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Molecular characterization of *bla*$_{\text{NDM-1}}$ in an *Acinetobacter baumannii* strain isolated in Germany in 2007

Yvonne Pfeifer[1],*, Gottfried Wilharm[1], Esther Zander[2], Thomas A. Wichelhaus[3], Stefan Göttig[3], Klaus-Peter Hunfeld[4], Harald Seifert[2], Wolfgang Witte[1] and Paul G. Higgins[2]

[1]Nosocomial Infections, Robert Koch Institute, Burgstraße 37, 38855 Wernigerode, Germany
[2]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Goldenfelsstrasse 19-21, 50935 Cologne, Germany
[3]Hospital of Johann Wolfgang Goethe-University Frankfurt/Main, Frankfurt/Main, Germany
[4]Nordwest Medical Center Frankfurt, Frankfurt/Main, Germany

Abstract

Objectives *To investigate the genetic environment of the metallo-β-lactamase gene* bla$_{NDM-1}$ *in an* Acinetobacter baumannii *isolated in 2007 in a German hospital.*

Methods *Antimicrobial susceptibility testing was performed and resistance genes were characterized by PCR amplification and sequencing. Transferability of β-lactam resistance was tested by broth mating assays and transformation of plasmids. The genetic background of* bla$_{NDM-1}$ *was analysed by primer walking. Typing of the* A. baumannii *strain was performed by repetitive extragenic palindromic sequence-based PCR (rep-PCR) using the DiversiLab system.*

Results *The multidrug-resistant* A. baumannii *isolate harboured β-lactamase genes* bla$_{NDM-1}$ *and intrinsic* bla$_{OXA-64}$*, but without the insertion sequence IS*Aba1 *often located upstream. Transfer of carbapenem resistance by conjugation and transformation failed. Hybridization of isolated plasmid DNA with* bla$_{NDM}$ *probes was not successful. Shotgun cloning of whole genomic DNA and sequence analyses revealed that* bla$_{NDM-1}$ *was located between two insertion elements of IS*Aba125*. Furthermore, this* bla$_{NDM-1}$*-containing transposon structure was integrated into a chromosomal gene encoding a putative* A. baumannii *major facilitator superfamily (MFS) metabolite/*H^+ *symporter.*

Conclusions *The metallo-β-lactamase gene* bla$_{NDM-1}$ *in this* A. baumannii *strain was integrated in the chromosome on a new transposon structure composed of two copies of insertion sequence IS*Aba125*. The variability of the genetic environment of* bla$_{NDM-1}$ *likely facilitates the rapid dissemination of this gene within many Gram-negative bacterial species.*

Introduction

In recent years an increasing number of reports on the emergence of multidrug-resistant Gram-negative pathogens have been published and in particular it is the emergence of *bla*$_{\text{NDM-1}}$ that has created the most concern.[1,2] Indeed, since its first description in 2009, this metallo-β-lactamase (MBL) has now been found worldwide.[3,4] NDM-1 has been identified in various Enterobacteriaceae due to localization of *bla*$_{\text{NDM-1}}$ on conjugative plasmids, enabling transfer and rapid dissemination of multidrug resistance.[4] In 2010 *bla*$_{\text{NDM-1}}$ and the related *bla*$_{\text{NDM-2}}$ gene were also found in *Acinetobacter baumannii*, whereby either plasmid transfer of these genes was confirmed or the genetic location was not further investigated in detail.[5–8] Infections with carbapenemase-producing *A. baumannii* are a serious threat because colistin is often the only treatment option. Carbapenem resistance in *A. baumannii* is mainly due to expression of various OXA β-lactamases, such as OXA-23, OXA-58, OXA-40 and OXA-143-related enzymes, as well as overexpression of the intrinsic OXA-51-like enzyme.[9] Non-OXA-mediated carbapenem resistance is still rare. Here we report the molecular characterization of the genetic environment of $bla_{\text{NDM-1}}$ detected in a clinical *A. baumannii* strain from a German hospital.

Materials and methods

Bacterial strains

A. baumannii strain 161/07 was isolated from a patient who had been repatriated to Germany from Serbia in 2007. The case history surrounding this $bla_{\text{NDM-1}}$-positive *A. baumannii* strain 161/07 has been described previously.[6] Standard *A. baumannii* ATCC 19606 and ATCC 17978 were used as recipients for plasmid transformation. Sodium azide-resistant *Escherichia coli* J53 was used as the recipient for transformation and broth mating assays.

Antimicrobial susceptibility

Antimicrobial drug susceptibilities were determined according to the guidelines of the CLSI by broth microdilution (Table S1, available as Supplementary data at *JAC* Online) and Etest (bioMérieux, Nürtingen, Germany).[10] The MBL phenotype was confirmed with MBL-Etest and a combined disc diffusion test CDDT (KPC+MBL Confirm ID Kit; Alere GmbH, Cologne, Germany).

Presence of carbapenem resistance genes

Detection of OXA-type carbapenemases and associated insertion elements was performed by PCR and sequence analyses were performed as previously described.[11,12] Detection of the $bla_{\text{NDM-1}}$ gene was performed by PCR using primers ndm1_F (5′-CTGAGCACCGCATTAGCC-3′) and ndm-1_R (5′-GGGCCGTATGAGTGATTGC-3′). For detection of other common bla_{OXA} genes ($bla_{\text{OXA-1,2,9,10}}$), the following primers were used: oxa1_ F (5′-TATCTACAGCAGCGCCAGTG-3′); oxa1_ R (5′-TAAATTCGACCCCAAGTTTCC-3′); oxa2_ F (5′-GCAAGAAGGCACGCTAGAAC-3′); oxa2_ R (5′-CAGCGTCCGAGTTGACTG-3′); oxa9_ F (5′-TTTGCTGCTGCATATGTTGG-3′); oxa9_ R (5′-CCCATCAACACGGGTAATTC-3′); oxa10_ F (5′-TTCGAGTACGGCATTAGCTG-3′); and oxa10_ R (5′-CAATGATGCCCTCACTTTCC-3′).

Molecular typing

Strain typing was performed by rep-PCR using the DiversiLab system (bioMérieux) and results were compared with our database of worldwide clonal lineages.[9] In addition, multiplex PCR-based typing in combination with ApaI PFGE was performed.[13]

Characterization of *bla*$_{\textit{NDM-1}}$

Transfer of β-lactam resistance was tested by broth mating assays with *E. coli* J53 as the recipient. Selection of transconjugants was performed on Mueller–Hinton agar plates that contained sodium azide (200 mg/L) and ampicillin (100 mg/L). Plasmid DNA was isolated using the QIAGEN Plasmid Mini Kit (QIAGEN, Hilden, Germany).

Transformation of plasmids into an electrocompetent *E. coli* J53 and *A. baumannii* ATCC 19606 and ATCC 17978 recipients was performed using standard procedures.[14] Plasmid size was determined by performing S1 nuclease restriction of whole genomic DNA combined with PFGE.[15] Southern hybridization using digoxigenin-dUTP-labelled probes and signal detection using CDP-*Star* were performed following the manufacturer's guidelines (Roche Diagnostics Ltd, West Sussex, UK).

Whole genomic DNA was isolated using the QIAGEN DNeasy kit and digested with EcoRV, ligated into EcoRV-cut plasmid pBBR1MCS, transformed into *E. coli* NEB 5-alpha and selected on ticarcillin

(25 mg/L).[16] Inserts of isolates that grew on ticarcillin were amplified by PCR using M13 primers and sequenced. Insert DNA was sequenced on both strands by primer walking.

Additionally, transfer of naked whole genomic DNA of *A. baumannii* 161/07 into an ampicillin-susceptible *A. baumannii* 102/07 recipient by natural transformation was performed. We have recently identified conditions promoting the uptake of naked DNA by a significant number of *A. baumannii* isolates. These results will be published elsewhere (E. Skiebe and G. Wilharm, unpublished results).

Nucleotide sequence accession number

The nucleotide and protein sequences of the *bla*$_{\text{NDM-1}}$-containing transposon and gene *aphA-6* have been registered in GenBank under accession numbers HQ857107 and JF949760, respectively.

Results and discussion

Strain typing by rep-PCR (DiversiLab) revealed that the NDM-1-producing multidrug-resistant *A. baumannii* strain 161/07 clustered with isolates of the clonal lineage WW7. Previous work has shown that *A. baumannii* strains belonging to the WW7 cluster harboured the carbapenemase genes *bla*$_{\text{OXA-58}}$ or *bla*$_{\text{OXA-23}}$ and were from different countries in South America, Europe and Asia.[9] Furthermore, all *A. baumannii* of the WW7 cluster harboured *bla*$_{\text{OXA-64}}$, a variant of the intrinsic *bla*$_{\text{OXA-51}}$.[17] It was confirmed by PCR that *A. baumannii* 161/07 was positive for the chromosomally located *bla*$_{\text{OXA-64}}$, but this gene was not associated with insertion element IS*AbaI*. Other *bla*$_{\text{OXA}}$ genes were not detected. PCR-based *A. baumannii* typing in combination with ApaI PFGE analysis confirmed that *A. baumannii* 161/07 was not related to European clonal lineages 1–3.[13]

Transfer of resistance genes by *in vitro* conjugation and transformation of plasmids into *E. coli* or *A. baumannii* recipients was not successful. Analysis of S1-digested DNA fragments revealed the presence of two plasmids (125 kb, 75 kb) in the NDM-1-producing *A. baumannii* 161/07 strain. However, hybridization signals with a *bla*$_{\text{NDM-1}}$ probe were not detected for these plasmids, and PCR with *bla*$_{\text{NDM-1}}$-specific primers failed to amplify a PCR product, suggesting a chromosomal location of *bla*$_{\text{NDM-1}}$.

Using naked whole genomic DNA from *A. baumannii* 161/07 to transform the naturally competent ampicillin-susceptible *A. baumannii* recipient 102/07, the gene *bla*$_{\text{NDM-1}}$ was successfully transferred, as confirmed by PCR. All β-lactams tested against the transformant 161/07-102 showed an increase in MIC, with imipenem and meropenem MICs increasing from 0.25 mg/L to >32 mg/L, and 0.5 mg/L to 32 mg/L, respectively (Table S1). No other class of antibiotic was affected.

Sequence analysis of shotgun cloned *A. baumannii* 161/07 genomic DNA revealed a 3.9 kb insert containing the *bla*$_{\text{NDM-1}}$ gene and parts of the plasmid sequence pKpANDM-1 described previously (Figure 1C).[3] However, the IS*26* transposase upstream of *bla*$_{\text{NDM-1}}$ in previously characterized plasmids pKpANDM-1 and pNDM-HK was not present in *A. baumannii* 161/07. Instead, the insertion sequence IS*Aba125* was identified (Figure 1B). Interestingly, pKpANDM-1 includes a partial sequence of IS*Aba125* adjacent to *bla*$_{\text{NDM-1}}$. BLAST analysis of IS*Aba125* revealed seven copies of IS*Aba125* on the chromosome of *A. baumannii* strain ACICU in the GenBank database, and IS*Aba125* was found disrupting the *carO* gene, leading to carbapenem resistance.[18] Furthermore, IS*Aba125* can also be plasmid located, and has been described recently upstream of the *bla*$_{\text{OXA-58}}$ gene.[19]

To determine whether *bla*$_{\text{NDM-1}}$ was located in a transposon, PCR was performed with inverse primers to IS*Aba125* [TRANSIS_R (5′-AAACAACGGATCGCTTCAAC-3′) and TRANSIS_F (5′-CGAGCATTACCAAAGGGTGA-3′)] using genomic DNA of *A. baumannii* 161/07 as template. Two products of 2 kb and 9 kb were amplified. Sequencing of the 2 kb amplicon revealed *aphA-6*, an aminoglycoside resistance gene bracketed between two copies of IS*Aba125*.

Primer walking from both ends of the 9 kb amplicon revealed a composite transposon structure containing *bla*$_{\text{NDM-1}}$ bracketed between two copies of IS*Aba125* (Figure 1B). Both copies of IS*Aba125* were flanked by 17 bp inverted repeats. Both 5′ inverted repeats and both 3′ inverted repeats were identical, respectively, but there were two nucleotide differences between 5′ and 3′ inverted repeats. The two IS*Aba125* transposase genes differed by four nucleotides whereby one resulted in amino acid substitution, Arg-41→Gln. Including both IS*Aba125* insertion elements, the composite transposon was 10093 bp in length. Furthermore, it was integrated into a chromosomal gene encoding a putative *A. baumannii* major facilitator superfamily (MFS) metabolite/H^+ symporter (Figure 1A) that has previously been disrupted by IS*Aba1*-bla_{OXA-23}.[20] Further PCR and sequencing with primers for IS*Aba125* and the disrupted MFS transporter confirmed the chromosomal location of the bla_{NDM-1}-containing transposon. Evidence of this being a transposition event was an 8 bp target site duplication at the point of insertion in the gene encoding MFS (Figure 1E). Adjacent to the MFS gene we identified a chromosomal homoserine lactone synthase gene (*cepI*). In addition, inside the bla_{NDM-1}-containing transposon there was a 4 kb element 93% similar to that described in *E. coli* plasmids pEH4H and pAR060302, encoding the chaperonin subunits *groS* and *groL*, and the transposase *insE*, which was not associated with inverted repeats (Figure 1D). The 8 bp target site duplication was also present at the 5′-end of the *insE* gene (Figure 1E). Further BLAST analysis revealed a 297 bp sequence, including 41 bp of the 3′-end of *insE* and extending towards IS*Aba125*, that showed 99% identity to insertion sequence IS*CR19*-like and *ori*IS previously described in a *Pseudomonas aeruginosa* isolate.[21] Deletions were found in gene *trpF* (79 bp, 3′-end) and gene *groS* (154 bp, 5′-end). No significant DNA homology or open reading frames were detected in the 1.6 kb region between the truncated *trpF* and *groS* genes.

In conclusion, analysis of the genetic environment of bla_{NDM-1} in *A. baumannii* 161/07 revealed a transposon structure composed of two copies of insertion sequence IS*Aba125* that is integrated into the bacterial chromosome. However, since bla_{NDM-1} is flanked by these insertion elements, we cannot discount the possibility of integration into a plasmid and subsequent horizontal spread. The variability of the genetic environment of bla_{NDM-1}, as evidenced by the transposon structure described here, with DNA seemingly originating from Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*, may explain the observed rapid dissemination of this gene within many Gram-negative bacterial species and across genera. Thus there is an urgent need for further investigations to find the origin of this gene and its mechanisms of spread.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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Figure

Figure 1. Schematic diagram showing the genetic environment of *bla*$_{\text{NDM-1}}$ in *A. baumannii* 161/07. (A) Chromosomal genes *cepI* and the truncated *mfs*; (B) IS*Aba125*; (C) *bla*$_{\text{NDM-1}}$ and truncated *trpF*; (D) truncated chaperonin subunit *groS*, chaperonin *groL* and the transposase *insE*; (E) 8 bp target duplication.