ampicillin/sulbactam were not tested.\(^3,6\) Notably, the isolate remained intermediately susceptible to minocycline with an MIC of 8 mg/L. Interestingly, previous studies reported *in vitro* activity of minocycline, an old, rarely used tetracycline derivative, against \(\approx 70\%\) of multidrug-resistant (MDR) *A. baumannii*.\(^7\)

Detection of carbapenemases by PCR for acquired \(\beta\)-lactamase \((\text{bla})\) genes encoding VIM, IMP, NDM, KPC and OXA-23, -40, -48, -58, -143 and -235 using whole-cell DNA and subsequent sequencing revealed the presence of the carbapenemase \text{bla}_{OXA-23}.\(^8\) PFGE and subsequent \text{bla}_{OXA-23} detection by Southern hybridization suggested a chromosomal location for the OXA-23 gene. A 3.5 kb sequence obtained by primer walking revealed that it was located in transposon Tn\(\text{0082358}\) and embedded in the chromosomal \text{IysE} gene (locus tag ADU58354).

Molecular strain typing was performed by semi-automated, repetitive sequence-based PCR (rep-PCR) using the DiversiLab platform (bioMérieux, Nürtingen, Germany). Our isolate grouped with the international clone (IC) 2, also known as European clone 2.\(^7\) Furthermore, subtyping of the chromosomally located intrinsic OXA-51-like \text{bla} gene identified \text{bla}_{OXA-66}, which is also associated with IC2. IC2 accounts for \(\approx 50\%\) of all isolates worldwide and can therefore be considered as the most successful and widespread clonal lineage of *A. baumannii*.\(^7,9\) There are no German national surveys of *A. baumannii* carbapenem resistance rates. However, in smaller surveys it has been shown that carbapenem resistance, although not prevalent, is most often associated with IC2 isolates.\(^10\) Furthermore, in contrast to the PDR isolate, carbapenem-resistant isolates belonging to IC2 are usually susceptible to minocycline, tigecycline and polymyxins.\(^7,10\)

This is the first report of a PDR *A. baumannii* isolate in Germany. Since PDR Gram-negatives will further disseminate and no treatment options are left, rigorous screening and pre-emptive isolation of patients with a history of medical treatment in a country where MDR Gram-negatives are endemic seems indispensable.

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Transparency declarations
None to declare.

References
1 EUCAST. *Clinical Breakpoint Tables (v 3.1).* http://www.eucast.org/clinical_breakpoints/ (4 April 2014, date last accessed).

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**IMP-45-producing multidrug-resistant *Pseudomonas aeruginosa* of canine origin**

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Sir,

The metallo-\(\beta\)-lactamases (MBLs), which hydrolyse all \(\beta\)-lactams except aztreonam, present a serious challenge to public health,
largely because the genes coding for MBLs can be disseminated horizontally among Gram-negative bacteria, including major pathogens for humans and animals. IMP-type carbapenemases represent one of the clinically most relevant MBL groups in terms of epidemiological dissemination; they have been reported worldwide in *Pseudomonas aeruginosa*, several members of the family Enterobacteriaceae and Acinetobacter species of human clinical origin. So far, bacterial isolates from non-human sources that harbour bla<sub>IMP</sub> genes have only been reported from river water in Tunisia (*Klebsiella pneumoniae*) and from sewage in Germany (identified by PCR without exact knowledge of the bacterial host). Here, we report the isolation of a bla<sub>IMP-45</sub>-carrying multidrug-resistant *P. aeruginosa* isolate from a dog in China during a routine surveillance study of carbapenem-resistant Gram-negative bacteria from pet animals in 2013. Using a Mueller–Hinton agar plate containing 100 mg/L vancomycin and 2 mg/L meropenem, isolate M140A was obtained from the anal swab of a healthy 3-month-old female German Shepherd dog admitted in June 2013 to the Animal Teaching Hospital of the China Agricultural University in Beijing, China, for routine vaccination against canine parvovirus infection. The isolate was identified as *P. aeruginosa* by both the API 20NE system (bioMérieux, Marcy-l’Étoile, France) and 16S rDNA sequencing as described previously. MICS of various antimicrobial agents were determined by broth microdilution and interpreted using the clinical breakpoints given in CLSI document M100-S24.IMP-13-producing isolates from patients in France. IMP-type carbapenemases, including IMP-13-producing isolates from patients in France.

The imipenem-EDTA double-disc synergy test and Etest using a Mueller–Hinton agar plate confirmed that this isolate produced an MBL. The presence of known mobile MBL-encoding genes for NDM-, VIM-, SIM-, SPM-, GIM-, AIM- and DIM-type β-lactamases was investigated using previously described PCR assays. Only bla<sub>IMP</sub> was detected using the primers F 5'-GAAGGTYTTTAGTCTCAC-3' and R 5'-GTAAGTTTCAAAGAGTGA TGC-3'. The complete bla<sub>IMP</sub> gene was obtained by primer walking using primers located inside the bla<sub>IMP</sub> gene. The resulting sequence contained an open reading frame of 738 bp, which showed 99.9% nucleotide sequence identity (737/738 bp, A640G nucleotide substitution) to the bla<sub>IMP-9</sub> gene from plasmid pOZ176 (GenBank accession no. NC_022344) of a clinical *P. aeruginosa* of human origin isolated during a multicentre surveillance study in Guangzhou, China. The corresponding 245 amino acid protein differed from that of IMP-9 by a single amino acid exchange (Gly214Ser). The bla<sub>IMP</sub> gene from *P. aeruginosa* M140A showed 100% nucleotide sequence identity to a bla<sub>IMP-45</sub> gene of not further specified origin, listed in the database of the nomenclature centre (http://www.lahey.org/studies). S1 nuclease PFGE and Southern blot analysis indicated that the bla<sub>IMP-45</sub> gene was located in the chromosomal DNA (Figure S1, available as Supplementary data at JAC Online).

Multilocus sequence typing (MLST) of isolate M140A was performed according to published protocols (http://pubmlst.org/paeruginosa). Comparison with the allelic profiles available in the aforementioned MLST database identified isolate M140A as sequence type (ST) 308. This ST has been associated with multidrug-resistant *P. aeruginosa* isolates carrying different types of carbapenemases, including IMP-13-producing isolates from patients in France.

The flanking regions of the bla<sub>IMP-45</sub> gene were sequenced using a modified random primer walking strategy, resulting in a 10991 bp fragment (GenBank accession no. KJ510410; Figure 1). The bla<sub>IMP-45</sub> gene was located in a class 1 integron as part of a resistance gene cassette array consisting of aacA4-bl<sub>IMP-45</sub>-bla<sub>OXA-1</sub>-catB3, which confers resistance to gentamicin and amikacin, carbapenems, ampicillin and chloramphenicol, respectively. The 6149 bp segment that comprised the bla<sub>IMP-45</sub>-carrying
class 1 integron showed 100% nucleotide sequence identity to the corresponding segment of a plasmid from the human clinical P. aeruginosa strain B3 isolated in Guangzhou (accession no. EU588392). Moreover, the 3′ conserved region of the integron (qacE\textsubscript{\text{11}} and sul\textsubscript{I}) and the upstream region of the bla\textsubscript{IMP-45} gene including aac\textsubscript{4}\text{A}\text{4}, the 5′ conserved segment (int\textsubscript{IF}) of the integron and a Tn1403-like transposon (tnp\textsubscript{R} and tnp\textsubscript{A}) exhibited 99.9% (2348/2349) and 100% (5728/5728) nucleotide sequence identity, respectively, to corresponding regions of plasmid pOZ176 from a clinical P. aeruginosa isolate also found in Guangzhou (Figure 1).

In companion animals, carbapenemase-producing Escherichia coli carrying bla\textsubscript{NDM-1} or bla\textsubscript{OXA-48} and K. pneumoniae carrying bla\textsubscript{OXA-48} have been isolated from clinical infections.\textsuperscript{7,8} The future will show whether these observations represent ‘the tip of the iceberg’ or rare accidental findings.\textsuperscript{6,9} In the present case, the frequency of identification of P. aeruginosa ST308 from human clinical samples as well as the high level of similarity of the genetic environment of bla\textsubscript{IMP-45} from canine P. aeruginosa with integrons found in human P. aeruginosa strongly suggest a human-to-dog transfer of this IMP-45-producing isolate. Considering that (i) carbapenemases are not approved for use in animals and (ii) most of the carbapenemase genes found in animals have been shown to be part of multiresistance integrons or multiresistance gene regions,\textsuperscript{1,10–12} co-selection and co-transfer of carbapenemase genes under the selective pressure imposed by other antimicrobial resistance genes seem to play a major role in the dissemination of carbapenemase genes. Moreover, the close contact between humans and their pet and companion animals facilitates the acquisition of carbapenem-resistant isolates of human origin by pet and companion animals. Nevertheless, surveillance of bacteria of animal origin for carbapenemase producers and prudent use of antimicrobial agents to decrease the options for co-selection of carbapenemase genes in animals are urgently warranted.

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**Supplementary data**

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

**References**


**Once-daily dosed gentamicin is more nephrotoxic than once-daily dosed tobramycin in clinically infected patients**

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