Dissemination of New Delhi metallo-β-lactamase-1-producing *Acinetobacter baumannii* in Europe

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Abstract

Multidrug-resistant and New Delhi metallo-β-lactamase 1 (NDM-1) -producing *Acinetobacter baumannii* are increasingly reported. A collection of five NDM-1-positive *A. baumannii* isolates recovered in four European countries were analysed. Genotyping was performed by pulsed-field gel electrophoresis, multiplex PCR sequence typing, Diversilab and multilocus sequence typing. Three distinct sequence types were identified. All isolates harboured a chromosomally located bla_{NDM-1} gene within a Tn*125*-like transposon. One isolate co-expressed another unrelated carbapenemase OXA-23. This report constitutes the first epidemiological study of NDM-1-producing *A. baumannii* from four countries.

Keywords: Carbapenemase, Gram-negative rods, New Delhi metallo-β-lactamase, Tn*125*

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Acinetobacter baumannii is an opportunistic pathogen that is an important source of nosocomial infections, mostly pneumonia [1]. The treatment of infections due to this microorganism is becoming a serious clinical concern because *A. baumannii* is frequently resistant to multiple antibiotics [2]. Resistance to carbapenems in *A. baumannii* is mostly related to the production of carbapenem-hydrolysing class D β-lactamases and to a lesser extent to metallo-β-lactamases [2].

Recent reports showed that the bla_{NDM-1} gene encoding New Delhi metallo-β-lactamase 1 (NDM-1) is spreading worldwide among gram-negative bacteria [3]. The bla_{NDM-1} gene was initially identified in *Klebsiella pneumoniae* and *Escherichia coli* isolates but has been additionally reported in many other Gram-negative rods [3,4]. In particular, the bla_{NDM-1} and bla_{NDM-2} genes have been recently identified in *A. baumannii* [5–9] and in other *Acinetobacter* species [10,11]. Notably, both genes have been identified as located on the same composite transposon named Tn*125*, being 10 099-bp long and comprising two copies of an identical insertion sequence IS*Aba125* bracketing the $bla_{NDM-1/-2}$ genes [12,13].

Our study aimed to study the clonal relationship of NDM-1-producing *A. baumannii* and the genetic context of bla_{NDM-1} responsible for dissemination of this resistance trait, by analysing a collection of bla_{NDM-1}-positive *A. baumannii* isolates recovered from different European countries. Isolate 161/07 was recovered in Germany in 2007 with a Balkan origin [5], isolate Slo was from a respiratory sample taken in Slovenia in 2008, isolate JH was collected in Switzerland in 2010 with a Balkan origin [12], and the two remaining isolates were isolated in France in 2011, one of those two being imported in France from Algeria (Table 1) [14].

The isolates were identified by 16S rRNA gene sequencing [15]. Susceptibility testing was performed by disc-diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France) and interpreted according to updated CLSI guidelines [16]. The MICs of β-lactams including imipenem, meropenem and doripenem were determined by Etest (AB bioMérieux; Solna, Sweden) as described previously [17]. All isolates were resistant to β-lactams including carbapenems (Table 1). The production of metallo-β-lactamases was assessed using a combined disc-test, based on the inhibition of the metallo-β-lactamase activity by EDTA as described elsewhere [18]. All isolates were positive for the production of metallo-β-lactamases. The PCR experiments performed to detect carbapenemase genes as described previously [19], followed by sequence analysis, led to the identification of the bla_{NDM-1} gene in the five isolates in addition to naturally occurring bla_{OXA-51}-like genes (respectively encoding OXA-64, OXA-69 or OXA-94) (Table 1). In addition, the bla_{OXA-23} gene, coding for the

TABLE 1. Features of NDM-1 producing *Acinetobacter baumannii*

A. baumannii isolates	Country of isolation	Year of isolation	Sample	Carbapenemase genotyping	MICs[c]					EC[a]//ST[b]	Pulsotype	Support	Genetic vehicle
					CTX	CAZ	IPM	MEM	DOR				
JH	Switzerland	2010	Rectal swab	NDM-1, OXA-23, OXA-69	>256	>256	>32	>32	>32	I/1	A	Chromosomal	Tn*125*
Slo	Slovenia	2008	Respiratory sample	NDM-1, OXA-64	>256	>256	>32	>32	>32	nt/25	B	Chromosomal	Tn*125*
161/07	Germany	2007	Skin, respiratory sample	NDM-1, OXA-64	>256	>256	>32	>32	>32	nt/25	B	Chromosomal	Tn*125*
Ora-1	France	2011	Rectal swab	NDM-1, OXA-94	>256	>256	>32	>32	>32	nt/85	C	Chromosomal	*Δ*Tn*125*
StN	France	2011	Rectal swab	NDM-1, OXA-94	>256	>256	>32	>32	>32	nt/85	C	Chromosomal	*Δ*Tn*125*

[a]Multiplex PCR for determining the clonal complex [22].
[b]Multi-locus sequence typing [21].
[c]CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; DOR, doripenem.

unrelated carbapenemase OXA-23, was identified in isolate JH, as found previously in one Indian *A. baumannii* isolate [12].

To determine the genetic location of the bla_{NDM-1} gene, the plasmid DNA of the different *A. baumannii* isolates was extracted by using the Kieser technique [20] and Southern hybridization was performed by using internal PCR-obtained amplicons as probe for the bla_{NDM} gene. DNA hybridization gave negative results, suggesting that the bla_{NDM-1} gene was chromosomally located in all isolates (data not shown). Transfer of the ticarcillin-resistance marker into *A. baumannii* BM4547 was attempted by liquid mating-out assays at 37°C and by electroporation of a plasmid DNA suspension extracted from *A. baumannii* clinical isolate into *A. baumannii* BM4547. It gave negative results, reinforcing the hypothesis of a chromosomal location of the bla_{NDM-1} gene in all isolates.

The genetic context of bla_{NDM-1} gene was determined by PCR mappings or by shotgun clonings using *Hin*dIII-restricted genomic DNA and the *Hin*dIII-restricted pBK-CMV plasmid as described previously [17]. Recombinant plasmids were selected onto Trypticase soy agar plates containing ticarcillin (100 mg/mL) and kanamycin (30 mg/mL). Sequencing of the recombinant plasmids showed that the bla_{NDM-1} gene was located in a transposon Tn*125* [12]. In isolate JH, transposon Tn*125* was inserted into a chromosomal gene encoding a putative protein of *A. baumannii* and surrounded by a 3-bp duplication (TTG) being the signature of the transposition process. In isolates SLO and 161/07, Tn*125* was inserted in a gene encoding a major facilitator superfamily transporter protein (CTG duplication). In the two remaining isolates (StN and Ora-1), Tn*125* was truncated at its righthand extremity by insertion sequence IS*Aba14* located upstream of the *dct* gene, giving rise to a truncated Tn*125* (*Δ*Tn*125*) (Table 1; Fig. 1). Further PCR assays did not identify a second copy of IS*Aba125*, suggesting that Tn*125* was no more functional in that latter structure.

Genotypic comparison was performed by multilocus sequence typing as described by Diancourt *et al.* [21], by sequence-type multiplex PCR [22], by DiversiLab following

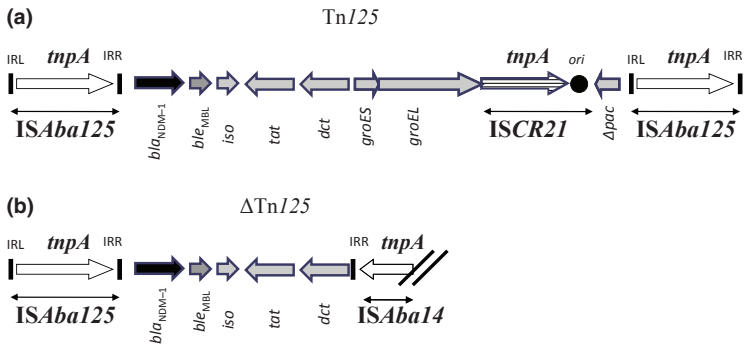

FIG. 1. Schematic representation of transposon Tn*125* carrying the bla_{NDM-1} gene. Genes and their transcription orientations are indicated by arrows. The lengths of the target genes and the exact location of the target site are not to scale. The ori*IS* of IS*CR21* is indicated by a circle. Gene names are abbreviated according to their corresponding proteins: *iso* for phosphoribosylanthranilate isomerase; *tat* for twin-arginine translocation pathway signal sequence protein; *dvt* for divalent cation tolerance protein; *Δpac* for truncated phospholipid acetyltransferase. IRL and IRR are for inverted repeat left and right, respectively. The Tn*125* complete was found in isolates JH, Slo and 161/07 and the truncated isoform of Tn*125* (ΔTn*125*) was found in isolates Ora-1 and StN.

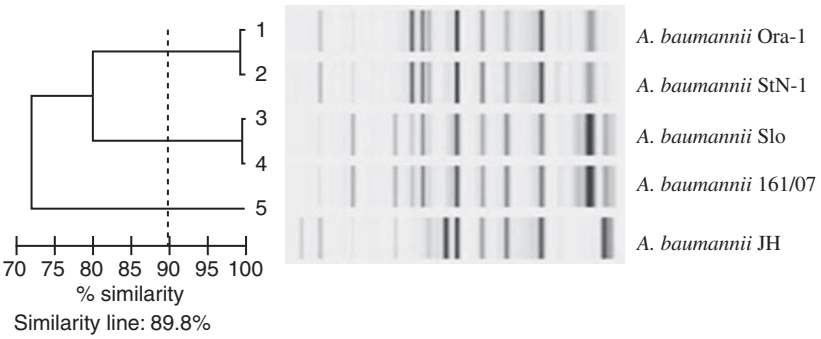

FIG. 2. Results of Diversilab analysis. Similarity line showed the cut-off to separate different clone.

the manufacturer's instructions (bioMérieux, La Balme-les-Grottes, France), and by pulsed-field gel electrophoresis as described [23]. The pulsed-field gel electrophoresis analysis showed that the five isolates were grouped into three distinct clones named A to C (Table 1), with strains Ora-1 and StN (from France) sharing identical patterns, strains Slo (from Slovenia) and 161/07 (from Germany) being clonally related according to the Tenover's criteria (two bands of difference) [24], and strain JH (from Switzerland) belonging to a different clone. Analysis by the Diversilab technique resulted in the same interpretation (Fig. 2). Further analysis with sequence-type multiplex PCR showed that isolate JH belonged to European clone I although other isolates did not correspond to any defined European clone (Table 1). Multilocus sequence typing analysis showed that isolate JH belonged to ST1, isolates Slo and 161/07 belonged to ST25, and the two remaining isolates belonged to ST85 (Table 1). ST1-type isolates are widely distributed throughout the world whereas ST25 and ST85 strains have been rarely reported (http://www.pasteur.fr/cgi-bin/genopole/PF8/mlstdbnet.pl?file=acin_isolates.xml).

In conclusion, this report highlights scattered diffusion of NDM-1 producing *A. baumannii* in Europe from the west to the east. This dissemination was neither due to a single clone nor to any plasmid diffusion but rather to different clones carrying the transposon Tn*125* or Tn*125*-derivatives which are truncated. While this study was in progress, two NDM-1-producing *A. baumannii* were isolated in Belgium and Czech Republic, [25,26]. The clinical isolate from the Czech Republic belonged to ST1, similar to isolate JH from Switzerland; whereas the clinical isolate from Belgium belonged to European clone II (whichcorresponds to ST2 in the multilocus sequence typing Pasteur Institute scheme) [25,26]. These two reports reinforce the fact that the spread of the bla_{NDM-1} gene in *A. baumannii* is not linked to a clonal spread but to the spread of a genetic structure. The spread of transposon Tn*125* in *Acinetobacter* species harbouring bla_{NDM} genes mirrors what has been observed with the bla_{KPC} carbapenemase gene, which is associated with transposon Tn*4401* [27]. Interestingly, we report here the first occurrence of an NDM-1 producer in Slovenia and therefore further confirm that Balkan countries constitute a significant reservoir for NDM-1-producing bacteria.

Nucleotide Sequence Accession Number

The nucleotide sequence data of the ΔTn*125* reported in this work has been deposited in the GenBank nucleotide database under accession no. JX000237.

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Transparency Declaration

Nothing to declare.

References

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008; 21: 538–582.
2. Poirel L, Bonnin RA, Nordmann P. Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. *IUBMB Life* 2011; 63: 1061–1067.
3. Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. *Trends Microbiol* 2011; 19: 588–595.
4. Kumarasamy KK, Toleman MA, Walsh TR *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010; 10: 597–602.
5. Göttig S, Pfeifer Y, Wichelhaus TA *et al.* Global spread of New Delhi metallo-β-lactamase 1. *Lancet Infect Dis* 2011; 10: 828–829.

6. Kaase M, Nordmann P, Wichelhaus TA, Gatermann SG, Bonnin RA, Poirel L. NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. *J Antimicrob Chemother* 2011; 66: 1260–1262.
7. Karthikeyan K, Thirunarayan MA, Krishnan P. Coexistence of bla_{OXA-23} with bla_{NDM-1} and *armA* in clinical isolates of *Acinetobacter baumannii* from India. *J Antimicrob Chemother* 2010; 65: 2253–2254.
8. Ghazawi A, Sonnevend A, Bonnin RA *et al.* NDM-2 carbapenemase-producing *Acinetobacter baumannii* in the United Arab Emirates. *Clin Microbiol Infect* 2012; 18: E34–E36.
9. Espinal P, Fugazza G, López Y *et al.* Dissemination of an NDM-2-producing *Acinetobacter baumannii* clone in an Israeli rehabilitation center. *Antimicrob Agents Chemother* 2011; 55: 5396–5398.
10. Chen Y, Cui Y, Pu F *et al.* Draft genome sequence of an *Acinetobacter* genomic species 3 strain harboring a bla_{NDM-1} gene. *J Bacteriol* 2012; 194: 204–205.
11. Hu H, Hu Y, Pan Y *et al.* Novel plasmid and its variant harboring both a bla_{NDM-1} gene and type IV secretion system in clinical isolates of *Acinetobacter lwoffii*. *Antimicrob Agents Chemother* 2012; 56: 1698–1702.
12. Poirel L, Bonnin RA, Boulanger A, Schrenzel J, Kaase M, Nordmann P. Tn*125*-related acquisition and expression of bla_{NDM}-like genes in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2012; 56: 1087–1089.
13. Pfeifer Y, Wilharm G, Zander E *et al.* Molecular characterization of bla_{NDM-1} in an *Acinetobacter baumannii* strain isolated in Germany in 2007. *J Antimicrob Chemother* 2011; 66: 1998–2001.
14. Boulanger A, Naas T, Fortineau N, Figueiredo S, Nordmann P. NDM-1-producing *Acinetobacter baumannii* from Algeria. *Antimicrob Agents Chemother* 2012; 56: 2214–2215.
15. Ibrahim A, Gerner-Smidt P, Liesack W. Phylogenetic relationship of the twenty-one DNA groups of the genus *Acinetobacter* as revealed by 16S ribosomal DNA sequence analysis. *Int J Syst Bacteriol* 1997; 47: 837–841.
16. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. CLSI M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
17. Bonnin RA, Potron A, Poirel L, Lecuyer H, Neri R, Nordmann P. PER-7, an extended-spectrum β-lactamase with increased activity toward broad-spectrum cephalosporins in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2011; 55: 2424–2427.
18. Bonnin RA, Naas T, Poirel L, Nordmann P. Phenotypical, biochemical and molecular techniques for detection of metallo-β-lactamase NDM in *Acinetobacter baumannii*. *J Clin Microbiol* 2012; 50: 1419–1421.
19. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011; 70: 119–123.
20. Kieser T. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* 1984; 12: 19–36.
21. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* 2010; 5: e10034.
22. Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. *Clin Microbiol Infect* 2007; 13: 807–815.
23. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
24. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis* 2010; 16: 35–40.
25. Bogaerts P, Rezende de Castro R, Roisin S *et al.* Emergence of NDM-1-producing *Acinetobacter baumannii* in Belgium. *J Antimicrob Chemother* 2012; 67: 1552–1553.
26. Krizova L, Bonnin RA, Nordmann P, Nemec A, Poirel L. Characterization of a multidrug-resistant *Acinetobacter baumannii* strain carrying the bla_{NDM-1} and bla_{OXA-23} carbapenemase genes from the Czech Republic. *J Antimicrob Chemother* 2012; 67: 1550–1552.
27. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011; 17: 1791–1798.