Emergence of Metallo-β-lactamase GIM-1 in a clinical isolate of *Serratia marcescens*

Heime Rieber¹,*,†, Andre Frontzek¹,†, Yvonne Pfeifer²

¹Medizinisches Versorgungszentrum Dr. Stein, Division of Microbiology, 41061 Mönchengladbach, Germany; ²Nosocomial Infections, Robert-Koch Institute, 38855 Wernigerode, Germany

*Corresponding author: Heime Rieber, Medizinisches Versorgungszentrum Dr. Stein, Division of Microbiology, Wallstraße 10, 41061 Mönchengladbach, Germany Tel: +49 (0) 2161-81940, Fax: +49 (0) 2161-819450, E-mail: hrieben@labor-stein.de

† authors contributed equally to the present work

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The Metallo-β-Lactamase GIM-1 (German imipenemase) has been found so far only in clinical isolates of *Pseudomonas aeruginosa* from Germany. Here we report the detection of *bla*GIM-1 in a clinical strain of *Serratia marcescens* isolated from urine, blood and wound samples over a period of 20 months. The strain was repeatedly isolated from one patient in two German hospitals and outpatient department located in the region where all previously described GIM-1 producing *P. aeruginosa* were identified.

Within *Serratia* spp. belonging to the *Enterobacteriaceae* family, *Serratia marcescens* is the most common detected species associated with nosocomial infections of respiratory tract, urinary tract and bloodstream infections. Outbreaks of *Serratia* spp. were caused by contamination of medical products (intravenous fluids, catheters) and equipments (apparatuses) mainly transmitted by the clinical personnel (11). Resistance to many β-lactams due to β-lactamase production in combination with resistance to various other antimicrobial agents have become a serious threat. In the last decade various extended-spectrum β-lactamases (TEM-, SHV-, CTX-M-, GES- and BES-type) and different carbapenemases (SME-, KPC-, OXA-48, VIM- and IMP-type) were identified in *S. marcescens* (2, 6, 7, 10, 15, 16, 18, 20, 22). Here we report the isolation of multidrug-resistant GIM-1-producing *S. marcescens* from Germany.

A 53 year old patient suffering from chronic renal insufficiency was hospitalized with urosepsis in January 2009. From blood culture and urine a *S. marcescens* strain (MG2504) was isolated. An empiric antimicrobial therapy with imipenem was successful and was not modified upon receiving the microbiology results. Altogether seven isolates (blood, n=1; urine, n=4; hypogastric wound, n=2) being clonally identical by *XbaI* macrorestriction followed by pulsed-field gel electrophoresis (PFGE; data not shown) were collected from this patient within a period of 20 months from two hospitals and one outpatient department in where the patient was hospitalized. All *S. marcescens* isolates, except the one from blood, did
not cause infections why further antibiotic treatment was not necessary. The three healthcare
facilities are located within a distance of 25 km in the federal state of North Rhine-
Westphalia, Germany.

The isolated strain MG2504 was identified as *S. marcescens* with the VITEK2 system
(VITEK2 GN-card, bioMérieux, Brussels, Belgium) and confirmed by using mass
spectrometry (MALDI-TOF, Bruker, Bellerica, USA). Antimicrobial susceptibilities were
determined according to the guidelines of the Clinical Laboratory Standards Institute (5) using
the VITEK2 AST-N118 and AST-N110 cards and by Etest (bioMérieux,
Nuertingen, Germany). The strain *S. marcescens* MG2504 was resistant to various β-lactams.

However, the strain was susceptible to aztreonam and cefepime (Table 1). The confirmatory
tests for carbapenemases (modified Hodge test, 8) and metallo-β-lactamases (Etest-MBL
bioMérieux, Nuertingen, Germany) showed ambiguous results and could not clearly confirm
the presence of a carbapenem-hydrolyzing enzyme. During occurrence of *S. marcescens* strain
MG2504 in 2009 we recovered 1024 further non-duplicate *S. marcescens* isolates from
different hospitals and outpatient departments in North Rhine-Westphalia. Altogether, 20
isolates (2.0%) were resistant to third generation cephalosporins (ceftazidime, cefotaxime). In
2010 we recovered 998 isolates with 59 (6%) isolates being resistant to ceftazidime and/or
cefotaxime and in 2011 the resistance rate of increased to 10% (102 of 1047 isolates)

However, *S. marcescens* MG2504, isolated in 2009 and 2010, was the only strain exhibiting
reduced susceptibility to carbapenems.

Molecular screening by PCR and sequencing was performed for different extended
spectrum β-lactamase (ESBL genes *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>BES</sub>) and carbapenemase
genes (*bla*<sub>OXA-48</sub>, *bla*<sub>SME</sub>, *bla*<sub>GES</sub>, *bla*<sub>KPC</sub>) as well as for the most commonly detected metallo-β-
lactamase (MBL) genes *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub> and for locally occurring types *bla*<sub>GIM</sub>, *bla*<sub>SPM</sub>
and *bla*<sub>SIM</sub> as described previously (2, 7, 9, 14, 15, 19). Using PCR and sequence analysis we
identified MBL-gene *bla*<sub>GIM-1</sub> in *S. marcescens* MG2504. Detailed investigation of the *bla*<sub>GIM</sub>-
genetic environment by PCR mapping (primer walking, 4) showed an integron structure comparable with structures that have been identified in \textit{bla}_{GIM}-harbouring \textit{P. aeruginosa} isolates collected in 2002 and 2009-10 in North Rhine-Westphalia, Germany (4, 21). In contrast to these \textit{P. aeruginosa} integron structures in \textit{S. marcescens} the \textit{aadA1} gene was not interrupted by a copy of the insertion sequence IS1394 (Figure 1A-B). \textit{S. marcescens} isolates producing MBL of VIM- and IMP-type have been reported from South Korea, Japan, Taiwan and Australia (10, 12, 13, 17). The GIM-1 enzyme that we found in \textit{S. marcescens} MG2504 was described so far only in six clinical \textit{P. aeruginosa} strains from a localized region in Germany (4, 21). Interestingly, in two hospitals where the patient was treated, we identified two different multidrug-resistant \textit{P. aeruginosa} strains harbouring \textit{bla}_{GIM-1}. One \textit{P. aeruginosa} was isolated four months before the first detection of the \textit{S. marcescens} strain in another ward from a patient with colonization. The second \textit{P. aeruginosa} isolate was recovered from lower respiratory tract specimens nine months after detection of the \textit{S. marcescens} strain in the same ward, isolated from a patient with pneumonia. However, in both \textit{P. aeruginosa} isolates \textit{bla}_{GIM-1} was located within widely different integron structures compared to \textit{S. marcescens} (Figure 1C-D).

Transferability of the \textit{bla}_{GIM-1} gene was tested by broth mating assays using a sodium azide resistant \textit{E. coli} K12J53 as the recipient. Selection of transconjugants was performed on Mueller-Hinton agar plates that contained sodium azide (200mg/L) and ampicillin (100mg/L) or meropenem (0.5mg/L). For determination of plasmid size whole genomic DNA was digested with S1 nuclease, subjected to pulsed-field gel electrophoresis (PFGE) as described previously (1). Southern-hybridisation using Digoxigenin-dUTP-labelled probes and signal detection using CDP-$\text{Star}$ were performed following the manufacturer’s guidelines (Roche Diagnostics Ltd, West Sussex, UK). The broth mate conjugation experiment in the present study was not successful but we detected \textit{bla}_{GIM-1} on a plasmid of ca. 22 kb size in the clinical
S. marcescens MG2504 strain. Replicon typing for identification of the plasmid was performed as described previously (3). However, a replicon type could not be determined. In the first GIM-1-positive P. aeruginosa isolate from 2002 blaGIM-1 was found to be located on a 22 kb plasmid (4). We speculate that a blaGIM-1-carrying plasmid was transferred between species P. aeruginosa to S. marcescens. Since the source of the blaGIM-1 gene and the blaGIM-1-carrying plasmid is still unknown we assume that the spread in other Gram-negative species is possible and probably ongoing. A problem are the slightly increased MIC values for meropenem and imipenem leading to ambiguous results of phenotypic carbapenemase tests which substantially complicates the diagnostics of these strains in common microbiological laboratories without having the possibility of using PCR. Therefore, the prevalence of GIM-1-producing S. marcescens and P. aeruginosa and the presence of GIM-1-possessing other Gram-negative species in Germany is most probably underestimated.

The present multidrug-resistant isolate of S. marcescens (MG2504) contained MBL gene blaGIM-1 previously described only in P. aeruginosa. This indicates the potential of transmission of blaGIM-1-carrying mobile genetic elements or plasmids within different Gram-negative species. Extensive resistance surveillance including molecular epidemiological investigations is needed to learn more about emergence and dissemination of GIM-1-producing bacteria in Germany. The nucleotide sequence of the S. marcescens MG2504 integron structure has been registered in the GenBank database under accession number JQ409537.

Acknowledgments

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**bla**<sub>KPC-2</sub> gene among clonal isolates of *Serratia marcescens*. J. Clin. Microbiol. 48:2546-
2549.
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<th>Antimicrobial agent</th>
<th>S. marcescens MG2504</th>
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<td>Ampicillin</td>
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<td>Ampicillin-sulbactam</td>
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\(^1\) tested by Etest (bioMérieux, Nuertingen, Germany)
Figure 1: Comparison of blaGIM-1-containing integron structures in *S. marcescens* and *P. aeruginosa*. Genes are indicated by boxes. The arrows in the boxes show the direction of transcription.
A  S. marcescens JO409537 (MG2504)

B  P. aeruginosa GU390399 (MG4737)

C  P. aeruginosa GU390404 (MG3918)

D  P. aeruginosa GU390403 (MG3404)