Case report

Characterization of a rare $\text{bla}_{\text{VIM-4}}$ metallo-β-lactamase-producing *Serratia marcescens* clinical isolate in Hungary

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ARTICLE INFO

Keywords:
- Microbiology
- Bacteria
- Microorganism
- Public health
- Infectious disease
- Pharmacology
- Antibiotic resistance
- β-lactam antibiotics
- Metallo-β-lactamases
- *Serratia marcescens*
- Sequencing

ABSTRACT

A carbapenem-resistant *S. marcescens* isolate was recovered from a patient with an inflamed pacemaker implantation pocket from a Cardiac Surgery ward in a Hungarian University Hospital. Phenotypic tests and polymerase chain reaction (PCR) confirmed a very rare gene responsible for production of a carbapenemase ($\text{bla}_{\text{VIM-4}}$), which was further characterized by Sanger-sequencing. The characterization of this *S. marcescens* strain emphasizes the ongoing emergence of novel or rare carbapenemases. Strains expressing a weak carbapenemase like this strain might go unrecognized by routine diagnostics due to low minimum inhibitory concentrations (MICs) for the bacterial strains producing such enzymes.

1. Introduction

*Serratia marcescens* (*S. marcescens*) is the most commonly isolated species within the genus, which was discovered by Bizio, an Italian pharmacist in 1819, as the cause of the bloody discolouration on cornmeal mush [1]. Members of this species are ubiquitous in the environment; this opportunistic pathogen is an important cause of hospital-acquired infections (HAIs), which are often associated with serious outbreaks: different contaminated medical devices, intravenous fluids and medical solutions are frequent sources of nosocomial infections [2,3]. *S. marcescens* is most frequently isolated from respiratory tract infections, including ventilator-associated bacterial pneumonia, meningitis and ocular infections [4, 5, 6, 7]. Other common sources include complicated and uncomplicated skin and soft tissue infections, urinary tract infections (UTIs), as well as bloodstream infections [8, 9]. The main risk factors for *Serratia* bacteraemia/sepsis are hospitalization, placement of intravenous, intraperitoneal and urinary catheters and prior instrumentation of the respiratory tract [9, 10, 11]. *S. marcescens* infections are a great concern, as this organism is intrinsically resistant to a large number of antibiotics including ampicillin, aminopenicillin/β-lactamase-inhibitor combinations, cefuroxime, nitrofurantoin and polymixins [12]. Resistance in *S. marcescens* is conferred by several chromosomal and plasmid-mediated resistance determinants, which facilitate the spread of resistance genes among the different species. During the last two decades, AmpC cephalosporinase–producing *S. marcescens*, *Enterobacter cloacae* and *Citrobacter freundii* have emerged as the most important causes of HAIs and the prevalence of extended-spectrum β-lactamase (ESBL)-producing enzymes in these organisms is very high globally [13, 14].

Carbapenem-resistance in *S. marcescens* is still very rare: the possible mechanisms include the production of plasmid-encoded (KPC, OXA-48, IMP, VIM, NDM) or chromosomal (SME) metallo-β-lactamases (MBLs) or porin-mutations. *S. marcescens* enzymes (SMEs) are class A carbapenemases found on the chromosome, which were first identified in the UK in 1982; these enzymes (NMC-A, Sme-1 to Sme-3, IMI-1) are very rare and they are inhibited by clavulanic acid [15]. Class B MBLs (i.e. IMP [Impenemase], VIM [Verona Integron-encoded Metallo-β-lactamase] and NDM [New Delhi metallo-beta-lactamase]) are the most common (they have been reported worldwide but most frequently from Southeast Asia).
Asia and different European countries) and clinically important as they can hydrolyze the broadest spectrum of β-lactams (except for aztreonam) and they can spread via plasmids and integrons of S. marcescens [16]. Only a few reports are available on carbapenem-resistant S. marcescens, where carbapenem-R was attributed to carbapenemase production. IMP-like MBL were the first reported transferable carbapenemases in Gram-negative bacteria [17]; in line with this, IMP-1 was the first carbapenemase identified as a source of acquired resistance to carbapenems in a S. marcescens isolate from a patient with a UTI from Japan in 1991 [18]. Although IMP-like MBL was the earliest transferable carbapenemases reported in Gram-negative bacteria, which was followed by VIM types, the Verona-integron encoded metallo-beta lactamas occurred mostly in P. aeruginosa isolations and very rarely in the members of Enterobacteriales [19].

2. Case presentation

A 65-year-old male patient was admitted to the Department of Internal Medicine, Albert Szent-Györgyi Clinical Center for a pacemaker implantation, due to a Grade III AV-block. According to the Institutional protocols for implantation procedures, a single-dose cefazolin-prophylaxis was administered. The patient was released home one week after the operation without any complaints, however, two weeks later, the patient returned to the Clinical Center with complaints of a permanent bleeding of the surgical wound. The pacemaker pocket was re-opened, and the pacemaker was subsequently removed. In the pacemaker pocket, a large amount of gore and gelatinous tissue-debris was found. The debris was removed and from this material, samples were sent to the microbiology laboratory for analysis. The pacemaker itself as well as the leads were ruled out as a source of infection. The pocket was drained by povidone-iodine and a single dose of vancomycin powder for solution was dispensed at the site locally. The patient received an empiric therapy of iv. cefprozil; after the microbiology and susceptibility results were available, therapy was switched to ceftazidime plus gentamicin for 14-d. Antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) determination were carried out on Mueller-Hinton agar plates (bioMérieux, Marcy-l’Étoile, France), using disk diffusion and gradient tests (Liofilchem, Roseto degli Abruzzi, Italy), respectively, as recommended by EUCAST Standard Procedures. Colistin susceptibility testing was performed by broth microdilution method (MERLIN Diagnostic, Bornheim-Hersel, Germany). Interpretation of the results was performed based on EUCAST breakpoints v.9.0.

ESBL-production was investigated by a modified double-disk synergy test, using cefazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg) and aztreonam (30 μg) disks, containing 3-aminocephalosporonic acid (APB) (300 μg) opposite to the amoxicillin/calulanic acid (20 μg/10 μg) disk [20]. The putative carbapenemase-production were detected phenotypically by the modified Hodge-test and a phenotypic method utilizing meropenem, cefepime and ceftazidime disks, either alone or combined with dipicolinic acid (835 μg). Additionally, confirmation of carbapenemase production was performed using a phenotypic inhibition assay with commercially available diagnostic disks (KPC + MBL Confirmation ID Kit; Rosco, Taastrup, Denmark).

The molecular characterization of the strain was performed at the National Institute of Public Health (Budapest, Hungary). The presence of different MBL genes (blaVIM, blabIM, blabIM, blabIM, blasPM, blasCM) and class-1 integron was tested by a PCR method, using primers and technical specifications described previously [21, 22, 23, 24, 25, 26]. PCR products were purified (GFX PCR DNA kit Amersham Biosciences). PCR amplions were further characterized by Sanger-sequencing. Sequence analysis and alignment was performed using Vector NTI suite 9 (InforMax Inc.). Resulting nucleotide sequences were compared with sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov/nuccore). The PCR mapping of integrons was conducted on the isolate using primers described previously [27].

3. Materials and methods

Sample processing in our Institute was carried out according to guidelines for routine clinical bacteriology. The wound samples were cultured on Columbia blood agar (bioMérieux, Marcy-l’Étoile, France), chocolate agar (bioMérieux, Marcy-l’Étoile, France) and eosin-methylene blue agar (bioMérieux, Marcy-l’Étoile, France). The identification of the isolate was carried out using the VITEK 2 Compact ID/AST system, according to the manufacturer’s recommendations (bioMérieux, Marcy-l’Étoile, France).

Antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) determination were carried out on Mueller-Hinton agar plates (bioMérieux, Marcy-l’Étoile, France), using disk diffusion and gradient tests (Liofilchem, Roseto degli Abruzzi, Italy), respectively, as recommended by EUCAST Standard Procedures. Colistin susceptibility testing was performed by broth microdilution method (MERLIN Diagnostik, Bornheim-Hersel, Germany). Interpretation of the results was performed based on EUCAST breakpoints v.9.0.

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4. Results of the laboratory analyses

From the wound samples, a S. marcescens strain was isolated in a high colony-forming unit (CFU). Based on disk diffusion, the strain was imipenem-R, ertapenem-R and R to 3rd and 4th generation cephalosporins, while gentamicin-S, ciprofloxacin-S, levofloxacin-S and meropenem-S. MIC values for the investigated strain are presented in Table 1. The results of phenotypic tests supposed that the strain did not produce extended-spectrum β-lactamase enzyme, but produced a metallo-β-lactamase.

The molecular investigations revealed that the blaVIM,4 metallo-β-lactamase gene harbored by this isolate was part of a class-1 integron. According to the INTEGRALL database (http://integrall.bio.ua.pt), this integron was designated as In238b. The nucleotide sequence of the integron was assigned to GenBank under accession number JF905459 (https://www.ncbi.nlm.nih.gov/nuccore/JF905459).

5. Discussion

blaIMP and blaVIM genes are horizontally transferable as they are inserted into integrons (In) and some of these integrons are located on conjugative plasmids. Due to this ability to spread, carbapenem resistance related to IMP and VIM β-lactamas has become a serious concern. Until now, several outbreaks of KPC-2 producing S. marcescens has been published [28, 29], however, reports on outbreaks caused by VIM-producing S. marcescens are infrequent, and most of them occurred in ICUs [30, 31, 32]. Nastro et al. published the first nosocomial outbreak (with three patients affected) caused by VIM16-producing S. marcescens strains in Argentina in 2013 [31]; neither blaCTX,M nor blaESBL genes (which represented the most prevalent ESBLs in Argentina at the time) were detected in these isolates. Iovene et al. reported the first documented nosocomial outbreak of VIM-producing S. marcescens in Europe in an adult ICU [32], with a fatality rate of 50%. Ghait et al. was first to report the detection of MBL-producing S. marcescens isolates (including strains harbouring the blaIMP,4 [42.5%] and blavim,2 [37.5%] genes) from inpatients with bacteremias in Egypt [30]. Pérez-Viso et al. investigated a total of 21 S. marcescens isolates which were displaying a multidrug-resistant phenotype in a Madrid University Hospital. Nineteen patients (most of them in the ICU; 89%) were colonized with VIM-1 producing S. marcescens strains; 17 of these isolates were clustered into a single clone (99% similarity) [23].

Carbapenems are usually used as safe last-line treatments for ESBL-producing Gram-negative bacterial infections; subsequently, acquired resistance to carbapenems due to carbapenemase production has been increasingly reported over the last 15 years in Pseudomonas aeruginosa, Acinetobacter spp., as well as members of the Enterobacteriales.
order [34, 35]. Some reports highlighted the emergence of multi-resistant *S. marcescens* strains: in these studies, *S. marcescens* isolates produced plasmid-mediated *blaIMP*-like MBLs or KPC-2 [36,37]. In contrast, other studies showed MDR *S. marcescens* strains that remained sensitive to carbapenems [38]. The strain in our case remained susceptible to gentamicin and fluoroquinolones, therefore still left clinicians with adequate treatment options. Estimating their incidence, complications, and attributable mortality associated with multidrug resistant *Serratia* is challenging [39]. Our results are consistent with the European survey on carbapenemase-producing *Enterobacteriales*, which highlighted the geographical heterogeneity of carbapenemase-producing *Enterobacteriales* in the EU and EEA, and the endemic situation in Italy and Greece, where the incidence of such infections per 100 000 patient-days was the highest of all EU and EEA countries [34, 39, 40, 41]. VIM-type MBLs occur more often in non-fermenting Gram-negative bacteria, while among *Enterobacteriales*, these enzymes were detected most frequently in *K. pneumoniae*, *E. coli* or *Enterobacter cloacae*. According to the results of the global survey regarding their prevalence in *Enterobacteriales* by Matsumura et al., VIM-1 has a global distribution, VIM-2 was present in Mexico and Spain, VIM-4 in Europe (only one *S. marcescens* strain from Czech Republic), VIM-5 and -31 in Turkey, VIM-19, -26, -27 and -33 were limited to Greece, VIM-23 in Mexico and VIM-29 was present in Saudi Arabia and the UK [42]. The structure of integron In238b found in this strain was identical with the integron previously characterized for a VIM-4–bearing *P. aeruginosa*: this was the first identified carbapenemase in Hungary in 2002 [43]. In238b was later found in *Aeromonas hydrophila*, *K. pneumoniae* sequence type 11 (ST11) and *Klebsiella oxytoca* [27, 44]. The common origin of these integrons was suspected. In all previously mentioned cases, low-level carbapenem-resistance was detected, which may prove to be difficult to detect using phenotypic methods.

### Table 1. MIC values of the tested *S. marcescens* strain.

| Antibiotics     | MIC (g/L) | Interpretation  |
|-----------------|-----------|-----------------|
| Imipenem        | 8         | Resistant       |
| Ertapenem       | 4         | Resistant       |
| Meropenem       | 1         | Resistant       |
| Cefotaxime      | 128       | Susceptible     |
| Cefazidime      | 4         | Resistant       |
| Gentamicin      | 2         | Susceptible     |
| Amikacin        | 32        | Resistant       |
| Ciprofloxacin   | 0.5       | Susceptible     |
| Tigecycline     | 4         | Resistant       |
| Trimethoprim/sulfamethoxazole | 4   | Intermediate    |
| Colistin        | ≥256      | Resistant       |

### 6. Conclusions

To the best of our knowledge, this is the first time that a VIM-type MBL-producing *S. marcescens* was isolated from a clinical sample in Hungary and characterized by sequencing; the isolate was only detected from the patient, no carrier or environmental source was identified. Additionally, the patient had no history of ever taking carbapenem antibiotics. The isolation of this VIM-4 producing *S. marcescens* is yet another chain in the event of emergence (in a *P. aeruginosa* strain in 2002) and subsequent dissemination of *blaVIM-4* gene in Hungary. The presented results show that the ongoing dissemination of carbapenem-resistant *Enterobacteriales* is further expanding across healthcare systems in Europe. This trend highlights the need for enhanced containment efforts within countries as well as concerted action at a European level.

### Declarations

**Author contribution statement**

Ákos Tóth: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Attila Makai: Analyzed and interpreted the data.

Laura Jánvári, Ivelina Damjanova: Performed the experiments.

Máró Gajdacs: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Edit Urban: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

**Funding statement**

This work was supported by the University of Szeged Open Access Fund (Grant number: 4701). M.G. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. M.G. was also supported by the ESMID’s “30 under 30” Award.

**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

Data associated with this study has been deposited at GenBank under the accession number JF905459.

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