Journal of Global Antimicrobial Resistance 26 (2021) 128–129

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

First report of NDM-1 in an *Acinetobacter baumannii* strain from a pet animal in Europe

Editor: Dr J. Hobman

Sir,

Multidrug-resistant *Acinetobacter baumannii*, including carbapenem-resistant strains carrying New Delhi metallo-β-lactamase 1 (NDM-1), have emerged as a major cause of healthcare-associated infections worldwide [1]. Although carbapenem-resistant *Acinetobacter* spp. strains have been reported from animals, NDM-1-producing strains are still rare. They mainly occurred in livestock animals in China and were often identified in non-*baumannii* strains [2–4].

Here we report an NDM-1-positive, carbapenem-resistant *A. baumannii* strain belonging to a globally distributed clonal lineage isolated from a dog suffering from a urinary tract infection in Europe. Strain IHIT38008, identified as *A. baumannii* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik, Bremen, Germany), was isolated from the urine of a dog from Italy during routine diagnostics in a veterinary microbiology laboratory in Germany in 2018. Antimicrobial susceptibility was determined using VITEK®2 (bioMérieux, Nürtingen, Germany; cards AST-GN38 and AST-N248), antibiotic gradient strips in the case of carbapenems (Liofilchem, Roseto degli Abruzzi, Italy) and a MICRONAUT system (Merlin Diagnostika GmbH, Bornheim-Hersel, Germany) for colistin. According to Clinical and Laboratory Standards Institute (CLSI) breakpoints, the isolate was resistant to all β-lactams, including the carbapenems imipenem [minimum inhibitory concentration (MIC) = 8 mg/L], doripenem (MIC > 32 mg/L) and meropenem (MIC = 8 mg/L). It was also resistant to enrofloxacin (MIC ≥ 4 mg/L), marbofloxacin (MIC ≥ 4 mg/L) and nitrofurantoin (MIC ≥ 512 mg/L) but showed susceptibility to amikacin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole and colistin. The genome was sequenced using MiSeq (Illumina Inc., San Diego, CA, USA) (Sequence Read Archive no. **SDSK00000000.1**), and de novo assembly and annotation of sequences were performed with SPAdes v.3.15.1 (http://cab.spbu.ru/software/spades/) and RAST v.2.0 (http://rast.nmpdr.org/), respectively. Using ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder/), we identified the carbapenemase gene $bla_{\text{NDM-1}}$ and the intrinsic β-lactamase genes $bla_{\text{OXA-64}}$ and $bla_{\text{ADC-25-like}}$. In accordance with the resistance observed for fluoroquinolones, we identified mutations in the quinolone resistance-determining region of DNA gyrase (GyrA; S83L) and topoisomerase IV (ParC; E84K). Other known resistance genes or mechanisms were not present.

NDM-producing *Acinetobacter* spp. strains have been frequently reported from humans [1,5], but there are much fewer findings in animals [2–4,6]. In China, the $bla_{\text{NDM-1}}$ gene was identified in *A. baumannii* and *Acinetobacter calcoaceticus* isolated from pigs, in *Acinetobacter lwoffii* from chicken and cat, and recently in *Acinetobacter indicus, Acinetobacter schindleri* and *A. lwoffii* from waterfowl [2,4]. So far, there is only one report regarding an NDM-1-positive *Acinetobacter* sp. strain in Europe, namely an *Acinetobacter radioresistens* strain isolated from a rectal swab of a hospitalised dog in Italy in 2014 [6].

The $bla_{\text{NDM-1}}$ gene in strain IHIT38008 was identified on a whole-genome sequencing contig of 345 993 bp in size. This, together with the results from mlplasmids (https://sarredondo.shinyapps.io/mlplasmids/) analysis, indicated the chromosomal location of the gene, which was confirmed by *S1* nuclease digestion and subsequent pulsed-field gel electrophoresis (PFGE) and Southern blot hybridisation. By analysing the genetic environment of $bla_{\text{NDM-1}}$ using Geneious v.R8.1 (Biomatters Ltd., Auckland, New Zealand), we could locate the gene inside the composite transposon Tn*125* (Fig. 1). This transposon structure was first described in a clinical *A. baumannii* strain (161/07) from a hospitalised patient in Germany with travel history to Serbia in 2007 [5]. Of note, the ~10.1-kb transposon structure of IHIT38008 (GenBank accession no. **MK467522.1**) revealed > 99.9% sequenctie identity with that of the human strain 161/07. In addition, it was also almost similar to transposon sequences previously identified in *A. baumannii* strain JH from Switzerland and on plasmid pNDM-Iz4b of feline *A. lwoffii* strain Iz4b [1,3,5]. In addition, the NDM-1-positive *A. radioresistens* strain from a hospitalised dog in Italy shows this genetic environment [6]. In contrast, plasmids pNDM-AB and pAL-1, both identified in *Acinetobacter* spp. from livestock animals in China, differed from the previous ones by carrying a partial Tn*125* (Fig. 1). In strain IHIT38008, Tn*125* was inserted into a gene encoding a protein of unknown function (GenBank no. **MK467522.1**, locus_id QBQ02716.1). This insertion site differs from those previously published (Fig. 1). These data further confirm that independent transposition events targeted the chromosomes and plasmids of different *Acinetobacter* spp. strains and that the Tn*125* transposon likely contributes to the global spread of NDM-1 [1,2].

Our strain was assigned to ST25$^{\text{Pasteur}}$ (https://cge.cbs.dtu.dk/services/MLST/) and international clone VII, an emerging genotype that has been associated with epidemics in humans worldwide.

A core genome comparison of 39 *A. baumannii* ST25 strains (Supplementary Table S1) revealed that IHIT38008 clustered closely [maximum of 568 single nucleotide polymorphisms (SNPs) among 2,222 orthologous genes] to strains previously isolated from human patients (Supplementary Fig. S1). Only 179 SNPs distinguished IHIT38008 from strain 1429530 obtained from a perirectal swab of a human in the USA. Strains from Europe were less related, as exemplified by 161/07 (Germany; 1,861 SNPs), RUH1486 (The Netherlands; 2,882 SNPs) and 4190 (Italy; 5,708 SNPs).

In summary, we describe the first case of an NDM-1-producing *A. baumannii* belonging to the successful clonal ST25 lineage from a pet in Europe. Together with the finding that $bla_{\text{NDM-1}}$ was integrated into transposon Tn*125*, which is a major vehicle for NDM-

https://doi.org/10.1016/j.jgar.2021.05.003

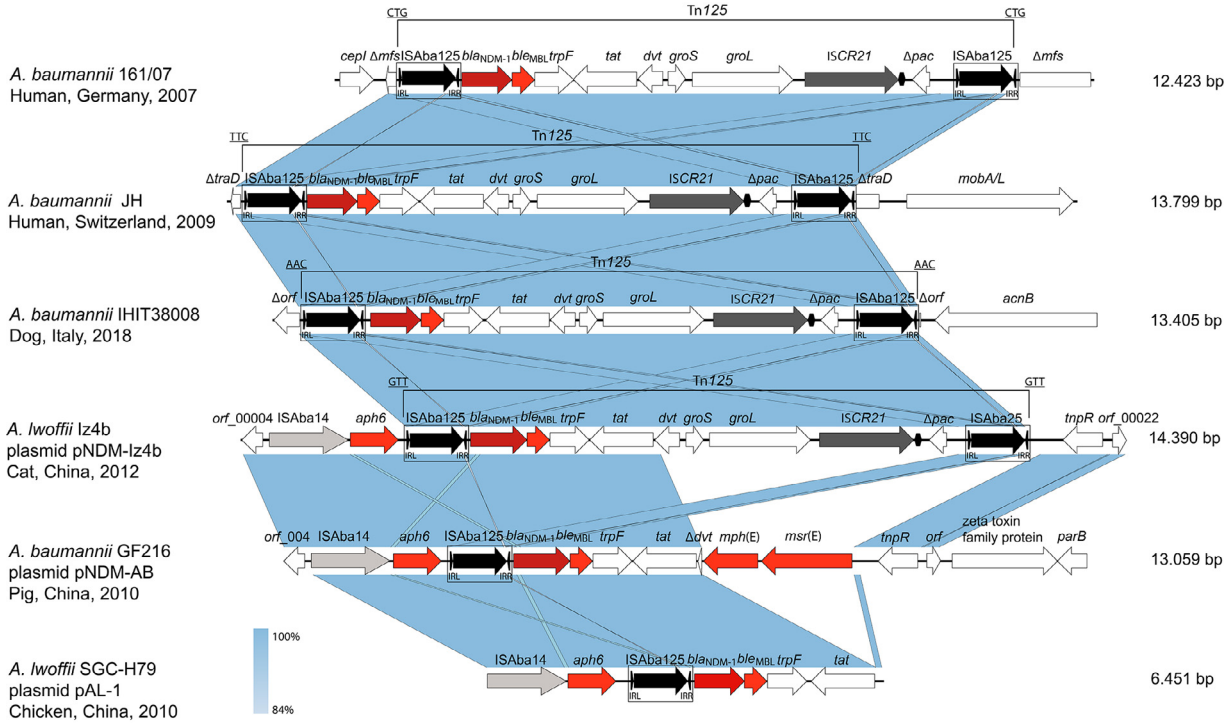

Fig. 1. Genetic environment of the bla_{NDM-1} gene in canine *Acinetobacter baumannii* strain IHIT38008 (GenBank assession no. **MK467522.1**) and comparison with the corresponding genetic regions in strains of human origin [*A. baumannii* 161/07 (**HQ857107** and **JZCA00000000.1**); *A. baumannii* JH, Switzerland (**JN872329.1**)] and animal origin [*Acinetobacter lwoffii* SGC-H79 plasmid pAL-1 (**JN616388.1**); *A. lwoffii* Iz4b plasmid pNDM-Iz4b (**KJ547696.1**); *A. baumannii* GF216 plasmid pNDM-AB (**KC503911.1**)]. Genes and their transcription orientations are presented by arrows. Resistance genes are indicated by red arrowed boxes and insertion sequences by grey and black arrowed boxes. In the four Tn*125*-carrying strains, the transposon is bracketed by a 3-bp target site duplication (transposon signature; underlined and uppercase). IRR and IRL indicate inverted repeats left and right, respectively. Tn*125* was inserted into different genes in these strains, encoding a protein of unknown function (IHIT38008), a transfer protein (*traD*; JH) and a putative major facilitator superfamily metabolite/H^+ symporter (*mfs*; 161/07), or an intergenic region (pNDM-Iz4b) located between *aphA6* and IS*Aba125* on the left site and between two copies of IS*Aba125*. Map generation was performed with Easyfig v.2.2.2 (http://mjsull.github.io/Easyfig/).

1 in human *Acinetobacter* spp. and *Enterobacterales*, our data suggest that companion animals may incidentally acquire NDM-1-producing strains from humans. These findings warrant further investigation of the epidemiology of carbapenem resistance in *Acinetobacter* spp. strains from animals and the processes that may favour the emergence and spread of such bacteria in veterinary settings.

Funding

LJ was financially supported by the 'Akademie für Tiergesundheit e.V.'.

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.05.003.

References

[1] Poirel L, Bonnin RA, Boulanger A, Schrenzel J, Kaase M, Nordmann P. Tn*125*-related acquisition of bla_{NDM}-like genes in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2012;56:1087–9.

[2] Cui C-Y, Chen C, Liu B-T, He Q, Wu X-T, Sun R-Y, et al. Co-occurrence of plasmid-mediated tigecycline and carbapenem resistance in *Acinetobacter* spp. from waterfowls and their neighboring environment. Antimicrob Agents Chemother 2020;64:e02502–19.

[3] Sun Y, Ji X, Liu Y, Liu Q, Guo X, Liu J, et al. New Delhi metallo-β-lactamase-1-producing *Acinetobacter lwoffii* of companion animal origin in China. Indian J Med Microbiol 2015;33:615–17.

[4] van der Kolk JH, Endimiani A, Graubner C, Gerber V, Perreten V. *Acinetobacter* in veterinary medicine, with an emphasis on *Acinetobacter baumannii*. J Glob Antimicrob Resist 2019;16:59–71.

[5] Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, Hunfeld K-P, 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.

[6] Gentilini F, Turba ME, Pasquali F, Mion D, Romagnoli N, Zambon E, et al. Hospitalized pets as a source of carbapenem-resistance. Front Microbiol 2018;9:2872.

Lisa Jacobmeyer
Institute of Hygiene and Infectious Diseases of Animals (IHIT), Giessen, Germany

Ivonne Stamm
IDEXX GmbH, Ludwigsburg, Germany

Torsten Semmler
Robert Koch Institute, Microbial Genomics, Berlin, Germany

Christa Ewers*
Institute of Hygiene and Infectious Diseases of Animals (IHIT), Giessen, Germany

*Corresponding author: Tel.: +49 641 993 8300.
E-mail address: christa.ewers@vetmed.uni-giessen.de (C. Ewers)
Revised 15 April 2021