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Genetic Characterization and Emergence of the Metallo-β-Lactamase GIM-1 in *Pseudomonas* spp. and *Enterobacteriaceae* during a Long-Term Outbreak

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Since the first isolation in 2002, the metallo-β-lactamase GIM-1 has not been detected outside Germany. The data presented here, for 50 clinical bla_{GIM-1}-positive isolates, including *Pseudomonas* spp. and *Enterobacteriaceae* (*Enterobacter cloacae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Escherichia coli*, and *Citrobacter freundii*), collected between 2007 and 2012 at the original site in an ongoing outbreak, demonstrate a diverse genetic background and dissemination of the gene conferring resistance to enteric bacteria.

Metallo-β-lactamases (MBLs) hydrolyze all β-lactams (except monobactams), including the carbapenems, and thus are an important emerging obstacle to the treatment of Gram-negative bacterial nosocomial infections. To date, at least 11 subgroups have been described, with IMP, VIM, and NDM-1 being the most geographically widespread, while others, like GIM-1, have not become globally established (1–3). The prevalence of MBLs in Germany is low and mainly due to VIM types (4; http://ecdc.europa.eu/en/activities/surveillance/EARS-net/database/Pages/database.aspx). GIM-1 was initially discovered in a *Pseudomonas aeruginosa* clone isolated from the surgical intensive care unit (ICU) of the University Hospital of Düsseldorf (Germany) in 2002, in which the bla_{GIM-1} gene was located on a small 22-kb nontransferable plasmid embedded in the class 1 integron In77 (5). Since then, GIM-1 has been described in only a few isolates of *Enterobacter cloacae*, *Serratia marcescens*, *Acinetobacter pittii*, and *P. aeruginosa* (6–9), nearly exclusively in the greater Düsseldorf region and never outside Germany.

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TABLE 1 Carbapenemase multiplex primers and probes

Gene	Primer/probe	Sequence (5′→3′)[a]	Reference	Nucleotide positions	Size (bp)
bla_{GIM-1}	GIM1-F	CGACACACCTTGGTCTGAAGAA	AJ620678	1232–1253	81
	GIM1-R	GATGCTAGCCATAACCTGGTATCC		1313–1290	
	GIM1-P	HEX-ACACGAAGTTGTTATTATCCTGGGCGACTGAC-BHQ-1		1255–1286	
bla_{VIM-1}	VIM1-F	TGCGCTTCGGTCCAGTAGA	FJ172675	698–716	76
	VIM1-R	TGACGGGACGTATACAACCAGAT		774–752	
	VIM-P	FAM-CTTCTATCCTGGTGCTGCGCATTCG-BHQ-1		720–744	
bla_{VIM-2}	VIM2-F	GCGCTTCGGTCCAGTAGAAC	AF191564	1810–1829	80
	VIM2-R	CTCGCAGACGGGACGTACA		1890–1872	
	VIM-P	Same probe as for bla_{VIM-1}		1831–1855	
bla_{IMP-1}	IMP1-F	GGGCGGAATAGAGTGGCTTA	AY168635	303–322	93
	IMP1-R	GGCTTGAACCTTACCGTCTTTTT		396–374	
	IMP1-P	HEX-CGATCTATCCCCACGTATGCATCTGAATTAACA-BHQ-1		328–360	
bla_{NDM-1}	NDM1-F	CGGCATCACCGAGATTGC	FN396876	2547–2564	73
	NDM1-R	CACCGACATCGCTTTTGGT		2620–2602	
	NDM1-P	FAM-GCGACTTGGCCTTGCTGTCCTTG-BHQ-1		2568–2590	

[a] HEX, hexachlorofluorescein; BHQ-1, black hole quencher 1; FAM, 6-carboxyfluorescein.

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TABLE 2 Characteristics of *bla*$_{GIM-1}$-positive strains ordered by species and isolation date[a]

Species and strain M no.	Date (yr or mo-yr)	First sample type	Ward	repPCR type	PFGE type	Integron type	Genetic location	Plasmid size(s) (kb)
Pseudomonas aeruginosa								
0	2002	Respiratory tract	SICU	PSA-1	PSA-A	In77	C/P	22
19	Oct-07	Wound	SW1	PSA-4	PSA-B	In853	C	
1	Dec-07	Respiratory tract	MICU2	PSA-5	PSA-C	In583*	C	
2	Jan-08	Stool	MW3	PSA-6	PSA-D	In851*	C	
4	Apr-08	Urine	MW2	PSA-1	PSA-A	In77*	C	
3	Apr-08	Respiratory tract	SICU	PSA-1	PSA-A	In77	C	
5	Jun-08	Respiratory tract	SICU	PSA-1	PSA-A	In77	C	
7	Sep-08	Respiratory tract	MICU1	PSA-2	PSA-E	In770	C	
8	Sep-08	Respiratory tract	MICU1	PSA-2	PSA-A	In770*	C	
10	Oct-08	Stool	MICU1	PSA-2	PSA-F	In770	C	
16	Feb-10	Respiratory tract	SICU	PSA-1	ND	In77	C	
25	Feb-10	Urine	MW2	PSA-1	PSA-A	In77	C	
28	May-10	Urine	MOP	PSA-7	PSA-G	In770	ND	
29	May-10	Respiratory tract	SICU	PSA-8	PSA-H	In851	C	
45	Nov-10	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
46	Dec-10	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
47	Dec-10	Urine	MW1	PSA-1	PSA-A	In77b	C	
48	Mar-11	Wound	SICU	PSA-1	PSA-A	In77b	C	
57	Apr-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
49	May-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
50	Jun-11	Urine	SICU	PSA-1	PSA-A	In77b	C	
51	Jun-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
53	Jun-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
52	Jun-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
55	Jul-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
58	Sep-11	Wound	SICU	PSA-1	PSA-A	In77b	C and P	25
63	Dec-11	Respiratory tract	SICU	PSA-1	PSA-A	In77b*	C	
64	Jan-12	Wound	SICU	PSA-1	PSA-A	In77b	C	
67	Mar-12	Wound	SICU	PSA-1	PSA-A	In77b	C	
68	Mar-12	Urine	MW1	PSA-3	PSA-A	In770	C	
69	Apr-12	Wound	SICU	PSA-1	PSA-A	In851	C	
70	May-12	Respiratory tract	SICU	PSA-1	PSA-A	In77b	C	
71	May-12	Respiratory tract	MW4	PSA-3	PSA-D	In851	C	
Enterobacter cloacae								
14	Jun-09	Urine	MW4	ENT-1	ND	In770	C and two P	25 and 220
15	Jan-10	Blood culture	MICU1	ENT-1	ND	In770*	C and two P	25 and 220
21	Mar-10	Wound	MW2	ENT-2	ND	In770	C and two P	25 and 220
35	Jul-10	Urine	MW3	ENT-2	ND	In770	C and two P	25 and 220
44	Aug-10	Urine	MW3	ENT-2	ND	In770	C and two P	25 and 220
60	Dec-11	Wound	MW3	ENT-1	ND	In770	C and two P	25 and 220
65	Dec-11	Urine	MW3	ENT-2	ND	In770	C and two P	25 and 220
Pseudomonas putida								
18	Sep-07	Urine	MW3	PUT-3	ND	In770*	C	
6	Sep-08	Stool	MW3	PUT-1	ND	In853*	C	
12	Dec-08	Urine	MW3	PUT-1	ND	In853	C	
37	Aug-10	Urine	MW3	PUT-2	ND	In853	C	
38	Aug-10	Urine	MW3	PUT-2	ND	In853	C	
Serratia marcescens								
9	Sep-08	Urine	MICU2	ND	SMA-A	In770*	P	140
11	Nov-08	Urine	SW2	ND	SMA-B	In770	C	
Escherichia coli 17	Mar-07	Blood culture	MW5	ND	ND	In852*	C and two P	50 and 130
Klebsiella oxytoca 43	Oct-10	Urine	MW3	ND	ND	In770*	C	
Citrobacter freundii 56	Jul-11	Wound	SICU	ND	ND	In851*	C	

[a] MBL-positive isolates were given consecutive numbers with the prefix M. DiversiLab repPCR cluster and PFGE types were given numbers and letters, respectively, with an acronym of the species. M0 (73-5671) was collected in 2002 and *bla*$_{GIM-1}$ was described by Castanheira et al. (5) on a 22-kb plasmid, while we detected it chromosomally. *, integron sequences were published in GenBank (JX566704 to JX566715) and given INTEGRALL numbers as shown (In77b being a variant of In77). SICU, surgical intensive care unit; MICU, medical intensive care unit; SW, surgical ward; MW, medical ward (numbers indicate different wards); ND, not determined (M28 and M16 strains lost for further analysis); C, chromosome; P, plasmid.

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A total of 230 clinical isolates collected between 2007 and 2012 in one hospital, fulfilling the criteria of nonsusceptibility to piperacillin-tazobactam or ceftazidime and imipenem/meropenem, were further screened for MBL by using MBL MIC test strips (Liofilchem, Roseto degli Abruzzi, Italy) and real-time in-house PCR targeting the bla_{IMP-1}, bla_{VIM-1}-type, bla_{VIM-2}-type, bla_{GIM-1}, and bla_{NDM-1} genes (Table 1). The bla_{GIM-1} gene was detected in 50 isolates (*Pseudomonas aeruginosa* [33], *Enterobacter cloacae* [7], *Pseudomonas putida* [5], *Serratia marcescens* [2], *Escherichia coli* [1], *Klebsiella oxytoca* [1], and *Citrobacter freundii* [1]). Other isolates contained bla_{VIM-1} (2), bla_{VIM-2} (2), or bla_{NDM-1} (1). All bla_{GIM-1}-positive isolates except the single *E. coli* isolate (MIC, 8 mg/liter) were highly resistant to imipenem and meropenem (MIC, >32 mg/liter), and all bla_{GIM-1}-positive isolates were additionally resistant to quinolones and aminoglycosides. The genetic relatedness of the *P. aeruginosa*, *P. putida*, and *E. cloacae* isolates was investigated using DiversiLab repetitive-sequence-based PCR (repPCR) (bioMérieux, Nürtingen, Germany) according to the manufacturer's protocol. A similarity of >95% between isolates was defined as representing a genetic cluster. Pulsed-field gel electrophoresis (PFGE) was performed on all *P. aeruginosa* and *Serratia marcescens* isolates by using SpeI (Fermentas, ThermoFisher, Schwerte, Germany) as described previously (10) and interpreted according to the criteria of Tenover et al. (11). One isolate of the "original" *P. aeruginosa* clone, 73-5671 (5), was included. These fingerprinting data (Table 2) revealed one main PFGE cluster of 24 *P. aeruginosa* isolates (PSA-A), including isolates from 2002 to 2012, nearly all of which (19 of 24) were isolated from the surgical ICU, suggesting an ongoing clonal spread. The genetic relatedness of all species is shown in Table 2.

We determined the genetic environment of the bla_{GIM-1} gene by targeting conserved sequences of class 1 integrons by PCR and using a walking sequencing strategy. In addition to detecting the three integron types described before (5, 6, 8, 9), we detected four new arrays (In77b, In851, In852, and In853) (Fig. 1). The bla_{GIM-1} gene cassette was fused to *aacA4* in all isolates, except in *P. aeruginosa* M1 (fused to *aadB*). Five integron arrays (In77, In77b, In770, In851, and In853) were related to each other, sharing identical elements (*aacA4*, *aadA1*, and bla_{OXA-2}). The integron found in *E. coli* M17 (In852) had a unique structure with different elements (Δ*IS1600* and bla_{OXA-10}) suggesting an enhanced degree of mobility of the bla_{GIM-1}-*aacA4* gene pair. All integron types (except In852) were found in *P. aeruginosa*, and the appearance of the same types, e.g., In851 and In770, in other *Pseudomonas* spp. and *Enterobacteriaceae* (Table 2) clearly demonstrates horizontal gene transfer of a large block of genetic information rather than an individual gene.

S1 restriction and in-gel detection, performed as previously described (10), using a ^{32}P-radiolabeled bla_{GIM-1} probe (product of primers 5.1.R2 [CCAAGCAGCAAGCGCGTTAC] and GIMR [ACTCATGACTCCTCACGAGG] [5]), demonstrated the chromosomal location of the gene in all isolates other than *S. marcescens* M9, in which it was found only on a 140-kb plasmid. In addition to being localized on the chromosome, the gene was present on two plasmids in all *E. cloacae* isolates (approximately 25 kb and 220 kb) and in *E. coli* M17 (approximately 50 kb and 130 kb). Interestingly, we were able to detect bla_{GIM-1} on a plasmid (25 kb), similar in size to that originally described in 2004 (5), in only one *P. aeruginosa* isolate (M58, belonging to PFGE type PSA-A). S1 nuclease treatment, uncommon at the time of the first description of GIM-1, should detect plasmids of this small size, and thus we suggest that the plasmid originally described is probably unstable and has been lost during cultivation or storage. As there is a dominant chromosomal bla_{GIM-1}-mediated resistance in our isolates and as the bla_{GIM-1} gene cassette has moved into plasmids of different sizes, transfer options besides plasmid-mediated transfer, such as transfer of transposons or integrative and conjugative elements (ICE) (12), must be considered. Horizontal gene transfer was demonstrated by conjugation experiments using the recipient *E. coli* rifampin-resistant C600 or sodium azide-resistant J53 strain on sheep blood agar at a recipient/donor ratio of 1:10 at three temperatures (18°C, 30°C, and 37°C) as previously described (13). Selective media contained 100 mg/liter ampicillin and either 100 mg/liter rifampin or 100 mg/liter sodium azide. Successful transfer was confirmed by real-time PCR targeting bla_{GIM-1} and by use of random amplified polymorphic DNA (RAPD) with four primers: Eric1-R, ERIC2 (14), 272 (15), and 1254 (16). We were able to prove only horizontal gene transfer *in vitro* for the *E. coli* M17 isolate; the acquired resistance had no impact on carbapenem susceptibility in the transconjugant.

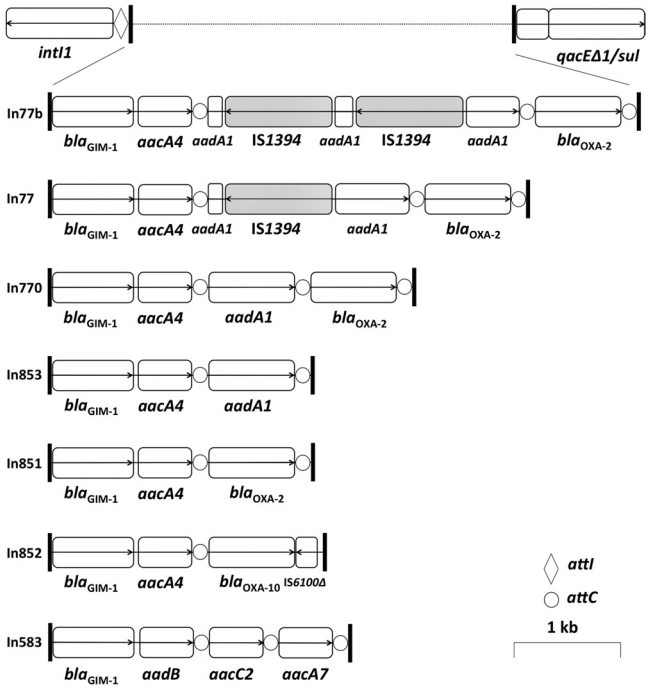

FIG 1 Class 1 integron arrays associated with bla_{GIM-1} found in this study. Open reading frames are represented by open boxes with arrows indicating the reading direction. IS*1394* inserting into gene *aadA1* is shown in gray.

The emergence of metallo-β-lactamase genes, first recovered in nonfermenters and recently spread to *Enterobacteriaceae*, poses a serious threat, since these bacteria are a common cause of severe nosocomial infections. This is the first description of a large number of isolates expressing the bla_{GIM-1} gene, including a much broader range of clinically relevant enteric bacteria than previously described. The originally described *P. aeruginosa* clone (5) may have acted as a genetic pool in the hospital environment over 10 years for the bacterial community, since it was the first described host of GIM-1. Although there is a considerable transfer of patients between hospitals in the greater Düsseldorf area, there does not seem to be an equivalent spread of our bla_{GIM-1}-carrying

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organisms, as there are only few isolates described elsewhere (6, 8, 9). Despite the data presented here, carbapenemase production in Gram-negative bacteria remains relatively uncommon in Düsseldorf, but these data may herald a rise in GIM-1-mediated carbapenem resistance in this region, which is of great concern.

Nucleotide sequence accession numbers. Integron sequences are published in GenBank under accession numbers JX566704 to JX566715.

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We have no transparency declarations.

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