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Emergence of Metallo-β-Lactamase GIM-1 in a Clinical Isolate of *Serratia marcescens*

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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* that was 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 an outpatient department located in the region in which all previously described GIM-1-producing *P. aeruginosa* strains were identified.

Within *Serratia* spp. belonging to the *Enterobacteriaceae* family, *Serratia marcescens* is the most commonly detected species associated with nosocomial infections of the respiratory tract, urinary tract, and bloodstream. Outbreaks of *Serratia* spp. were caused by contamination of medical products (intravenous fluids, catheters) and equipment (apparatuses), transmitted mainly by the clinical personnel (11).

Resistance to many β-lactams due to β-lactamase production in combination with resistance to various other antimicrobial agents has become a serious threat. In the last decade, various extended-spectrum β-lactamases (ESBLs) (TEM, SHV, CTX-M, GES, and BES types) and different carbapenemases (SME, KPC, OXA-48, VIM, and IMP types) were identified in *S. marcescens* (2, 6, 7, 10, 14, 15, 17, 19, 21). 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, an *S. marcescens* strain (MG2504) was isolated. An empirical 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, $n = 2$) that were 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 which the patient was hospitalized. No *S. marcescens* isolate except the one from blood caused an infection, so further antibiotic treatment was not necessary. The three health care 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 (matrix-assisted laser desorption ionization–time of flight [MALDI-TOF]; Bruker, Bellerica, MA). Antimicrobial susceptibilities were determined according to the guidelines of the Clinical and 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 an occurrence of *S. marcescens* strain MG2504 in 2009, we recovered 1,024 further nonduplicate *S. marcescens* isolates from different hospitals and outpatient departments in North Rhine-Westphalia. Altogether, 20 isolates (2.0%) were resistant to expanded-spectrum 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 increased to 10% (102 of 1,047 isolates). However, *S. marcescens* MG2504, isolated in 2009 and 2010, was the only strain exhibiting reduced susceptibility to carbapenems.

TABLE 1 *S. marcescens* MG2504 MICs

Antimicrobial agent	MIC (μg/ml)
Ampicillin	>32
Ampicillin-sulbactam	>32
Piperacillin-tazobactam	>128
Cefepime	≤1
Cefotaxime	>64
Ceftazidime	32
Aztreonam	≤1
Gentamicin	4
Amikacin	≤2
Ciprofloxacin	>32
Trimethoprim-sulfamethoxazole	>320
Fosfomycin	64
Tigecycline	4
Meropenem	1
Imipenem	2
Ertapenem[a]	1.5

[a] Tested by Etest (bioMérieux, Nuertingen, Germany).

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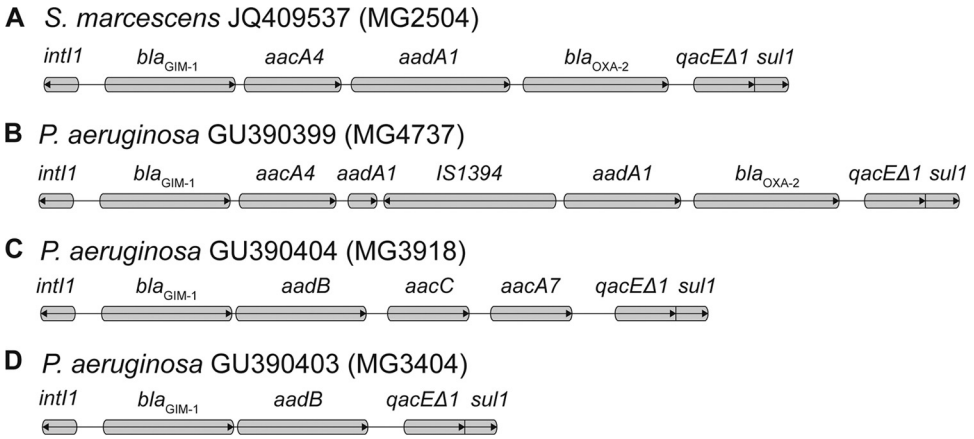

FIG 1 Comparison of bla_{GIM-1}-containing integron structures in *S. marcescens* and *P. aeruginosa*. Genes are indicated by boxes. Arrows in the boxes show the direction of transcription.

Molecular screening by PCR and sequencing was performed for different extended-spectrum β-lactamase (ESBL) genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{BES}) and carbapenemase genes (bla_{OXA-48}, bla_{SME},bla_{GES}, and bla_{KPC}) as well as for the most-commonly detected metallo-β-lactamase (MBL) genes (bla_{IMP}, bla_{VIM}, and bla_{NDM}) and for locally occurring types (bla_{GIM}, bla_{SPM}, and bla_{SIM}) as described previously (2, 6, 9, 13, 14, 18). Using PCR and sequence analysis, we identified the MBL gene bla_{GIM-1} in *S. marcescens* MG2504. Detailed investigation of the bla_{GIM-1} genetic environment by PCR mapping (primer walking [4]) showed an integron structure comparable to structures that have been identified in bla_{GIM}-harboring *Pseudomonas aeruginosa* isolates collected in 2002 and 2009 to 2010 in North Rhine-Westphalia, Germany (4, 20). In contrast to these *P. aeruginosa* integron structures, in *S. marcescens*, the *aadA1* gene was not interrupted by a copy of the insertion sequence IS*1394* (Fig. 1A and B).

S. marcescens isolates producing MBLs of the VIM and IMP types have been reported from South Korea, Japan, Taiwan, and Australia (10, 22, 12, 16). The GIM-1 enzyme that we found in *S. marcescens* MG2504 was described so far in only six clinical *P. aeruginosa* strains from a localized region in Germany (4, 20). Interestingly, in two hospitals in which the patient was treated, we identified two different multidrug-resistant *P. aeruginosa* strains harboring bla_{GIM-1}. One *P. aeruginosa* strain was isolated 4 months before the first detection of the *S. marcescens* strain in another ward from a patient with colonization. The second *P. aeruginosa* isolate was recovered from lower-respiratory-tract specimens 9 months after detection of the *S. marcescens* strain in the same ward, isolated from a patient with pneumonia. However, in both *P. aeruginosa* isolates, bla_{GIM-1} was located within integron structures widely different from those of *S. marcescens* (Fig. 1C and D).

Transferability of the bla_{GIM-1} gene was tested by broth mating assays using sodium azide-resistant *Escherichia coli* K12J53 as the recipient. Selection of transconjugants was performed on Mueller-Hinton agar plates that contained sodium azide (200 mg/liter) and ampicillin (100 mg/liter) or meropenem (0.5 mg/liter). For determination of plasmid size, whole genomic DNA was digested with S1 nuclease and subjected to pulsed-field gel electrophoresis (PFGE) as described previously (1). Southern hybridization using digoxigenin-dUTP-labeled probes and signal detection using CDP-*Star* were performed following the manufacturer's guidelines (Roche Diagnostics Ltd., West Sussex, United Kingdom). The broth mate conjugation experiment in the present study was not successful, but we detected bla_{GIM-1} on a plasmid with a size of ca. 22 kb 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, bla_{GIM-1} was found to be located on a 22-kb plasmid (4). We speculate that a bla_{GIM-1}-carrying plasmid was transferred between species, from *P. aeruginosa* to *S. marcescens*. Since the source of the bla_{GIM-1} gene and the bla_{GIM-1}-carrying plasmid is still unknown, we assume that the spread in other Gram-negative species is possible and probably ongoing. A problem is 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 other GIM-1-possessing Gram-negative species in Germany are most probably underestimated.

The present multidrug-resistant isolate of *S. marcescens* (MG2504) contained the MBL gene bla_{GIM-1}, previously described only in *P. aeruginosa*. This indicates the potential of transmission of bla_{GIM-1}-carrying mobile genetic elements or plasmids between different Gram-negative species. Extensive resistance surveillance, including molecular epidemiological investigations, is needed to learn more about the emergence and dissemination of GIM-1-producing bacteria in Germany.

Nucleotide sequence accession number. The nucleotide sequence of the *S. marcescens* MG2504 integron structure has been registered in the GenBank database under accession number JQ409537.

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