Emergence of *Citrobacter freundii* carrying IMP-8 metallo-β-lactamase in Germany

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**Abstract**

Metallo-β-lactamases (MBLs) in *Enterobacteriaceae* are an increasing problem worldwide. This report describes the isolation of *Citrobacter freundii* carrying IMP-8 MBL from three patients during the period from March 2012 until March 2013 in Germany. The *bla*

**IMP-8** enzyme is predominantly found in Asia, where IMP-8 has spread to various enterobacterial species causing serious infections. To our best knowledge, this is the first report of *bla*

**IMP-8** harbouring *Enterobacteriaceae* in Europe.

**Keywords:** Antimicrobial resistance, IMP, infection control, laboratory surveillance, metallo-β-lactamases, multidrug resistance

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**Introduction**

The emergence and spread of carbapenemase-producing *Enterobacteriaceae* is an increasing problem of global dimensions. Originally, metallo-β-lactamases (MBLs) were associated with resistance in Gram-negative non-fermenters, but they have become increasingly important regarding carbapenem resistance in *Enterobacteriaceae*. MBLs confer resistance to almost all β-lactam antibiotics and are not inactivated by β-lactamase inhibitors, hence limiting treatment options in the individual patient and presenting a major challenge for infection control within the hospital setting [1].

To date, the occurrence of carbapenem-resistant *Enterobacteriaceae* in Germany is still a rare event; however, outbreaks involving KPC and VIM-1 carrying *Klebsiella pneumoniae* isolates have been described [2,3]. In the year 2012, VIM-1 was the most prevalent MBL detected in *Enterobacteriaceae* in Germany, followed by NDM-1 and GIM-1 [2]. IMP type MBLs were first identified in *Pseudomonas aeruginosa* in Japan [4] and have since been reported predominantly from Asia [5]. With the exception of Italy, IMP-type enzymes have rarely been reported from other countries in Europe [1,5,6].

Here, we report the isolation of *Citrobacter freundii* harbouring MBL IMP-8 from three patients between March 2012 and March 2013. All isolates were obtained from rectal swabs. All three patients had underlying haematological conditions (acute myeloid leukaemia *n* = 2 and myelodysplastic syndrome *n* = 1) and underwent haematopoietic stem cell transplantation. They were screened from rectal swabs for colonization with multidrug resistant Gram-negative bacteria on a weekly routine schedule.

**Laboratory Analysis**

Identification of the isolates was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF-MS) (AXIMA Assurance, bioMérieux SA, Marcy l’Étoile, France; Saramis Database Version 4.09) and the VITEK 2 identification system (bioMérieux SA). Antimicrobial susceptibility testing was initially performed with the VITEK 2 system (bioMérieux SA) and confirmed by Etest (bioMérieux SA). Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.euCAST.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf). The detection of extended-spectrum β-lactamase genes *bla*

**CTX-M, bla**

**TEM, bla**

**SHV** [7] and carbapenemase genes *bla*

**OXA-48** [8], *bla*KPC [9], *bla*NDM [10], *bla*IMP and *bla*VIM [11] was performed as described previously. Sequencing of the IMP genes was performed using class 1 integron primer 3CS (5′-AAC CAG ACT TGA CCT GA-3′) in combination with primer IMP-A, and 3CS (5′-GGC ATC CAA GCA GCA AG-3′) in combination with primer IMP-B [11]. Sequence identification was determined by comparison with the sequences available in GenBank (http://www.ncbi.nlm.nih.gov/BLAST) and with the reference sequences of...
the Lahey database (http://www.lahey.org/studies). Alignment was performed using BioEdit version 7.1.11 (Ibis Biosciences, Carlsbad, CA, USA). Genomic fingerprinting of the isolates was done by the enterobacterial repetitive intergenic consensus (ERIC) method using the ERIC2 primer as described previously [12]. Plasmids were extracted using the Qiagen large construct kit (Qiagen, Hilden, Germany) and digested using EcoRI and BamHI to allow for size estimation of the plasmids. Southern blotting was performed following a standard protocol. Briefly, DNA was blotted onto positively charged nylon membranes (Roche Biochemicals, Basel, Switzerland) in denaturation solution (3 M NaCl, 0.4 M NaOH) by capillary transfer. High stringency hybridization was performed in accordance with the instructions given by the manufacturer of the digoxigenin labelling and detection kit (Roche Biochemicals). Digoxigenin-labelled DNA probes were generated with a digoxigenin-labelling PCR kit as described in the manufacturer’s instructions (Roche Biochemicals) using the oligonucleotides IMP-A and IMP-B.

Results

The characteristics of the isolates are summarized in Table 1. All three isolates were resistant to piperacillin-tazobactam, cefturoxime and cefotaxime. The MIC of meropenem was >32 mg/L for the isolate of patients two and three, whereas meropenem MIC for patient one was intermediate with an MIC of 8 mg/L. All three strains were susceptible to tigecycline, colistin and amikacin. Molecular detection of ESBL genes \( \text{bla}_{\text{CTX-M}} \), \( \text{bla}_{\text{TEM}} \), \( \text{bla}_{\text{SHV}} \) and carbapenemase genes \( \text{bla}_{\text{OXA-48}} \), \( \text{bla}_{\text{KPC}} \), \( \text{bla}_{\text{NDM}} \) and \( \text{bla}_{\text{VIM}} \) was negative in all three isolates. The IMP PCR gave a positive result and subsequent determination of the nucleotide sequence revealed an MBL of the IMP-8 type. The sequence was compared with the reference sequence (GenBank accession number AF322577) [13]. The \( \text{bla}_{\text{IMP-8}} \) gene detected in the three isolates harboured a non-coding point mutation at position 18 (T→C) as shown in Fig. 1. Additionally, an IMP-8 carrying plasmid of approximately

| TABLE 1. Characteristics of IMP-8 carrying \( \text{Citrobacter freundii} \) isolates from rectal swabs of three hospitalized patients in Germany |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patient         | Source          | Isolation date  | TETP | CXM | CTX | CAZ | FEP | ATM | MEM | TIG | CIP | AN | COL |
| 1               | Rectal swab     | March 2012      | 128 R | >256 R | 16 R | >256 R | 16 R | 2 I | >32 R | 8 I | 0.75 S | 8 R | 2 S | 0.75 S  |
| 2               | Rectal swab     | June 2012       | 96 R  | >256 R | 16 R | >256 R | 24 R | 3 I | >32 R | 32 R | 0.75 S | 16 R | 2 S | 1 S       |
| 3               | Rectal swab     | March 2013      | >256 R | 24 R | >256 R | 24 R | >256 R | 24 R | >32 R | 32 R | 0.75 S | 12 R | 2 S | 1 S       |

I, intermediate; R, resistant; S, susceptible; AN, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; CTX, cefotaxime; CXM, cefturoxime; ERT, ertapenem; MEM, meropenem; TIG, tigecycline; TZP, piperacillin-tazobactam.

Interpretation according to the EUCAST clinical breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf).

FIG. 1. Sequence of the \( \text{bla}_{\text{IMP-8}} \) gene of three \( \text{Citrobacter freundii} \) strains isolated from hospitalized patients in Germany. Compared with the sequence of the IMP-8 reference strain (GenBank accession number AF322577 [13]), a non-coding point mutation at position 18 was identified in the IMP-8 from the three \( \text{Citrobacter freundii} \) strains (grey shaded).
Genomic fingerprinting by the ERIC method revealed indistinguishable PCR patterns in all isolates (data not shown).

Conclusion

This is the first report of IMP-8 MBL in Enterobacteriaceae in Germany. IMP-8 is very uncommon in Europe, only once reported from Portugal in a Pseudomonas mendocina strain [6, 14]. In contrast, IMP-8 is frequently encountered in Asia, especially in Taiwan, where IMP-8-producing Enterobacteriaceae are involved in serious infections. Yan et al. reported on a case series of 37 patients with bloodstream infections caused by a large variety of IMP-8-producing enterobacterial species including Escherichia coli, K. pneumoniae, Enterobacter cloacae and C. freundii [15]. Furthermore, aggravating the issue, phenotypic screening for IMP-8-positive Enterobacteriaceae is extremely difficult because of the lack of distinctive phenotypes. Investigation of 95 IMP-8-positive Enterobacteriaceae revealed susceptibility to ertapenem in 21% and to meropenem in 45%, whereas phenotypic combined disk tests using EDTA and phenylboronic acid were positive in only 40% of the isolates [16]. These observations from Taiwan suggest that IMP-8 is capable of spreading between enterobacterial species, causing serious problems in terms of infection control measures and limiting therapeutic options in critically ill patients.

The role of faecal carriage of MBL-producing Enterobacteriaceae remains unclear and has not been investigated in a large-scale epidemiological study. Faecal carriage of one C. freundii VIM-1 has been reported from Spain in an outpatient, who was also colonized with two different VIM-1-carrying K. pneumoniae isolates [17]. In an Italian hospital, eight VIM-1-carrying C. freundii strains were isolated from rectal swab samples during active screening following the detection of a K. pneumoniae carbapenemase (KPC) -positive patient [18].

None of our three patients became infected by the IMP-8 C. freundii strains, but nevertheless it is alarming that IMP-8 MBL circulates in the gut flora of patients at high risk of nosocomial infections. Even more worrisome is the fact, that the IMP-8 gene is located on a plasmid, which might facilitate the transfer of the resistance gene within enterobacterial species. Our findings emphasize the importance of establishing screening schemes and laboratory diagnostic algorithms to ensure the implementation of efficient infection control measures and therapeutic strategies, not only in high-prevalence countries, but also in countries with a low incidence of MBL producing Enterobacteriaceae.

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Conflict of Interest

None declared.

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