Case report

Characterization of a rare bla_{VIM-4} metallo-β-lactamase-producing *Serratia marcescens* clinical isolate in Hungary

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A B S T R A C T

A carbapenem-resistant *S. marcescens* isolate was recovered from a patient with an inflamed pacemaker inplantation 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 (bla_{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 of 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 [Imipenemase], 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

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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 lactamases occured mostly in *P. aeruginosa* isolations and very rarely in the members of *Enterobacterales* [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 cefazoline-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 drainaged by povidone-ioidine and a single dose of vancomycin powder for solution was dispersed 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. According his anamnestic data, the patient has never received any carbapenem-therapy earlier in life. After treatment as an inpatient, the patient surgical site healed and no further complications were noted; subsequently, the patient was discharged. The environment of the Clinical Ward, the patients and the medical staff were screened in line with national and international infection control guidelines, however, the strain could not be found in the hospital environment, and none of the patients or medical staff was found to be a carrier of the strain. Thus, it was assumed that the strain was introduced to the Ward by the patient.

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 eosine-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.

ESBL-production was investigated by a modified double-disk synergy test, using ceftazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg) and aztreonam (30 μg) disks, containing 3-aminophenylboronic 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 discs (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 (bla_{VIM}, bla_{IMP}, bla_{SIM}, bla_{GIM}, bla_{SPM}, bla_{NDM}) 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 amplicons were further characterized by Sanger-sequencing. Sequence analysis and alignment was performed using Vecton NTI suite 9 (InforMax Inc.). Resulting nucleotide sequences were compared with sequences obtained from GenBank (http://www.lahey.org/studies/webt.html). The PCR mapping of integrons was conducted on the isolate using primers described previously [27].

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 bla_{VIM-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

bla_{IMP} and bla_{VIM} 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-lactamases 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 bla_{CTX-M} nor bla_{PER} 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 bla_{IMP-4} [42.5%] and bla_{VIM-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* stains; 17 of these isolates were clustered into a single clone (>95% similarity) [33].

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 aeruginosa*, *Acinetobacter* spp., as well as members of the *Enterobacterales*

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

Antibiotics	MIC	Interpretation
Imipenem	8 mg/L	Resistant
Ertapenem	4 mg/L	Resistant
Meropenem	1 mg/L	Susceptible
Cefotaxime	128 mg/L	Resistant
Ceftazidime	4 mg/L	Resistant
Gentamicin	2 mg/L	Susceptible
Amikacin	32 mg/L	Resistant
Ciprofloxacin	0.5 mg/L	Susceptible
Tigecycline	4 mg/L	Resistant
Trimethoprim/sulfamethoxazole	4 mg/L	Intermediate
Colistin	≥256 mg/L	Resistant

order [34, 35]. Some reports highlighted the emergence of multi-resistant *S. marcescens* strains: in these studies, *S. marcescens* isolates produced plasmid-mediated bla_{IMP}-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 *Enterobacterales*, which highlighted the geographical heterogeneity of carbapenemase-producing *Enterobacterales* 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 *Enterobacterales*, 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 *Enterobacterales* 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 were suspected. In all previously mentioned cases, low-level carbapenem-resistance was detected, which may prove to be difficult to detect using phenotypic methods.

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 bla_{VIM-4} gene in Hungary The presented results show that the ongoing dissemination of carbapenem-resistant Enterobacterales 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

References

[1] C.P. Merlino, Bartholomeo Bizios letter to the most eminent priest, Anggelo Bellani, concerning the phenomenon of the red coloured polenta, J. Bacteriol. 9 (1924) 527. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC379088/.

[2] A. Hejazi, F.R. Falkiner, Serratia marcescens, J. Med. Microbiol. 46 (1997) 903–912.

[3] A.H. Byrne, B. Boyle, C.M. Herra, R. Hone, C.T. Keane, Serratia marcescens causing hospital-acquired lower respiratory tract infection, J. Hosp. Infect. 45 (2000) 242–244.

[4] M.M. Cullen, A. Trail, M. Robinson, M. Keaney, P.R. Chadwick, Serratia marcescens outbreak in a neonatal intensive care unit prompting review of decontamination of laryngoscopes, J. Hosp. Infect. 59 (2005) 68–70.

[5] A. Hejazi, H.M. Aucken, F.R. Falkiner, Epidemiology and susceptibility of Serratia marcescens in a large general hospital over an 8-year period, J. Hosp. Infect. 45 (2000) 42–46.

[6] Pursell K. Liangpunsakul, Community-acquired necrotising fasciitis caused by Serratia marcescens: case report and review, Eur. J. Clin. Microbiol. Infect. Dis. 20 (2001) 509–521.

[7] A.Y. Peleg, D.C. Hooper, Hospital-acquired infections due to gram-negative bacteria, N. Engl. J. Med. 362 (2010) 1804–1813.

[8] E. Us, H.H. Kutlu, A. Tekeli, D. Ocal, S. Cipran, K.O. Memikoglu, Wound and soft tissue infections of Serratia marcescens in patients receiving wound care: a health care–associated outbreak, Am. J. Infect. Contr. 45 (2017) 443–447.

[9] H.J. Yoon, J.Y. Choi, Y.S. Park, C.O. Kim, J.M. Kim, D.E. Yong, et al., Outbreaks of Serratia marcescens bacteriuria in a neurosurgical intensive care unit of a tertiary care teaching hospital: a clinical, epidemiological and laboratory perspective, Am. J. Infect. Contr. 33 (2005) 595–601.

[10] S.D. Mahlen, Serratia infections: from military experiments to current practice, Clin. Microbiol. Rev. 24 (2011) 755–791.

[11] V. Crivaro, M. Bagattini, M.F. Salza, F. Raimondi, F. Rossano, M. Triassi, et al., Risk factors for extended spectrum beta lactamase producing Serratia marcescens and Klebsiella pneumoniae acquisition in a neonatal intensive care unit, J. Hosp. Infect. 67 (2) (2007) 135–141.

[12] I. Stock, T. Grueger, B. Wiedemann, Natural antibiotic susceptibility of strains of Serratia marcescens and the S. liquefaciens complex: S. liquefaciens sensu stricto, S. proteamaculans and S. grimesii, Int. J. Antimicrob. Agents 22 (2003) 35–47.

[13] W.E. Sanders, C.C. Sanders, Circumventing antibiotic resistance in specialized hospital units, Clin. Microbiol. Infect. 3 (1997) 272.

[14] Y.J. Park, S.Y. Park, E.J. Oh, J.J. Park, K.Y. Lee, G.J. Woo, K. Lee, Occurrence of extended-spectrum beta-lactamases among chromosomal AmpC-producing Enterobacter cloacae, Citrobacter freundii, and Serratia marcescens in Korea and investigation of screening criteria, Diagn. Microbiol. Infect. Dis. 51 (2005) 265–269.

[15] Y.J. Yang, P.J. Wu, D.M. Livermore, Biochemical characterization of a β-lactamase that hydrolyzes penems and carbapenems from two *Serratia marcescens* isolates, Antimicrob. Agents Chemother. 34 (1990) 755–758.

[16] M. Gajdács, E. Urbán, Resistance trends and epidemiology of *Citrobacter-Enterobacter-Serratia* in urinary tract infections of inpatients and outpatients (RECESUTI): a 10-year survey, Medicina 55 (2019) e285.

[17] M. Watanabe, S. Iyobe, M. Inoue, S. Mitsuhashi, Transferable imipenem resistance in *Pseudomonas aeruginosa*, Antimicrob. Agents Chemother. 35 (1991) 147–151.

[18] E. Osano, Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F. Yoshimura, N. Kato, Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance, Antimicrob. Agents Chemother. 38 (1994) 71–78.

[19] L. Lauretti, M. Riccio, L. Mazzariol, G. Cornalgia, G. Amicosante, R. Fontana, et al., Cloning and characterization og blaVIM, a new integrin-borne metallo-beta-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate, Antimicrob. Agents Chemother. 43 (1999) 1548–1590. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC89328/.

[20] J.D. Pitout, M.D. Reisbig, E.C. Venter, D.L. Church, N.D. Hanson, Modification of double-disk test for detection of *Enterobacteriaceae* producing extended spectrum and AmpC b-lactamases, J. Clin. Microbiol. 41 (2003) 3933–3935.

[21] G. Arlet, M. Rouveau, A. Philippon, Substitution of alanine for aspartate at position 179 in the SHV-6 extended-spectrum β-lactamase, FEMS Microbiol. Lett. 152 (1997) 163–167.

[22] H. Hasman, D. Mevius, K. Veldman, I. Olesen, F.M. Aarestrup, β-lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands, J. Antimicrob. Chemother. 56 (2005) 115–121.

[23] R.S. Hendriksen, M. Mikoleit, C. Kornschober, R.L. Rickert, S.V. Duyne, C. Kjelso, H. Hasman, et al., Emergence of multidrug-resistant *Salmonella* Concord infections in Europe and the United States in children adopted from Ethiopia, 2003–2007, Pediatr. Infect. Dis. J. 28 (2009) 814–818.

[24] I. Olesen, H. Hasman, F.M. Aarestrup, Prevalence of β-lactamases among ampicillin-resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark, Microb. Drug Resist. 10 (2004) 334–340.

[25] F.J. Perez-Perez, N.D. Hanson, Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR, J. Clin. Microbiol. 40 (2002) 2153–2162.

[26] L. Poirel, T.R. Walsh, V. Cuvillier, P. Nordmann, Multiplex PCR for detection of acquired carbapenemase genes. 2011, Diagn. Microbiol. Infect. Dis. 70 (2011) 119–123.

[27] K. Kristóf, Á. Tóth, I. Damjanova, L. Jánvári, M. Konkoly-Thege, B. Kocsis, R. Koncan, G. Cornalgia, E. Szegő, K. Nagy, D. Szabó, Identification of a blaVIM-4 gene in the internationally successful *Klebsiella pneumoniae* ST11 clone and in a *Klebsiella oxytoca* strain in Hungary, J. Antimicrob. Chemother. 65 (2010) 1303–1305.

[28] A. Tsakris, E. Voulgari, A. Poulou, M. Kimouli, S. Pournaras, K. Ranellou, O. Kosmopoulou, D. Petropoulou, In vivo acquisition of a plasmid-mediated bla(KPC-2) gene among clonal isolates of *Serratia marcescens*, J. Clin. Microbiol. 48 (2010) 2546–2549.

[29] J.C. Cai, H.W. Zhou, R. Zhang, G.X. Chen, Emergence of *Serratia marcescens*, *Klebsiella pneumoniae*, and *Escherichia coli* Isolates possessing the plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC-2 in intensive care units of a Chinese hospital, Antimicrob. Agents Chemother. 52 (2008) 2014–2018.

[30] D.M. Ghaith, M.M. Zafer, D.K. Ismail, M.H. Al-Agamy, M.F.F. Bohol, A. Al-Qahtani, M.N. Al-Ahdal, S.M. Elnagdy, I.Y. Mostafa, First reported nosocomial outbreak of *Serratia marcescens* harbouring bla$_{IMP-4}$ and bla$_{VIM-2}$ in a neonatal intensive care unit in Cairo, Egypt, Infect. Drug Resist. 11 (2018) 2211–2217.

[31] M. Nastro, R. Monge, J. Zintgraff, L.G. Vaulet, M. Boutureira, A. Famiglietti, C.H. Rodriguez, First nosocomial outbreak of VIM-16-producing *Serratia marcescens* in Argentina, Clin. Microbiol. Infect. 19 (2013) 617–619.

[32] M.R. Iovene, V. Pota, M. Galdiero, G. Giusy Corvino, F.M. Di Lella, D. Stelitano, M.B. Passavanti, et al., First Italian outbreak of VIM-producing *Serratia marcescens* in an adult polyvalent intensive care unit, August-October 2018: a case report and literature review, World J. Clin. Cases 7 (2019) 3535–3548.

[33] B. Pérez-Viso, V. Turrientes, N.L. Fresneña, C. Díaz-Agero, M.I.M. Reilly, P. Ruiz-Garbajosa, R.M. Cantón, P2349 Carbapenemase-producing *Serratia marcescens* in a university hospital in Madrid (Spain) is dominated by VIM producers due to the spread of a single clone. 28th ECCMID Madrid, Spain. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=2ahUKEwjsmpXR5K3pAhXJ4KYKHSXZANMQFjAAegQIAxAC&url=https%3A%2F%2Fwww.escmid.org%2Fescmid_publications%2Fescmid_elibrary%2Fmaterial%2F%3Fmid%3D62762&usg=AOvVaw1Ku1wLs7lgngNBsFSLg1YC.

[34] M. Gajdács, The concept of an ideal antibiotic: implications for drug design, Molecules 24 (2019) 892.

[35] B. Albiger, C. Glasner, M.J. Struelens, H. Grundmann, D.L. Monnet, European Survey of Carbapenemase-Producing Enterobacteriaceae Working Group, Carbapenemase-producing *Enterobacteriaceae* in Europe: assessment by national experts from 38 countries, May 2015, Euro Surveill. 20 (2015) 30062.

[36] A.C. Da Costa Guimares, A.C. Almeida, A.G. Nicoletti, Clonal spread of carbapenem-resistant *Serratia marcescens* isolates sharing an IncK plasmid containing blaKPC-2, Int. J. Antimicrob. Agents 42 (2013) 369–370.

[37] W. Jamal, V.O. Rotimi, M.J. Albert, High prevalence of VIM-4 and NDM-1 metallo-beta-lactamase among carbapenem-resistant *Enterobacteriaceae*, J. Med. Microbiol. 62 (2013) 1239–1244, 2013.

[38] M. Sisirak, M. Hukic, An outbreak of multidrug-resistant *Serratia marcencens*: the importance of continuous monitoring of nosocomial infections, Acta Medica Academica 42 (2013) 25–31.

[39] J.D. Bard, J.F. Hindler, M.A. Lewinski, Carbapenemase-producing *Serratia marcescens*: the treatment conundrum, Clin. Microbiol. Newsl. 32 (2010) 77–78.

[40] A. Brolund, N. Lagerqvist, S. Byfors, M.J. Struelens, D.L. Monnet, B. Albiger, Kohlenberg, European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) Capacity Survey Group, Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, Euro Surveill. 24 (2019) 1900123.

[41] A. Cassini, L.D. Högberg, D. Plachouras, A. Quattrocchi, A. Hoxha, G.S. Simonsen, M. Colomb-Cotinat, M.E. Kretzschmar, B. Devleesschauwer, M. Cecchini, D.A. Ouakrim, T.C. Oliveira, M.J. Struelens, C. Suetens, D.L. Monnet, Burden of AMR Collaborative Group, Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis, Lancet Infect. Dis. 19 (2019) 56–66.

[42] Y. Matsumura, G. Peirano, R. Devinney, P.A. Bradford, M.R. Motyl, M.D. Adams, L. Chen, B. Kreiswirth, J.D.D. Pitout, Genomic epidemiology of global VIM-producing Enterobacteriaceae, J. Antimicrob. Chemother. 72 (2017) 2249–2258.

[43] B. Libisch, M. Gacs, K. Csiszar, M. Muzslay, L. Rokusz, M. Fuzi, Isolation of an integron-borne blaVIM-4 type metallo-β-lactamase gene from a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate in Hungary, Antimicrob. Agents Chemother. 48 (2004) 3576–3578.

[44] B. Libisch, C.G. Giske, B. Kovacs, T.G. Toth, M. Fuzi, Identification of the first VIM metallo-β-lactamase-producing multiresistant *Aeromonas hydrophila* strain, J. Clin. Microbiol. 46 (2008) 1878–1880.