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Letters to the Editor

Emergence of OXA-72-producing *Acinetobacter pittii* clinical isolates

Sir,

The genus *Acinetobacter* comprises 47 characterised genomic species, among which species belonging to the *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex are the most clinically relevant. Within this complex, *A. baumannii*, *Acinetobacter nosocomialis* (formerly genomic species 13TU) and *Acinetobacter pittii* (formerly genomic species 3) are frequently associated with hospital-acquired infections [1]. Carbapenem resistance is being increasingly reported in *Acinetobacter* spp. isolates and this resistance trait is often related to the production of acquired carbapenem-hydrolysing class D β-lactamases (CHDLs) that are disseminating worldwide [2]. Five groups of acquired CHDLs have been identified to date in *A. baumannii*, namely OXA-23, OXA-24/-40, OXA-58, OXA-143 and OXA-235 [2]. OXA-72 is a point mutant of OXA-40 that was first described in carbapenem-resistant *A. baumannii* clinical isolates in China [2]. It was then reported in Colombia from a clinical isolate (*A. pittii* 2688), which has been used here as a reference strain [3].

This study was initiated by the isolation of three imipenem-non-susceptible *Acinetobacter* spp. isolates recovered in three hospitals in France in 2011–2013. Isolate RA1 was from the sputum of a patient hospitalised in November 2011, isolate RA2 was from pus of an 84-year-old patient in December 2011, and isolate RA3 was recovered after rectal screening of a 56-year-old patient in May 2013. These isolates were resistant to penicillins and penicillin–inhibitor combinations and were of intermediate susceptibility to carbapenems according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org/clinical_breakpoints/). By contrast, they remained susceptible to ceftazidime and cefepime as well as to amikacin, rifampicin, colistin, fluoroquinolones, tetracycline and tigecycline according to the EUCAST guidelines. The isolates were identified using the API32GN system (bioMérieux, Marcy l'Étoile, France), partial sequencing of their 16S rDNA genes and matrix-assisted laser desorption/ionisation time-of-flight mass (MALDI-TOF) analysis. Identification results showed that the three *Acinetobacter* spp. strains belonged to the *A. pittii* species.

Since the resistance phenotype to β-lactams suggested the production of a CHDL, corresponding genes were searched by PCR as described previously [2]. Interestingly, PCR followed by sequencing analysis identified the bla_{OXA-72} gene in the three isolates.

To determine the genetic location of the bla_{OXA-72} gene, transfer of the ticarcillin resistance marker into *A. baumannii* BM4547 was attempted by liquid mating-out assays at 37 °C and by electrotransformation of a plasmid DNA suspension extracted from the three clinical isolates and the reference strain (*A. pittii* 2688). Conjugation remained unsuccessful; nevertheless, transformants were obtained for the three clinical isolates and the reference strain, revealing

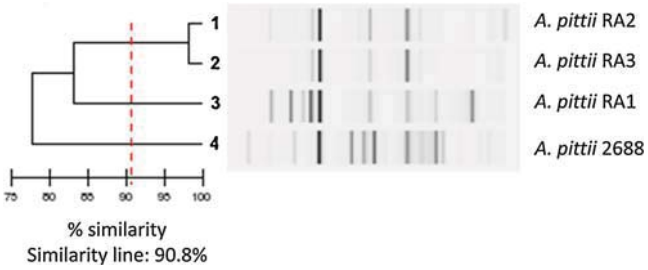

Fig. 1. Results of DiversiLab (bioMérieux, La Balme-les-Grottes, France) analysis. The horizontal similarity line showed the cut-off to separate different clones.

that bla_{OXA-72} was plasmid-located in all isolates. Plasmid analysis using the *A. baumannii* PCR-based replicon typing (AB-PBRT) scheme revealed that all French isolates possessed a plasmid of ca. 20 kb in size belonging to the GR12 family plasmid, as defined previously [4], whereas the Colombian isolate was negative for this PCR. Shotgun DNA cloning was then performed to identify the genetic structure surrounding the bla_{OXA-72} gene. It revealed very similar structures to those identified on the GR12 plasmid-type and bla_{OXA-72}-positive plasmid pMMD identified in a clinical isolate of *A. baumannii* from Spain [5]. Altogether, these data indicated that these three plasmids, although originating from different strains, were likely the same.

Genotypic comparison was performed by DiversiLab following the manufacturer's instructions (bioMérieux, La Balme-les-Grottes, France). The clinical isolate of OXA-72-producing *A. pittii* 2688 from Colombia was used as a reference strain for comparison [3]. Genotyping analysis showed that the four isolates corresponded to three distinct clones (A–C) (Fig. 1), with the Colombian isolate being distantly related to the French isolates. Two isolates, namely RA2 and RA3, were closely related. These two isolates have been recovered, respectively, in northern and southern suburb hospitals of Paris in 2011 and 2013. The remaining isolate recovered in another city in France was not related to the others.

This study constitutes the very first report of OXA-72-producing *A. pittii* in Europe following the initial identification of an OXA-72-producing *A. pittii* in Colombia. The fact that the same clone has been recovered in two different hospital settings 2 years apart likely indicated that this clone might be more widespread than expected. The difficulties in identifying *A. pittii* species might underestimate their clinical relevance, in accordance with a series of recent studies showing that non-*baumannii Acinetobacter* spp. were more clinically significant than expected.

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