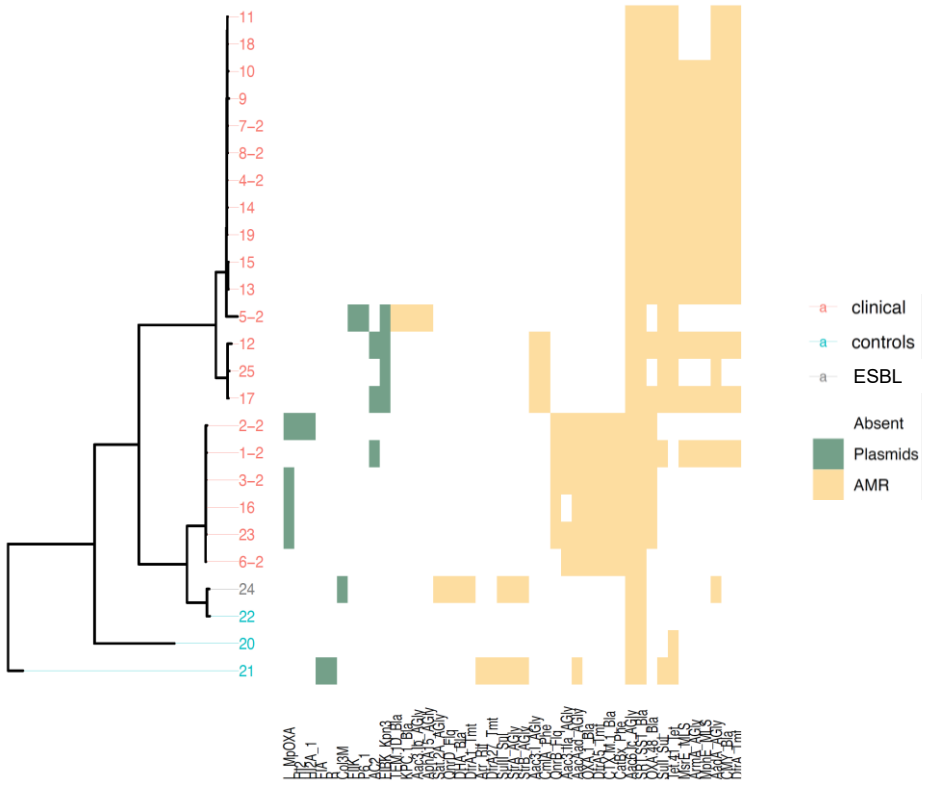

Figure 2. Dendrogram and heatmap of the sequenced isolates, indicating genetic relatedness, and the presence or absence of plasmids and resistance elements. ESBL = Extended Spectrum β-Lactamase, AMR = Antimicrobial Resistance Element.

Antibiotic resistance genes

A total of 24 different resistance genes were present in the CRSM isolates and only 9 in the controls (Figure 2). These included genes conferring resistance to β-lactams, aminoglycosides, cotrimoxazole, chloramphenicol, fluoroquinolones, and macrolides (Table 1). All isolates had at least two resistance genes with 17 isolates harbouring at least three or more.

Eight different β-lactamase genes were identified. OXA-48-like carbapenemases were detected in 18/21 (85.71%) of the CRSM, of which 14 were *bla*$_{\text{OXA-181}}$ (78%) and 4 were *bla*$_{\text{OXA-48}}$ (22%). KPC (*bla*$_{\text{KPC-2}}$) was found in a single isolate (5), which also harboured *bla*$_{\text{TEM-206}}$. No

known carbapenemases were detected in 2 of the CRSM isolates. All 21 CRSM isolates harboured 1 or more genes encoding for AmpC β-lactamases (21 bla_{SRT-1} and 14 bla_{CMY-4}). The rest of the β-lactamases consisted of 6 (29%) $bla_{CTX-M-15}$, an ESBL gene, which was present in 5 of the isolates concurrent to a carbapenemase-encoding gene. The bla_{DHA-9} AmpC β-lactamase was detected in the control ESBL isolate (24) alone.

Eight different genes conferring aminoglycoside resistance were identified. All CRSM harboured the *aac*(6)-Ic gene in combination with one or more of *aac*(3)-IIa*, aac*(3)-I*, aac*(3)-Ib*, aacaad, aad*A*, aph*A15 *and arm*A. One or more of *dfr*A5*, dfr*A12 *and sul*I*,* which confer resistance to co-trimoxazole and its components, were identified in all 21 CRSM isolates. *Tet*-41, a tetracycline resistance gene was present in 15 isolates, with no genes coding for tigecycline resistance. The macrolide resistance genes *mph*E *msr*E, were present in 12 isolates. The chloramphenicol resistance genes *cat*B4 *and cml*A1 were present in 9 isolates. The fluoroquinolone resistance gene *qnr*B was only present in 5 isolates. The resistance genes identified correlated with phenotypic resistance profiles, except for aminoglycosides and tigecycline.

In cluster 1, all isolates had tet-41 and the majority had *aad*A, *arm*A, bla_{CMY-4}, *dfr*A12, *mph*E, $bla_{OXA-181}$, *sul*I, and bla_{SRT-1}, however there were no common plasmids found. Cluster 2 all isolates harboured *aac*(3)-I, *aac*(6)-Ic, bla_{SRT-1}, *sul*I, *tet*-41, and plasmid FIBK_1. Isolates in cluster 3 contained *aac*(6)-1b, $bla_{CTX-M-15}$, *dfr*A14, bla_{OXA-1}, bla_{SRT-1}. The majority also contained *aac*(3)-IIa, bla_{OXA-48}, and *qnr*B. This cluster also included plasmid L-MpOXA identified in 4 isolates, and is known to carry bla_{OXA-48} and additional resistance elements[23] (Table 1).

Four control isolates collected during the study period were included for comparison. Isolates 20, 21, and 22 were phenotypically WT, and isolate 24 was an ESBL producer. Isolate 20 contained *aac*(6)-Ic*, sst*-1 which encode aminoglycoside resistance and an AmpC β-lactamase with S22 also having *tet*-41*, a* tetracycline efflux pump gene. One of the isolates (21) also had *aac(6)*-Ib*, arr*-3*, dfr*A*, str*A*, stre*B*, sul*II, and *tet*-41 which encodes resistance to aminoglycosides, rifamycin, cotrimoxazole, and tetracycline, respectively. The ESBL isolate (S24) had *aac(6)*-Ic, *aad*A, *bla*$_{DHA-9}$, *dfr*A, *qnr*D, *bla*$_{SRT}$, *sat*-2A, *stra*A, *stra*B, and *sul*II which encoded for resistance to aminoglycosides, β-lactams, cotrimoxazole and fluoroquinolones.

Table 1. Antibiotic resistance genes detected in CRSM.

Antibiotic enzyme class	Genes	Number of isolates	Proportion of isolates
β-lactamases	Total	21/21	100
	bla$_{CMY-4}$	14/21	67
	bla$_{SRT-1}$	18/21	86
	bla$_{SST-1}$	3/21	14
	bla$_{OXA-1}$	6/21	29
	bla$_{CTX-M-15}$	6/21	29
	bla$_{TEM-206}$	1/21	5
	bla$_{KPC-2}$	1/21	5
	bla$_{OXA-48}$	4/21	19
	bla$_{OXA-181}$	14/21	48
Aminoglycosides	Total	21/21	100
	Aac(3)-IIa	5/21	24
	Aac(3)-I	3/21	14

	Aac(6)-Ic	21/21	100
	Aac(3)-Ib	1/21	5
	AacAad	6/21	29
	AadA	15/21	71
	AphA15	1/21	5
	ArmA	12/21	57
Fluoroquinolone	Total	5/21	24
	QnrB	5/21	24
Co-trimoxazole	Total	21/21	100
	DfrA5	6/21	29
	DfrA	14/21	67
	SulI	16/21	76
MLS	Total	12/21	57
	MphE	12/21	57
	MsrE	12/21	57
Tetracycline	Total	15/21	71
	Tet-(41)	15/21	71

Plasmid types

Ten different plasmid replicon types were identified by srst2[18] using PlasmidFinder. Seven of these were in the CRSM isolates (Table 2). FIBK_1_Kpn3 in 4/21 (19.04%), L_MpOXA in 4/21 (19.04%), AC2 in 3/21 (14.29%), FIIK_1 in 1/21 (4.76%), HI2_1 in 1/21 (4.76%), HI2A_1 in 1/21 (4.76%), and P6_1 in 1/21 (4.76%). The remaining 3 plasmids were Col3M in the ESBL isolate, FIAHI1_1_HI1 in a wild type and R_1 in another wild type.

Table 2: Plasmid replicon types identified.

Plasmid	Number	Percentage	Cluster
FIBK_1_Kpn3	4	19	1, 3
L__MpOXA-48	4	19	3
A__C2_1	3	14	2, 3
FIIK_1	1	5	1
HI2_1	1	5	3
HI2A_1	1	5	3
P6_1	1	5	1
Col3M	1	-	Control
FIAHI1_1_HI1	1	-	Control
R_1	1	-	Control

Virulence factors

Based on the statistical presence/absence analysis, putative virulence genes which were only present in CRSM and not in control isolates included *aae*A_3, which encodes for an efflux pump and was present in all the CRSM isolates, *fim*D_4 which encodes for fimbriae, *puu*B3 a gene involved in energy production and conversion, and *yfm*J, a NADP-dependent oxidoreductase, which were present in the majority of CRSM (Figure 3).

Genes detected in the majority of CRSM, and in a minority of control strains, included *fim*D_3 which encodes fimbriae*, dsd*X, a D-serine transporter protein, *dnt*K which in conjunction with *pdx*A2 is involved in carbohydrate metabolism, and *Icf*B which is part of long chain fatty acid

metabolism. *Mar*A is a transcriptional activator of antibiotic resistance genes and *ybb*H is another transcription regulator (Figure 3).

There were variations detected in the following genes which were present in both CRSM, and control isolates namely *add* which is involved in nucleotide metabolism, *bvg*A which activates the transcription of virulence genes, *cdi*A a component of a cellular contact dependent growth inhibitor which inhibit growth of closely related bacteria, *adt*A which encodes an efflux pump, *pho*B which is involved in swarming motility, and *zit*B which is a zinc transporter (Figure 3).

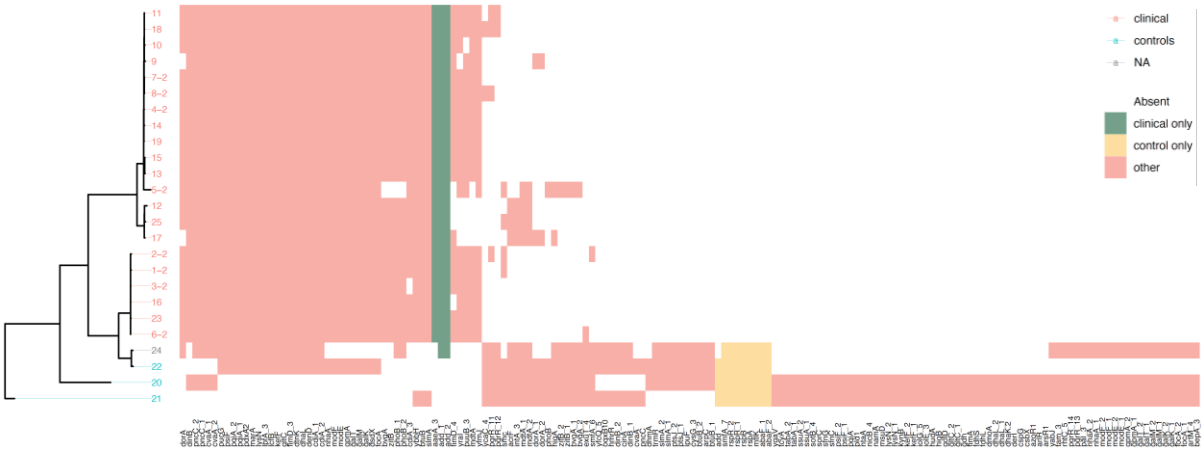

Figure 3. Dendrogram and heatmap of the CRSM and control isolates, indicating the presence or absence of potential virulence elements.

Discussion

A total of 1396 (1.09%) bacteria were identified as *S.* marcescens over the study period. Phenotypic reduced susceptibility to carbapenems was noted in 12.11% of the isolates. Twenty-one were available for sequencing and inclusion in the study. CRSM isolates exhibited varying degrees of resistance to the non-β-lactam antibiotics, but no single agent displayed less than 50% resistance.

The isolates could be grouped into three clusters with the third cluster harbouring the OXA-48 plasmid. A total of 24 different resistance genes were present in the CRSM isolates including genes conferring resistance to β-lactams, aminoglycosides, cotrimoxazole, chloramphenicol, fluoroquinolones, and macrolides. All isolates had at least two resistance genes. Ten different plasmid replicon types were identified.

Virulence genes present in CRSM included those for efflux pumps, fimbriae, metabolism, transporter proteins, and transcriptional activators of antibiotic resistance genes. There were variations detected in the following genes present in both CRSM, and control isolates. These were for nucleotide metabolism, virulence gene transcription activator, cellular contact dependent growth inhibitor, efflux pumps, motility, and a zinc transporter.

Carbapenem-resistant Enterobacterales are an increasing global problem, including in South Africa. *S. marcescens* was previously shown to be the third most common CRE isolated in this country by two studies undertaken by the National Institute of Communicable diseases (NICD)[11,12]. Despite this, limited genomic data for carbapenem resistant *S. marcescens* (CRSM) is available for South Africa. WGS is an invaluable tool, especially in outbreak and

epidemiological investigations, but is underutilised in our setting due to resource constraints, despite a progressive drop in price and availability. This study is one of the few to use WGS to examine CRSM and the first to examine a cohort of CRSM in South Africa.

Carbapenem resistance is usually attributed to a combination of factors including defined antibiotic resistance genes, efflux pumps, and altered cell membrane permeability[7]. This study investigated the genetic occurrence of resistance and potential virulence elements in a collection of CRSM. Most of the CRSM isolates in this study harboured *bla*$_{\text{oxa-48-like}}$ enzymes, with 1 carrying *bla*$_{\text{KPC2}}$. In contrast, Xu *et al*., reported CRSM from a tertiary hospital in China[24], and identified *bla*$_{\text{KPC2}}$ as the only carbapenemase. KPC was also the most common carbapenemase identified in two CRSM studies from Brazil[25] and the USA[26]. Notably, in Madrid, *bla*$_{\text{VIM1}}$ and *bla*$_{\text{OXA-48}}$ predominated[27], and *bla*$_{\text{NDM}}$ caused an CRSM outbreak in Romania[28].

There were 2 isolates where no known carbapenemase genes were identified. One of these isolates harboured *bla*$_{\text{SRT-1}}$, and was *bla*$_{\text{OXA-48}}$ positive using the carbapenemase lateral flow assay (LFA). This isolate likely harboured a novel carbapenemase gene or genotype. Since the *bla*$_{\text{OXA-48}}$ family is large and diverse, new genotypes are frequently described. Since no *bla*$_{\text{OXA-48-like}}$ gene was identified, the presence of a novel carbapenemase gene cannot be excluded, and should be investigated in future work. The second isolate harboured *bla*$_{\text{SRT-1}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{CTX-M}}$, and was mCIM negative, yet phenotypically resistant to ertapenem. This isolate harboured an ESBL gene (*bla*$_{\text{OXA-1}}$) which has been shown to confer varying resistance to the carbapenems with phenotypic resistance to ertapenem more common than meropenem, albeit in *Klebsiella pneumoniae.* [29] Since the mCIM is performed using meropenem, this isolate

was likely mCIM negative but phenotypically ertapenem resistant based on the specific activity of this *bla*$_{OXA-1}$ gene.

Multiple virulence genes were identified in this study including those promoting resistance such as efflux pumps, which export antimicrobials out of the bacterium, and transcriptional activators of resistance genes. Other virulence genes include those for fimbriae which facilitate adherence and subsequent colonization, and those involved in metabolism either through nutrient transport or energy production. Variations in common genes were found and these genes may provide a colonization advantage through inhibiting growth of closely related bacteria, or by increased motility, although their significance is yet to be confirmed.

Core SNP analysis indicated that all CRSM isolates grouped into one of three clusters, with significant homology within the clusters although no clonality was observed, as was originally suspected. It was initially theorised that these isolates may have been clones due to the peaks observed in isolation of these organisms. Since these isolates were spread over a 4-year period, there may have been an environmental reservoir rather than point source contributing to the dissemination of these isolates, or the resistance and putative virulence factors identified. This is even more likely when considering some CRSM were part of a mixed culture included other CRE which may have acted as reservoirs. The patients were located in different ICUs and/or wards, which may imply a common ancestor that evolved in the specific environments or be due to other genetic elements that could not be identified. Alternatively, patient referral from other hospitals may have introduced different strains.

Interestingly, the third cluster on the core SNP difference tree comprised six isolates which all harboured the *bla*$_{OXA-1}$ gene. These isolates also carried several resistance genes against several classes of antibiotics, which were not detected in the other strains in the study. Previous studies have indicated that *bla*$_{OXA-1}$ and *aac*(6)-Ib are often co-carried[30]. This is further compounded by the fact that four of these strains carried the MpOXA-48 plasmid, known to carry *bla*$_{OXA-48}$ and ESBLs, such as *bla*$_{CTX-M-15}$ identified in our study. These finding likely indicate a shared resistance plasmid between the strains which confers a MDR phenotype. Other plasmid replicons were also detected, but their relevance is unclear.

S. marcescens accounted for approximately 1% of organisms isolated in our laboratory during the study period, but exhibited a high rate of carbapenem resistance compared with contemporary surveillance studies elsewhere[31]. Of the CRSM isolates collected, the majority were from patients in ICU, particularly the surgical ICU, which is in accordance with previous reports[24,27,32]. Our CRSM patients had several multidrug-resistant (MDR) organisms including other CREs, ESBL producing organisms, carbapenem resistant *Acinetobacter baumannii* (CRAB), carbapenem resistant *Pseudomonas aeruginosa* (CRPA) and methicillin resistant *Staphylococcus aureus* (MRSA), isolated previously. This may have represented an opportunity for genetic exchange between the organisms, possibly plasmid mediated, or may reflect the high environmental burden of difficult-to-treat resistance in gram-negative pathogens in our setting[33,34].

Antibiotic utilization data showed extensive use of broad-spectrum antibiotics prior to isolating a CRSM. This was not surprizing, since prior antibiotic exposure is a major risk factor for colonization and infection with carbapenemase-producing Enterobacterales[35].

Considering the range of resistance genes identified in our study, any of these antibiotics may have contributed to a selection for MDR strains. The administering of colistin, to which *S. marcescens* is intrinsically resistant, may have played a role in selecting for CRSM, although it was only prescribed in the minority of our patients, prior to isolating a CRSM. Notably, overall colistin consumption data over the study period did show an increase, which coincided with the CRSM isolation peaks noted (data not shown).

Based on antimicrobial susceptibility results, extremely limited treatment options remain for CRSM in our institution. For non-β-lactam antibiotics, tigecycline resistance was phenotypically detected in isolates that contained the *tet*-41 gene, which encodes a tetracycline efflux pump, however it does not confer resistance, but may be associated with other resistance determinants which lead to tigecycline resistance. With regards to the aminoglycosides, phenotypic non-susceptibility did not correlate with genotypic aminoglycoside resistance determinants. Therefore, due to the presence of multiple resistance genes and a highly resistant phenotypic profile, treatment of CRSM, particularly in ICU patients in our setting remains challenging. In this regard, since $bla_{\text{oxa-48-like}}$ carbapenemases predominated, ceftazidime-avibactam may represent a therapeutic option. However, it is currently expensive and difficult to access in our public sector setting[36].

Limitations

This was a small descriptive retrospective study and not all isolates identified were available for sequencing, therefore it is underpowered to draw any statistically significant conclusions. Notably, there are no genomic databases for *S. marcescens* which hindered the search for virulence factors and other *in silico* analyses. Future WGS studies should include more non-

CRSM isolates, to expand the comparison for virulence factors. We also did not include rectal screening isolates which may have had IPC implications. Furthermore, environmental sampling to exclude institutional contamination were not performed.

Due to the nature of high throughput short read sequencing, plasmid reconstruction could not be conducted. However, of the ten different plasmid types identified, all isolates with the L_MpOXA plasmid also carried the *bla*$_{\text{OXA-48}}$ gene, indicating that the carbapenemase genes were likely plasmid encoded. Although this plasmid was found in a minority of isolates, this mobile genetic element does have the potential to spread to other organisms including other strains of *S. marcescens* and may therefore prove a transmission risk.

Conclusion

This study is the first in South Africa and one of only a handful internationally to examine the genomic profile of CRSM using WGS. The isolates clustered into three groups and since there may have been limited shared plasmids between them, this could suggest a common lineage with differentiation within specific environments over time. Extensive prior use of broad-spectrum antibiotics, as well as a complex resistome in our isolates, highlights both AMS and therapeutic challenges in our setting. Resistance elements in combination with putative virulence factors may have provided a survival advantage. This study highlights the importance of ongoing WGS studies on *S marcescens*, which could potentially inform data-driven local patient management principles, AMS, and IPC strategies.

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Transparency declaration

No conflicts to declare.

Author contributions

C.M., E.P., and A.B., conceptualised the study. C.M., and A.O., conducted the laboratory testing, data analysis, and interpretation. K.L. performed all bioinformatic analyses. C.M., E.P., K.L., A.B., and A.O., contributed to the manuscript preparation.

Supplementary data

Tables S1 and S2 are available as supplementary data online.

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Supplementary material

Supplemental Table S1: Patient demographics and epidemiological risk factors.

Patient	Age (yrs)	Gender	Ward	Sample type	Days in hospital prior to CRSM	Other CREs cultured	Other MROs	Prior antibiotics
1	60	Male	Surgical Ward	Blood culture	17	-	-	Piperacillin-tazobactam
2	51	Male	Surgical Ward	Pus	11	-	CRAB	Co-amoxiclav Ceftriaxone
3	42	Male	Medical Ward	Fluid	18	-	-	ceftriaxone
4	14	Female	Surgical ICU	Blood culture	14	-	CRAB	Piperacillin-tazobactam Amikacin
5	64	Male	Surgical ICU	Urine	27	*K. pneumoniae*	-	-
6	55	Male	Transplant Unit	fluid	1	*E. cloacae*	CRAB, CRPA	Ertapenem Vancomycin
7	35	Male	Surgical ICU	Urine	2	-	-	NIL
8	51	Male	Medical ICU	Urine	6	-	-	cefazolin
9	54	Female	Surgical ICU	CVP tip	18	*K. pneumoniae*	CRAB	Amoxicillin-clavulanic acid Imipenem Ciprofloxacin
10	33	Male	Surgical ICU	Blood culture	31	-	CRAB, ESBL	Amoxicillin-clavulanic acid Meropenem Clindamycin Colistin Cefepime
11	41	Male	surgical ICU	CVP tip	11	-	-	Cefazolin
12	57	Male	Haematology ICU	Blood culture	8	*K. pneumoniae*	ESBL	Amoxicillin-clavulanic acid Piperacillin-tazobactam Amikacin
13	23	Female	Surgical ICU	Blood culture	56	-	CRAB	Amoxicillin Metronidazole Piperacillin-tazobactam Ciprofloxacin Ertapenem imipenem Erythromycin Meropenem Colistin

14	22	Male	surgical ICU	Blood culture	18	-	-	Piperacillin-tazobactam Amikacin Cefazolin
15	23	Male	Surgical ICU	Blood culture	11	-	-	Piperacillin-tazobactam Meropenem
16	47	Male	Surgical ICU	Blood culture	6	*C. freundii*	CRAB	Amoxicillin-clavulanic acid
17	57	Male	Surgical ICU	Blood culture	20	-	CRAB, CRPA	Amoxicillin-clavulanic acid Ertapenem
18	30	Male	Medical	Blood culture	104	-	CRAB	Rifafour Piperacillin-tazobactam Amikacin ertapenem Colistin Imipenem Cotrimoxazole Azithromycin Cefepime
19	21	Female	Haematology ICU	Blood culture	23	*K. pneumoniae*	-	Ciprofloxacin Piperacillin-tazobactam Amikacin Amoxicillin-clavulanic acid
23	32	Male	Surgical ICU	Blood culture	110	*E. cloacae*	CRAB ESBL CRPA	Amoxicillin-clavulanic acid Ceftriaxone Imipenem Meropenem Ertapenem Vancomycin Ampicillin Gentamycin Tigecycline Piperacillin-tazobactam Amikacin Erythromycin Ciprofloxacin Azithromycin Colistin
25	63	Male	Haematology ICU	Blood culture	47	-	MRSA	Piperacillin-tazobactam Amikacin Vancomycin Ciprofloxacin

								Meropenem

CRE: carbapenem resistant Enterobacterales, CRAB: carbapenem resistant *Acinetobacter baumannii*, CRPA: carbapenem resistant *Pseudomonas aeruginosa*, ICU: intensive care unit, ESBL: extended spectrum β-lactamase, MRO: multidrug resistant organisms, MRSA: methicillin resistant *Staphylococcus.*

Supplementary Table S2: Carbapenem antimicrobial susceptibility results.

	Ertapenem	Meropenem	Imipenem
Susceptible	2 (9.50%)	5 (23.81%)	2 (9.5%)
Intermediate	9 (42.86%)	7 (33.33%)	5 (21.81%)
Resistant	10 (47.62%)	9 (42.46%)	6 (28.57%)
Unavailable	0 (0%)	0 (0%)	8 (38.10%)