J Antimicrob Chemother 2015
doi:10.1093/jac/dku405
Advance Access publication 27 October 2014

Detection of NDM-1 carbapenemase-producing Acinetobacter calcoaceticus and Acinetobacter junii in environmental samples from livestock farms

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Keywords: plasmids, transposases, single nucleotide polymorphisms

Sir,

Recently, a new carbapenemase, NDM-1, encoded by blaNDM-1, has become a global concern.1 Previous work reported the prevalence of blaNDM-1 in animal farms in Hangzhou (Zhejiang province, China) (Table S1, doi:10.1093/jac/dku405). However, the occurrence of blaNDM-1 in soil has not been documented. Here, we screened for blaNDM-1 in soil and other environmental samples from animal farms and analysed the molecular features of regions around blaNDM-1 from soil and animal farms.

We screened for blaNDM-1 from 83 soil samples in and around animal farms in Hangzhou (Zhejiang province, China) (Table S1, available as Supplementary data at JAC Online), including surface soil and soil from deeper layers (depth 15 cm). With primers specific to blaNDM-1, the presence of blaNDM-1 was detected in isolates ABZN41 (from a pig farm) and ABZN73 (from around a cow farm) in deeper soil layers, but not from any isolates from surface soil. We also examined the presence of blaNDM-1 in 30 animal faecal and 6 waste water samples from the farms and no blaNDM-1-positive isolates were detected. ABZN41 and ABZN73 were identified as Acinetobacter junii and Acinetobacter calcoaceticus, respectively, by 16S rDNA analysis, amplified rDNA restriction analysis (ARDRA) and partial RNA polymerase β-subunit (rpoB) analysis. The antimicrobial susceptibilities of ABZN41 and ABZN73 were tested with Mueller–Hinton medium and the results were interpreted as recommended by the CLSI guideline.9 Both were highly resistant to the broad-spectrum β-lactam antibiotics (e.g. imipenem and meropenem) and susceptible to colistin and tigecycline (Table S2).

The plasmids were extracted from ABZN41 and ABZN73 and named pNDM-WS1 and pNDM-WS2, respectively. PFGE and Southern blot hybridization showed that the sizes of pNDM-WS1 and pNDM-WS2 were both ~40 kb (Figure S1). To further detail blaNDM-1 and its surrounding structure in each of the two plasmids (GenBank accession numbers KJ018153 and KJ018154 for pNDM-WS2 and pNDM-WS1, respectively), we sequenced a 13.94 kb fragment containing blaNDM-1 by primer walking. The nucleotide sequences of the two DNA fragments were identical. As in other reports,10–12 in both pNDM-WS1 and pNDM-WS2, blaNDM-1 was located downstream of the transposon ISAba125 (Figure 1a). Sequence comparisons revealed identical promoter sequences and 5′ untranslated regions of blaNDM-1 among pNDM-WS1 and pNDM-WS2 from soil and plasmids from other sources (Figure 1b).

However, the coding region of ISAba125 contained 14 SNP sites, distinguishing pNDM-WS1 and pNDM-WS2 from other blaNDM-1-carrying plasmids (Table S3). All of these SNP sites showed two-allele substitution (e.g. G to C, T to A) within or between species. The SNP sites in the coding region may lead to amino acid substitution (non-synonymous) or no amino acid substitution (synonymous) in the corresponding protein. In the ISAba125 coding region from 14 blaNDM-1-carrying bacteria, eight sites were synonymous and six sites were non-synonymous (Table S3). The Ks/Ka ratio is an indicator of selective pressure on a protein-coding gene. Ka being the number of non-synonymous substitutions per non-synonymous site and Ks being the number of synonymous substitutions per synonymous site.13 Ks/Ka <1 means that a gene is under purifying selection, indicating that selection eliminates deleterious mutations.14 Using DNASPv5 software, we calculated the Ks/Ka of the ISAba125 transposase gene for 14 blaNDM-1-carrying bacteria and discovered that all values were <1 (Table S4), indicating that it was affected by purifying selection during evolution.15 Thus, a non-synonymous mutation that changes the corresponding amino acid in the ISAba125 transposase is unlikely to change the evolutionary function of the transposase. Phylogenetic analysis indicated that ISAba125 in pNDM-WS1 and pNDM-WS2 from soil bacteria belongs to a new branch, closely related to that in the plasmid or the chromosome from Acinetobacter, Escherichia and Providencia (Figure S2). To examine transferability of pNDM-WS1 and pNDM-WS2, conjugation was carried out with ABZN41 and ABZN73 as the donors and Escherichia coli J53 (resistance to azide) as the recipient. Conjugation frequencies were 6.84 ± 1.11 × 10−7 and 73.3 ± 3.53 × 10−7, respectively, showing that pNDM-WS1 and pNDM-WS2 were transferable through conjugation.

In summary, we detected two blaNDM-1-positive bacteria from soil in and around animal farms, further expanding the...
Figure 1. (a) Schematic representations of genetic elements surrounding \( \text{bla}_{\text{NDM}-1} \) from different isolates (Acinetobacter ABZN41 pNDM-WS1, Acinetobacter ABZN73 pNDM-WS2, Acinetobacter lwoffii pNDM-BJ01, Acinetobacter baumannii pNDM-AB, Acinetobacter calcoaceticus XM1570, Pseudomonas aeruginosa MMA83 and Providencia stuartii pMR0211; their GenBank accession numbers are KJ018154, KJ018153, JQ001791, KC503911, NZ_KB465429, HF546976 and JN687470, respectively). \( \text{IS}_{\text{Aba125}} \) is represented by grey arrows and \( \text{bla}_{\text{NDM}-1} \) is represented by black arrows. (b) Comparison of the promoter sequences and the 5′ untranslated regions of \( \text{bla}_{\text{NDM}-1} \) among Acinetobacter ABZN41 pNDM-WS1, Acinetobacter ABZN73 pNDM-WS2, A. lwoffii pNDM-BJ01, A. baumannii pNDM-AB, A. calcoaceticus XM1570, P. aeruginosa MMA83 and P. stuartii pMR0211. The –35 region, the –10 region, the ribosome binding site (RBS) and the start codon of the \( \text{bla}_{\text{NDM}-1} \) ORF are represented by underlined letters.
environmental reservoir of blaNDM-1. Analysis of the molecular features of ISAb125 in host and soil bacteria carrying blaNDM-1 indicates that ISAb125 has been under purifying selective pressure during its evolution. Since the carbapenems are not approved for use in food-producing animals worldwide, we propose that the presence of blaNDM-1 in animals and animal surroundings (e.g., the farm environment) most likely originates from either contamination by humans and/or co-selection due to other resistance genes located on the same blaNDM-1-carrying mobile genetic element.

Acknowledgements

We thank Dr. Ying Fu, Dr. Miaomiao Li and Jumei Zhan for providing technical assistance, Dr. Bo Deng for providing assistance in sample collection, Dr. Yan Jiang, Dr. Hua Jiang and Dr. Danfeng Jin for providing useful suggestions and Dr. Yanmei Zhang for critically reading the manuscript prior to submission.

Funding

This work was supported by the Natural Science Foundation of China (grant number 31100071), the China Postdoctoral Science Foundation (grant number 2012MS2119), the Natural Science Foundation of Zhejiang Province (grant number Y13110237), a Zhejiang Postdoctoral Grant (grant number BS1202079), the Zhejiang Open Foundation of the Most Important Subjects and the Science and Technology Innovation Ability Promotion Project of the Zhejiang Academy of Agricultural Sciences (grant number 2014CX018).

Supplementary data

Tables S1 to S4, Figure S1 and Figure S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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Characterization of the genetic environment of the ribosomal RNA methylase gene erm(B) in Campylobacter jejuni

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Keywords: foodborne pathogens, multidrug resistance, food safety, campylobacteriosis

Sir,

Campylobacter jejuni is the leading cause of foodborne bacterial gastroenteritis in humans. C. jejuni is increasingly resistant to clinically important antibiotics, particularly fluoroquinolones and macrolides. Because of the lack of ribosomal RNA methylases