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Characterization of a Novel Metallo-β-Lactamase Variant, GIM-2, from a Clinical Isolate of *Enterobacter cloacae* in Germany

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The metallo-β-lactamase (MBL) GIM-1, first identified in *Pseudomonas aeruginosa* isolates in Germany in 2002 (1), has since been described sporadically and never outside Germany in *Pseudomonas* spp. (2, 3), *Acinetobacter pittii* (4), and a range of *Enterobacteriaceae* (3, 5, 6). No genetic variations of bla_{GIM-1} have been reported (7).

The *Enterobacter cloacae* isolate described here was first identified in June 2014 from the rectal screening of a 49-year-old male patient from Saudi Arabia previously hospitalized in Germany and in Saudi Arabia.

Results of phenotypic detection of a carbapenemase using a combined disk test (10 μg meropenem with and without 930 μg EDTA [8]) and a modified Hodge test (9) were both positive. The MICs of the following relevant antibiotics were determined by Etest (bioMérieux): piperacillin (>256 mg/liter), piperacillin-tazobactam (>256 mg/liter), ceftazidime (>256 mg/liter), cefepime (>12 mg/liter), imipenem (1 mg/liter), meropenem (4 mg/liter), aztreonam (>256 mg/liter), gentamicin (4 mg/liter), amikacin (2 mg/liter), ciprofloxacin (0.5 mg/liter), and colistin (0.5 mg/liter).

Multiplex PCRs were performed for the following β-lactamase genes: bla_{IMP-1}, $bla_{VIM-1-type}$, $bla_{VIM-2-type}$, bla_{GIM-1}, and bla_{NDM-1} (3); bla_{KPC} and bla_{OXA-48} (10); bla_{CTX-M} groups 1, 2, 9, and 8/25 (11); and bla_{TEM} and bla_{SHV} (12). PCR detected bla_{GIM-1} and bla_{CTX-M} group 9 genes. Sequencing of the bla_{GIM} gene (GIM-F-flanking, 5′-TCCAGAACCTTGACCGAACG-3′, and GIM-R-flanking, 5′-GCCACTCATAGAGCATCGCA-3′) revealed a new variant of the metallo-β-lactamase bla_{GIM-1} gene (given the name bla_{GIM-2}) with one nucleotide substitution, A290G, causing an amino acid substitution of glutamic acid to glycine at position 97. The sequenced class 1 integron, In1101, is identical in the order of the gene cassettes to integrons previously described in bla_{GIM-1}-positive *E. cloacae* (3, 5). The genes were located downstream of the *attI1* recombination site in the following order: bla_{GIM-2} and aminoglycoside acetyltransferase gene *aacA4* in one common (fused) gene cassette, aminoglycoside acetyltransferase gene *aadA1*, and β-lactam resistance gene bla_{OXA-2}.

Genetic localization of the bla_{GIM-2}-containing integron was determined by S1-nuclease digestion and in-gel hybridization with a ^{32}P-labeled bla_{GIM} probe as previously described (13). As a template, the amplicon of primers (5′ to 3′) 5.1.R2 (CCAAGCA GCAAGCGCGTTAC) and GIMR (ACTCATGACTCCTCACG AGG) (1), which bind to bla_{GIM-2}, was used. No bla_{GIM-2}-containing plasmid was detected, and hybridization of the probe occurred only on chromosomal DNA. Conjugation experiments were carried out using the bla_{GIM-2} strain and sodium azide-resistant *Escherichia coli* J53 on sheep blood agar at a recipient/donor ratio of 1:10. The selective media contained 4 mg/liter ceftazidime and 100 mg/liter sodium azide. The bla_{GIM-2} gene was nonconjugative.

Genotyping was carried out together with bla_{GIM-1}-positive *E. cloacae* isolates previously described (3) using three methods: pulsed-field gel electrophoresis (PFGE) (XbaI, in accordance with the Tenover criteria [14]), repetitive sequence-based PCR (rep-PCR) (DiversiLab) (with a similarity cutoff of 95%), and multilocus sequence typing (MLST) (15). The bla_{GIM-2}-positive strain, confirmed to be sequence type 108, was shown to be unrelated to the other isolates by all genotyping methods.

In conclusion, the isolation of a new GIM-type MBL in Germany highlights the ongoing spread and evolution of this local metallo-β-lactamase gene. The isolate presented here may be easily missed in routine microbiology laboratories since isolates carrying the gene can show relatively low MICs for carbapenems; however, results of phenotypic tests for carbapenemases were positive.

Nucleotide sequence accession number. The integron whose sequence was determined in this work has been allocated GenBank accession number KM659858.

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We declare that we have no conflicts of interest.

REFERENCES

1. **Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR.** 2004. Molecular characterization of a beta-lactamase gene, blaGIM-1, encoding a new subclass of metallo-beta-lactamase. Antimicrob Agents Chemother **48:**4654–4661. http://dx.doi.org/10.1128/AAC.48.12.4654-4661.2004.
2. **Rieber H, Frontzek A, von Baum H, Pfeifer Y.** 2012. Emergence of metallo-beta-lactamases GIM-1 and VIM in multidrug-resistant Pseudomonas aeruginosa in North Rhine-Westphalia, Germany. J Antimicrob Chemother **67:**1043–1045. http://dx.doi.org/10.1093/jac/dkr579.
3. **Wendel AF, Brodner AH, Wydra S, Ressina S, Henrich B, Pfeffer K,**

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Toleman MA, Mackenzie CR. 2013. Genetic characterization and emergence of the metallo-beta-lactamase GIM-1 in Pseudomonas spp. and Enterobacteriaceae during a long-term outbreak. Antimicrob Agents Chemother **57:**5162–5165. http://dx.doi.org/10.1128/AAC.00118-13.

4. **Kaase M, Szabados F, Pfennigwerth N, Anders A, Geis G, Pranada AB, Rossler S, Lang U, Gatermann SG.** 2014. Description of the metallo-beta-lactamase GIM-1 in Acinetobacter pittii. J Antimicrob Chemother **69:**81–84. http://dx.doi.org/10.1093/jac/dkt325.
5. **Hamprecht A, Poirel L, Gottig S, Seifert H, Kaase M, Nordmann P.** 2013. Detection of the carbapenemase GIM-1 in Enterobacter cloacae in Germany. J Antimicrob Chemother **68:**558–561. http://dx.doi.org/10.1093/jac/dks447.
6. **Rieber H, Frontzek A, Pfeifer Y.** 2012. Emergence of metallo-beta-lactamase GIM-1 in a clinical isolate of Serratia marcescens. Antimicrob Agents Chemother **56:**4945–4947. http://dx.doi.org/10.1128/AAC.00405-12.
7. **Diene SM, Rolain JM.** 2014. Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, Pseudomonas and Acinetobacter species. Clin Microbiol Infect **20:**831–838. http://dx.doi.org/10.1111/1469-0691.12655.
8. **Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL.** 2005. Detection of Pseudomonas aeruginosa producing metallo-beta-lactamases in a large centralized laboratory. J Clin Microbiol **43:**3129–3135. http://dx.doi.org/10.1128/JCM.43.7.3129-3135.2005.
9. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing; 22nd informational supplement M100–S22. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Swayne RL, Ludlam HA, Shet VG, Woodford N, Curran MD.** 2011. Real-time TaqMan PCR for rapid detection of genes encoding five types of non-metallo- (class A and D) carbapenemases in Enterobacteriaceae. Int J Antimicrob Agents **38:**35–38. http://dx.doi.org/10.1016/j.ijantimicag.2011.03.010.
11. **Dallenne C, Da Costa A, Decre D, Favier C, Arlet G.** 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother **65:**490–495. http://dx.doi.org/10.1093/jac/dkp498.
12. **Schlesinger J, Navon-Venezia S, Chmelnitsky I, Hammer-Munz O, Leavitt A, Gold HS, Schwaber MJ, Carmeli Y.** 2005. Extended-spectrum beta-lactamases among Enterobacter isolates obtained in Tel Aviv, Israel. Antimicrob Agents Chemother **49:**1150–1156. http://dx.doi.org/10.1128/AAC.49.3.1150-1156.2005.
13. **Patzer JA, Walsh TR, Weeks J, Dzierzanowska D, Toleman MA.** 2009. Emergence and persistence of integron structures harbouring VIM genes in the Children's Memorial Health Institute, Warsaw, Poland, 1998–2006. J Antimicrob Chemother **63:**269–273.
14. **Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **33:**2233–2239.
15. **Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, Shimojima M, Kirikae T.** 2013. Multilocus sequence typing (MLST) for characterization of Enterobacter cloacae. PLoS One **8:**e66358. http://dx.doi.org/10.1371/journal.pone.0066358.