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Letter to the Editor

Detection of a new variant of OXA-23 carbapenemase in *Acinetobacter radioresistens* isolates from urban animals in Marseille, France

Sir,

Acinetobacter spp., naturally present in the environment but also in humans, are increasingly associated with human and animal infections [1]. Antimicrobial resistance in *Acinetobacter* spp. is mainly due to their great ability to acquire resistance determinants against last-resort drugs such as carbapenems. Moreover, the expression of carbapenem-hydrolysing class D β-lactamases (CHDLs), also called oxacillinases (e.g. OXA-23, OXA-24, OXA-58), is the main mechanism conferring resistance to carbapenems in *Acinetobacter* spp. [1]. The aim of the present study was to characterise carbapenemase-producing Gram-negative bacteria from chickens and yellow-legged gulls in Marseille, France.

A total of 41 faecal samples from chickens (*Gallus gallus domesticus*) ($n = 28$) and yellow-legged gulls (*Larus michahellis*) ($n = 10$) were collected from the Parc du 26e Centenaire and the beach of Estaque in Marseille, in July 2016. From two yellow-legged gulls and one chicken, three *Acinetobacter radioresistens* strains (B36, B57 and B62) were isolated on MacConkey agar supplemented with ertapenem (1 μg/mL) and were identified by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF). Antimicrobial susceptibility testing revealed that the three isolates were resistant to ticarcillin and ertapenem (ertapenem MIC = 2 μg/mL for strains B36 and B57; ertapenem MIC = 4 μg/mL for strain B62) but remained susceptible to ticarcillin/clavulanic acid, piperacillin/tazobactam, cefepime, ceftazidime, ceftriaxone, meropenem and imipenem. According to this phenotype, standard PCR targeting the bla_{OXA} genes (i.e. bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48} and bla_{OXA-58}) was positive only for bla_{OXA-23}. Sequencing of the PCR products and BlastN analysis revealed that all three *A. radioresistens* isolates harboured a $bla_{OXA-23-like}$ gene. Two of the three sequences (from strains B36 and B57) exhibited 100% nucleotide identity with the published $bla_{OXA-134}$ gene from *A. radioresistens* strain B472 (GenBank **FJ195387**), whilst the sequence from strain B62 exhibited 97% identity with the $bla_{OXA-134}$ gene (**FJ195387**) and 98% with the bla_{OXA-23} gene from *A. radioresistens* (**EU131372**). Sequence variation of the B62 $bla_{OXA-23-like}$ gene was due to six non-synonymous polymorphisms leading to six amino acid substitutions, including E27K, P29Q, V33I, T57A, A112G and A243T (Fig. 1A), therefore suggesting a new variant of the bla_{OXA-23} gene in *A. radioresistens.* Moreover, to investigate the phylogenetic relationship of this new bla_{OXA-23} variant from strain B62, a phylogenetic tree with proteins belonging to the five oxacillinase families was constructed. As shown in Fig. 1B, the three identified sequences as well as that annotated as OXA-134 (**FJ195387**) grouped together with proteins of the OXA-23 family, thus demonstrating their membership in this OXA-23 family. However, this analysis also showed that the published OXA-134 from *A. radioresistens* is falsely identified and should be renamed OXA-23-like protein. This analysis revealed that the three identified sequences form a separate clade with respect to the other OXA-23 family proteins (Fig. 1B) and thus confirms the new OXA-23 variant status of the CHDLs identified in the ertapenem-resistant *A. radioresistens* strains isolated in this study. Because of their new variant OXA-23 status, antibiotic hydrolysis activity was evaluated for strain B62 against ertapenem as previously described [2] by MALDI-TOF/MS using an autoflex™ mass spectrometer and FlexControl 3.0 software (Bruker Daltonics). As expected, complete degradation of ertapenem was noted following 2 h of incubation, revealed by disappearance of the ertapenem peak (m/z 498) (Fig. 1C). Moreover, to investigate the genetic environment of the $bla_{OXA-23-like}$ gene, whole-genome sequencing was performed for *A. radioresistens* strain B62 using an Illumina MiSeq sequencer (Illumina Inc., San Diego, CA). The sequenced genome was assembled into 58 contigs with an average contig size of 55 847 bp leading to a genome size of 3 239 127 bp and a GC content of 41.62%. Genome comparison with available sequences of *A. radioresistens* from the NCBI database revealed that the $bla_{OXA-23-like}$ gene is located on the chromosome and is not associated with any transposable elements (Fig. 1D), therefore suggesting that the bla_{OXA-23} gene is an intrinsic gene in this bacterial species.

To the best of our knowledge, we report here for the first time the presence of carbapenem-resistant *A. radioresistens* isolates harbouring the $bla_{OXA-23-like}$ carbapenemase gene in urban birds such as chickens and yellow-legged gulls. *Acinetobacter radioresistens* has been reported so far in the environment, such as cotton plant and urban riverine, or in human skin or urinary tract specimens [3]. It has emerged as an opportunistic pathogen responsible for human infections such as bacteraemia and pneumonia [3] as well as animal infections such as cystitis in dogs and conjunctivitis in cats [4]. In addition, this bacterial species has also been reported as a silent source of oxacillinase genes, such as the progenitor of the most widespread bla_{OXA-23} carbapenemase in *Acinetobacter* spp. [1,5]. Infections with multi-drug-resistant (MDR) OXA-23-producing *A. baumannii* have been reported in hospitals and companion animals [5]. In 2014, Pomba's team described an isolate of MDR *A. baumannii* producing OXA-23 in a urinary tract infection in a cat. OXA-23 enzymes have been identified in *Acinetobacter* genomospecies from cattle in France [6]. More recently, carbapenemase genes from pigeons stools have been reported in Mediterranean cities [7]. Therefore, the present study confirms these recent findings and describes the first detection of $bla_{OXA-23-like}$ genes in *A. radioresistens* isolates from chickens and yellow-legged gulls in France. These findings suggest

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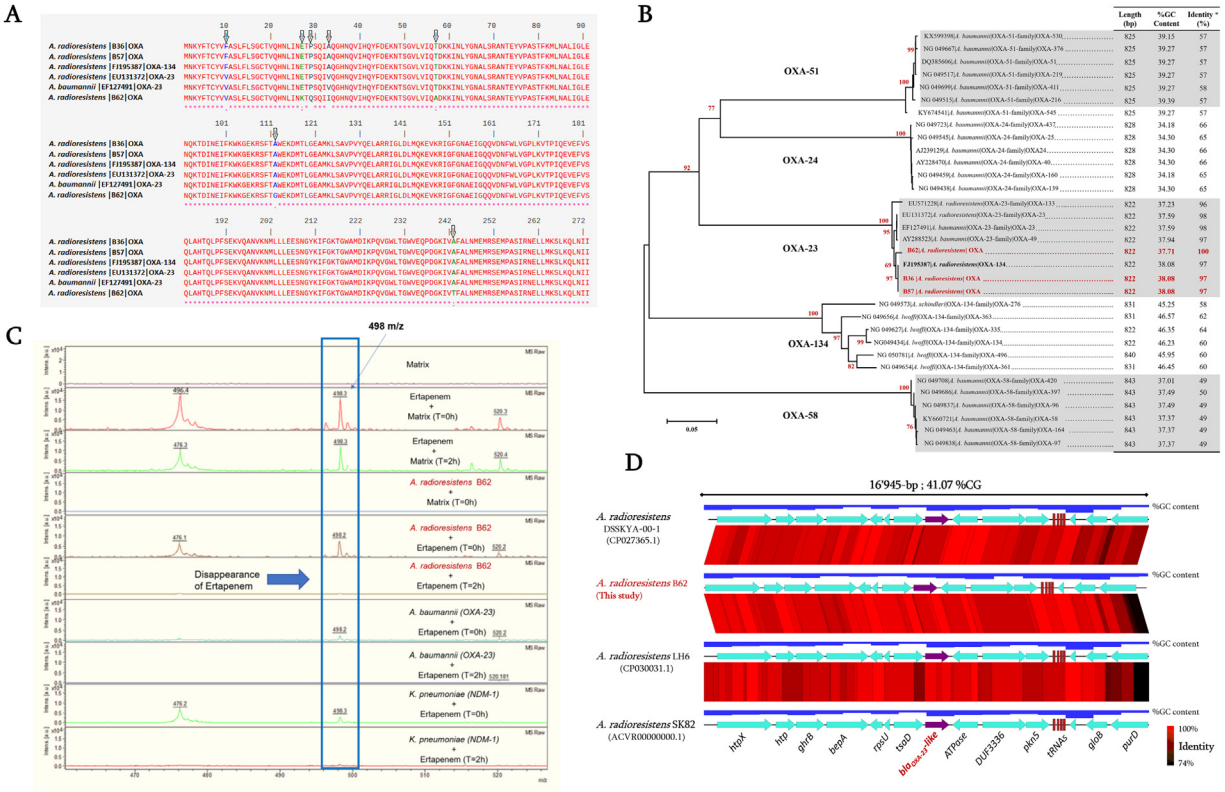

Fig. 1. Sequence analysis of the three identified $bla_{\text{OXA-23-like}}$ genes in *Acinetobacter radioresistens* in this study. (A) Protein alignment of the three OXA-23-like sequences with that of the published OXA-23 from *A. radioresistens* (**EU131372** and **FJ195387**) and that from *Acinetobacter baumannii* (**EF127491**). Positions and amino acid changes in the OXA-23-like proteins from the three strains in this study are indicated by arrows. (B) Phylogenetic tree of the three *A. radioresistens* strains in this study with the oxacillinase genes of the five OXA families from *Acinetobacter* spp. * All sequences presented here were compared with that of *A. radioresistens* strain B62. (C) Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) spectra of ertapenem hydrolysis activity of *A. radioresistens* strain B62 and two carbapenem-resistant strains (one *Klebsiella pneumoniae* carrying $bla_{\text{NDM-1}}$ and one *A. baumannii* carrying $bla_{\text{OXA-23}}$) used as positive controls. Strains were incubated at 37 °C for 2 h (NaCl 0.85%) and ertapenem was tested at a concentration of 0.25 mg/mL. Units on the *x*-axis represent the mass per charge (m/z in Da) and those on the *y*-axis represent the relative intensity. Incubation of ertapenem with the three resistant strains tested after 2 h ($T = 2$ h) resulted in disappearance of the peak at m/z 498.1 (ertapenem sodium salt, [M + Na]), thus emphasising hydrolysis of the antibiotic. *Acinetobacter baumannii* (OXA-23) and *K. pneumoniae* (NDM-1) are used as positive controls. For *A. baumannii* (OXA-23), ertapenem was quickly degraded at $T = 0$ h, thus the two characteristic molecular peaks at 476.1 ([M + H] native form) and 498.1 (ertapenem sodium salt, [M + Na]) were weakly detectable. (D) Comparison of the genetic environment of the $bla_{\text{OXA-23-like}}$ gene and comparison with available genomes of *A. radioresistens* retrieved from the NCBI database. The 16 kb surrounding the $bla_{\text{OXA-23-like}}$ gene was compared. The annotation of all genes is presented in Supplementary Table S1.

that urban animals, such as chickens and yellow-legged gulls that are migratory birds and predators, can be reservoirs and potential vectors of the dissemination of carbapenemase genes in the community.

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Competing interests

None declared.

Ethical approval

Not required.

Availability of data and material

The three sequenced $bla_{\text{OXA-23-like}}$ genes from this study have been deposited in the NCBI database under the following accession nos.: *A. radioresistens* B36 (**MG213846**); *A. radioresistens* B57 (**MG213847**); and *A. radioresistens* B62 (**MG213848**). The whole genome sequence of *A. radioresistens* strain B62 has been deposited in the GenBank database under accession no. **QZDR00000000.1**.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2019.01.021.

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