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Serratia marcescens enzyme SME-2 isolated from sputum in New Zealand

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Introduction: The *Serratia marcescens* enzymes (SMEs) are chromosomally encoded Ambler Class A carbapenem-hydrolysing β-lactamases, which distinctively express resistance to carbapenems while remaining susceptible to extended-spectrum cephalosporins. Global reports of SMEs are infrequent. Here we describe the isolation of an SME-2-producing *S. marcescens* from the sputum of a patient who was hospitalized at Christchurch Hospital, New Zealand.

Methods: An immunosuppressed asthmatic patient who presented with shortness of breath and hypoxia grew *S. marcescens* from a sputum culture. Antimicrobial susceptibilities were determined by Phoenix, with MICs of meropenem and imipenem determined by Liofilchem® MIC gradient strips and interpreted according to EUCAST breakpoints. Investigation for carbapenemase was performed using Carba NP, modified CIM (mCIM) and GeneXpert® Carba-R. WGS was performed using the Illumina DNA Prep library kit and sequenced using MiSeq.

Results: The isolate showed an unusual susceptibility profile, including high-level resistance to meropenem and imipenem, while remaining susceptible to extended-spectrum cephalosporins. The Carba NP and mCIM were positive and WGS demonstrated the presence of a bla_{SME-2} gene located on the chromosome within the SmarGI1-1 genomic island. In addition, a bla_{SRT}-like class C β-lactamase, *aac(6′)-Ic* aminoglycoside-modifying enzyme and various multidrug efflux mechanisms were found. Phylogenetic core-genome analysis indicated no matching genome with RefSeq database strains.

Conclusions: *S. marcescens* is an opportunistic pathogen of concern, harbouring a variety of intrinsic resistance mechanisms, including the potential for stable AmpC hyperproduction. Globally, SME-type carbapenemases have been infrequently reported; however, isolates carrying this mechanism could have limited treated options, having implications for patient management. To the best of our knowledge this is the first report of SME in New Zealand.

Introduction

The *Serratia marcescens* enzymes (SMEs) are Ambler Class A carbapenem-hydrolysing β-lactamases, produced by *S. marcescens*.[1] The SME carbapenemase belongs to the chromosomally encoded Bush–Jacoby 2f group of β-lactamases, and they distinctively express resistance to carbapenems and aztreonam, while remaining susceptible to extended-spectrum cephalosporins.[1]

Although SME-1 was detected decades ago, in England, in 1982, global reports of SME-1 and other SME variants remain infrequent, with only occasional small outbreaks and clusters.[2–4] Here we describe the isolation of an SME-2-producing *S. marcescens* from the sputum of a patient who was hospitalized at Christchurch Hospital. To the best of our knowledge, this is the first isolation of SME in New Zealand.

Methods

A 49-year-old patient presented at the emergency department, Christchurch Hospital, in late 2022, with shortness of breath and hypoxia. The patient had a medical history of asthma and was immunosuppressed. Their travel history was unknown. Initial investigations included a nasopharyngeal swab for a rapid PCR targeting influenza A, influenza B, parainfluenza, RSV and SARS-CoV-2. As part of a septic screen, a set of blood cultures was taken, with another two sets of blood cultures collected the following day. A sputum sample was sent to the laboratory for molecular detection of *Legionella* species and *Pneumocystis jirovecii*

and respiratory virus multiplex PCR. On Day 5, a second sputum sample was sent to the laboratory for bacterial culture. Isolate identification was performed using MALDI-TOF (Bruker) and antimicrobial susceptibilities were determined by broth microdilution (BMD) using Phoenix (BD, Sparks, USA), with interpretation according to EUCAST breakpoints.[5] Subsequent MIC testing against meropenem and imipenem was performed using Liofilchem® MIC gradient strips. Investigation for carbapenemase was performed using in-house Carba NP, modified CIM (mCIM) and GeneXpert® Carba-R, as previously described.[6]

WGS was performed using the Illumina DNA Prep library kit (Illumina, Australia). Libraries were sequenced using MiSeq (Illumina, Australia) with micro flow-cell, reagent kit v2, 300 cycles, according to the manufacturer's instructions. Bioinformatic analysis was performed with the following software using default settings (unless stated otherwise). FASTQ paired-end reads were quality trimmed and assembled into contigs using Shovill v0.9.0 (https://github.com/tseemann/shovill). Antimicrobial resistance genes were screened using AMRFinder software v3.11.11 and assembled sequences were annotated using Bakta v1.7.0.[7,8] Annotated genomes were viewed for gene synteny using Mauve (https://darlinglab.org/mauve/mauve.html). The NCBI reference sequence (RefSeq) database contains over 1000 *S. marcescens* genomes. *S. marcescens* genomes were downloaded (1 August 2023) and screened using mash distances.[9] Eight hundred and twenty-six genomes were selected for further analysis based on contig assembly number, purity and GC content. Sequences with more than one matching distance score were removed and the closest matching 100 sequences were used for phylogenetic analysis by Harvest Parsnp software.[10]

Results

The nasopharyngeal swab was negative for influenza A/B, parainfluenza, RSV and SARS-CoV-2. The initial sputum sample was negative for *Legionella* species and *P. jirovecii*, while the respiratory multiplex PCR was positive for only coronavirus OC43. Routine bacterial culture was not performed on the first sputum sample, so any bacterial pathogens that may have been present could not be detected. All three sets of blood cultures were negative after 5 days of incubation.

Culture from the second sputum sample revealed a moderate growth of a coliform bacillus, identified by MALDI-TOF as *S. marcescens*. Susceptibility results for the *S. marcescens* showed an unusual susceptibility profile, including high-level resistance to meropenem and imipenem and low-level aztreonam resistance, while remaining susceptible to extended-spectrum cephalosporins (ceftriaxone and ceftazidime), piperacillin/tazobactam, quinolones (norfloxacin and ciprofloxacin) and trimethoprim/sulfamethoxazole. MICs of aminoglycosides (amikacin, gentamicin, tobramycin) were below the epidemiological cut-off (ECOFF) values. *S. marcescens* is intrinsically resistant to ampicillin, amoxicillin/clavulanate, cefalexin, cefuroxime, cefoxitin, nitrofurantoin and colistin.[11] Susceptibility results are shown in Table 1. The patient was considered to have a coinfection with both *S. marcescens* and coronavirus OC43 and was successfully treated with piperacillin/tazobactam.

The Carba NP was positive within 15 min and the mCIM was also positive, producing no zone around the meropenem disc. The GeneXpert® Carba-R was negative for KPC, NDM, VIM, IMP and OXA-48-like enzymes. These results, together with the distinctive susceptibility profile in a *S. marcescens* isolate, were highly suggestive of an SME-type carbapenemase. The isolate was sent to the Microbiology Department, Middlemore Hospital (Otahuhu, Auckland), who initially confirmed the presence of SME using a TandemPlex CRE panel (AusDiagnostics, Australia). The isolate was also sent to the Institute for Environmental Science and Research (ESR, Wellington) for molecular confirmation.

Table 1. Antibiotic resistance profile of *S. marcescens*

Antimicrobial[a]	MIC (mg/L)	Category
Amikacin	≤4	
Ampicillin	>16	R
Amoxicillin/clavulanate	>32/2	R
Aztreonam	4	I
Cefepime	≤0.5	S
Cefoxitin	>16	R
Ceftazidime	≤0.5	S
Ceftriaxone	≤0.5	S
Cefuroxime	>16	R
Ciprofloxacin	≤0.125	S
Colistin	>4	R
Ertapenem	>2	R
Gentamicin	≤1	
Imipenem[b]	>32	R
Imipenem	>16	R
Meropenem[b]	32	R
Meropenem	16	R
Nitrofurantoin	>128	R
Piperacillin/tazobactam	≤4/4	S
Tigecycline	2	I
Tobramycin	2	
Trimethoprim/sulfamethoxazole	≤1/19	S

S, susceptible; I, susceptible, increased exposure; R, resistant.
[a]MIC determined by BD Phoenix BMD with the NMIC-422 panel.
[b]MIC determined by Liofilchem MIC Strips.

WGS detected a bla_{SME-2} gene, which was found on the chromosome near the *ampR* regulatory gene within a region known as *S. marcescens* genomic island SmarGI; see Figure 1.[12] A bla_{SRT}-like class C β-lactamase was found on the chromosome within the *ampR-ampC* operon. Other resistance mechanisms were detected including aminoglycoside-modifying enzyme *aac(6′)-Ic* gene and various multidrug efflux mechanisms. Phylogenetic core-genome analysis, as shown in Figure 2, indicates no matching genome with RefSeq database strains. There were only four other isolates containing bla_{SME-2} in the 826 strains downloaded from RefSeq and 11 bla_{SME} (2 bla_{SME-2} and 9 bla_{SME-4}) included in the phylogenetic analysis (blue nodes, Figure 2). These four bla_{SME-2} were from human isolates in the USA (blood culture, urine and unknown site) and Argentina (parotidectomy tissue). This Whole-Genome Shotgun BioProject ID: PRJNA1003958 has been deposited at DDBJ/ENA/GenBank under the accession JAVBXF000000000.

Discussion

S. marcescens is ubiquitous in the environment and can be found in a wide variety of sources including water (particularly in basins, sinks and showers in healthcare settings), soil, plants and animals. It is an important clinical pathogen, causing both community-acquired and nosocomial infections, most commonly involving the urinary tract, respiratory tract, wounds and sepsis.

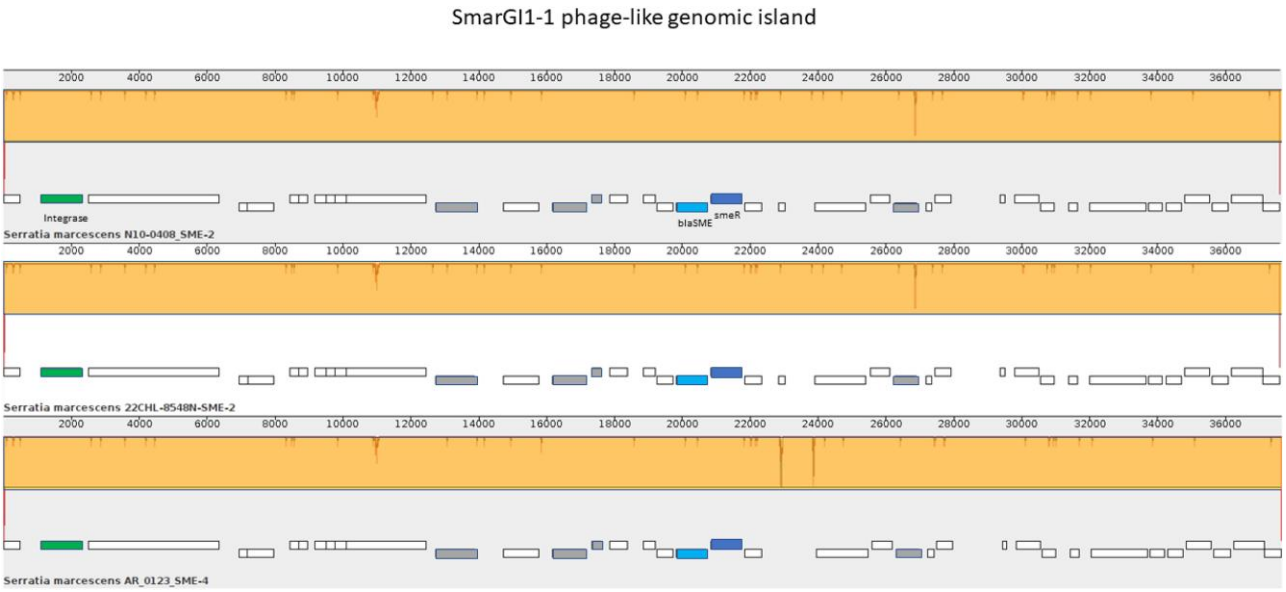

Figure 1. Shows conserved gene synteny within the *S. marcescens* genomic island SmarGI. The bla_{SME} and *smeR* genes are surrounded by mobile genetic elements (grey coloured boxes) and a phage-like integrase site (green box).

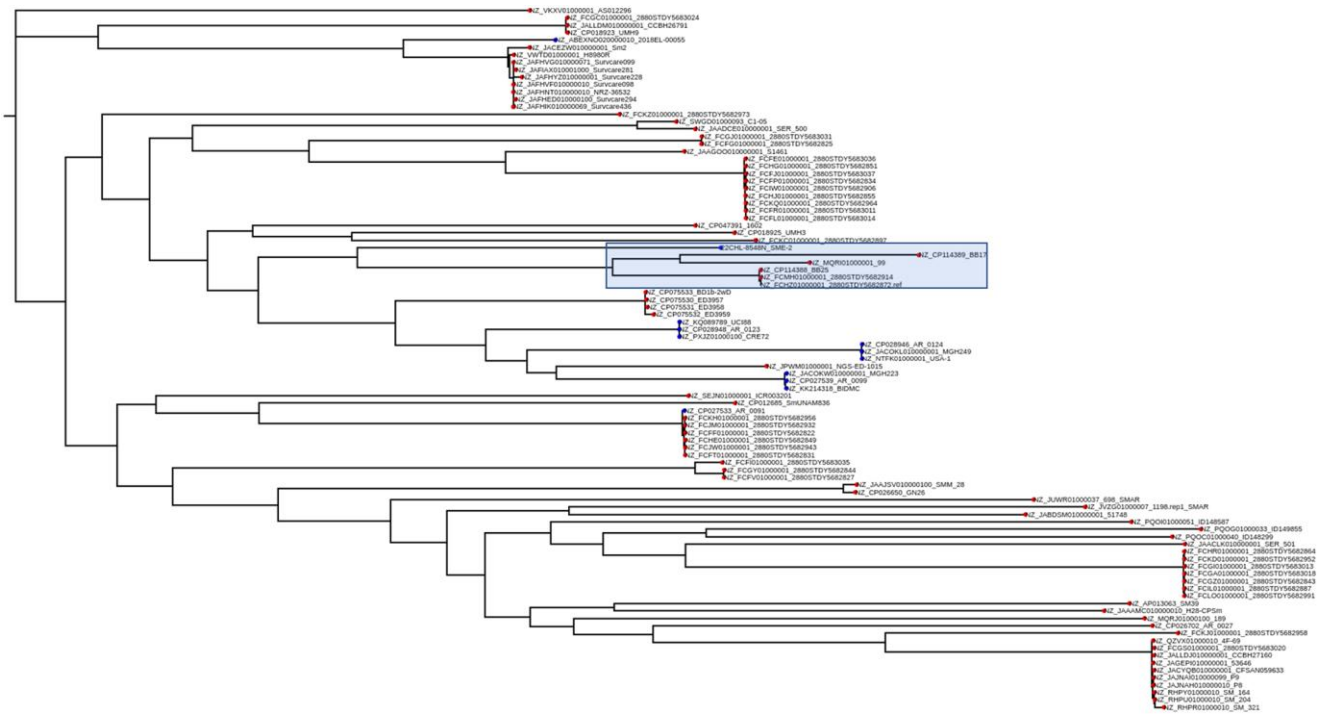

Figure 2. Phylogenetic analysis indicated that the genome for *S. marcescens* strain 22CHL-8548N-SME-2 is unique and does not cluster with other strains with bla_{SME} genes (blue coloured nodes). The highlighted region shows closest matching genomes in the RefSeq database.

In the hospital environment *S. marcescens* has been associated with nosocomial outbreaks, particularly involving ICUs or neonatal ICUs (NICUs).[13] Our isolate was detected in only one sputum sample from a single patient and was not recovered from any other routine patient samples from that ward. Due to the suspected chromosomal nature of the enzyme and multiple treatment choices, the infection control risk was regarded as low and standard infection control precautions were considered appropriate. No extra screening of other patients in the same ward nor environmental sampling was performed.

Antimicrobial resistance is acknowledged to be a substantial burden on health systems, involving significant morbidity and mortality, with lower respiratory tract infections estimated to contribute the highest toll.[14] *S. marcescens* is an opportunistic pathogen of concern, being both a respiratory tract pathogen and harbouring a variety of intrinsic resistance mechanisms. Furthermore, *S. marcescens* has the potential for stable AmpC hyperproduction, leading to extended-spectrum cephalosporin resistance. If the isolate is also harbouring an SME-type carbapenemase, treatment options would be severely limited. Recently, Hemarajata *et al.*[15] reported on such a case where prolonged antibiotic treatment with piperacillin/tazobactam and ciprofloxacin exerted selective pressure, leading to stable AmpC derepression in an SME-1-producing *S. marcescens*.

The aminoglycoside-modifying enzyme *aac(6′)-Ic* gene, which is chromosomally expressed in all *S. marcescens*, was detected in our isolate, but resistance was not phenotypically expressed.[2] The SME gene, bla_{SME}, was found to be carried on a genetic island, SmarGI1-1, that can exist in a circular form, enabling genetic transfer between *S. marcescens* strains.[12] However, the rate of propagation appears to remain low, perhaps due to an associated fitness cost and, to date, bla_{SME} has not been found outside of *S. marcescens*.[1,16] Analysis of *S. marcescens* strains in the NCBI RefSeq database shows a very limited number of strains with bla_{SME} and only four containing bla_{SME-2}.

Currently, SME-2 has been reported from only a limited number of countries, including Australia, USA, Canada, Switzerland and Argentina.[3,4] Indeed, Australia, as our nearest neighbour, have only scant reports of SMEs in their annual CARAlert reports.[17] SME-2 differs from SME-1 by a single amino acid change and shows similar hydrolysis kinetics against β-lactams, which aligns with our susceptibility results.[18] Our *S. marcescens* isolate with bla_{SME-2} gene was unique; however, even though SMEs appear to be present in only a small subset of *S. marcescens* isolates, it is possible that SMEs are underrecognized in clinical diagnostic laboratories causing ascertainment bias. The limited data may in part be due to the failure to include a carbapenem in antimicrobial susceptibility panels, particularly for direct susceptibility testing, or that bla_{SME} is not included in molecular detection tests.[19] It would be prudent for laboratories to follow EUCAST or CLSI guidelines for carbapenemase detection.

One limitation of our study was the use of Phoenix and MIC gradient strips to determine antimicrobial MICs rather than reference BMD; however, these approximate methods are widely used in clinical practice and the results concurred with WGS analysis.

Here we have described the first known detection of an SME, from an SME-2-producing *S. marcescens*, in New Zealand.

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Transparency declarations

None to declare.

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