Carbapenemase-producing *Acinetobacter baumannii* in two university hospitals in Algeria

The emergence of carbapenem resistance in *Acinetobacter baumannii* has become a global concern, as these β-lactams are often the only agents active against multiresistant strains (Kempf & Rolain, 2012). Over the last 10 years, an increase in carbapenem-resistant strains of *A. baumannii* has been observed worldwide; in particular, a north to south gradient has been reported in Europe (Kempf & Rolain, 2012). This north to south gradient in Europe likely suggests that the prevalence of carbapenem resistance in North Africa has increased over the last 10 years as well. The purpose of the present study was to investigate the prevalence and genetic background of carbapenem resistance in *A. baumannii* clinical isolates recovered over a 17-month period from March 2010 to July 2011 in two university hospitals in Algeria.

A total of 71 de-duplicated isolates (42 from Sétif hospital and 29 from Tizi-Ouzou hospital) were identified as *A. baumannii* using the API 20NE identification system (bioMérieux) and confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Seng et al., 2009). The peak profiles (mean of 4 mass spectra per isolate) were compared and analysed using Biotyper 2.0 software (Bruker Daltonics) to build a dendrogram of mass spectral data using the instructor default settings (Fig. 1). The strains were isolated essentially in the intensive care unit (54 %) followed by infectious and general surgery ward (25.4 %), and were recovered generally from tracheal swabs (32 %). Among the 71 strains, 34 (47.9 %) were found to be resistant to imipenem (MIC ≥ 12 mg l⁻¹, confirmed by Etest); 32 of these were isolated in Sétif and 2 in Tizi-Ouzou (Fig. 1). Interestingly, the majority of the strains from Sétif (28/32; 87.5 %) were recovered during a short period (July–December 2010) (Fig. 1) in different clinical wards, likely suggesting an outbreak and spread in this hospital. Conversely, the two cases from Tizi-Ouzou were not time-connected (Fig. 1).

Real-time PCR was performed to screen for the presence of *bla*OXA51-like, *bla*OXA23-like, *bla*OXA24-like, *bla*OXA58-like and *bla*OXA8-1 genes and conventional PCR was performed for *bla*IMP, *bla*SIM, *bla*VIM and *bla*GIM genes and ISAba1 (Diene et al., 2011; Kusradze et al., 2011; Mendes et al., 2007). Positive PCR products obtained were sequenced. All 71 strains were positive for the *bla*OXA-51 gene. The *bla*OXA-23-like gene (100 % similarity with GenBank accession no. HQ700358) was detected in 29 imipenem-resistant strains (MIC: >16 mg l⁻¹) isolated from Sétif between July 2010 and May 2011, as well as in 2 imipenem-resistant (MIC: ≥ 12 mg l⁻¹) strains isolated from Tizi-Ouzou in April 2010 and March 2011 (Fig. 1). Moreover, the *bla*OXA-72 gene (100 % similarity with GenBank accession no. HQ219688), a *bla*OXA-24-like gene, was detected in five imipenem-resistant strains from Sétif including in two isolates that harboured both *bla*OXA-23-like and *bla*OXA-72 genes isolated in the intensive care unit (ICU) in December 2010 and May 2011 (Fig. 1). For the three other strains, two strains were isolated in the ICU and infectious ward during the same period (September 2010), and one strain was isolated in the ICU in May 2011 (Fig. 1). Looking for the genetic location of *bla*OXA-23, *bla*OXA-72 and *bla*OXA-51 in association with insertion sequence (IS), we found that ISAba1 was located upstream of *bla*OXA-23 for 31 strains and not upstream of *bla*OXA-72 and *bla*OXA-51. Plasmids from three strains (one containing *bla*OXA-23, the second containing *bla*OXA-72 and the third containing *bla*OXA23/72) strain numbers 3310, 710 and 431, respectively, in Fig. 1) were extracted and purified (Kusradze et al., 2011) and the same PCR methods as above were performed. The results showed that the *bla*OXA-23 and *bla*OXA-72 genes from the strains tested were present in plasmids. Finally, a minimum spanning dendrogram generated by the Biotyper 2.0 program demonstrated, at an arbitrary distance level of 300, 400 or 500, that isolates were significantly separated in two clusters, one with imipenem-sensitive strains from Tizi-Ouzou and one with imipenem-resistant strains from Sétif (Fig. 1).

MALDI-TOF MS is an emerging tool for routine identification of bacteria at the species level in clinical microbiology laboratories (Seng et al., 2009, 2010) that has been used successfully recently for rapid identification of genomic species from the *A. baumannii* group (Espinal et al., 2011; Kempf et al., 2012). Here we show for what we believe to be the first time that MALDI-TOF MS was useful to characterize our strains according to their geographical origin and susceptibility to imipenem, i.e. at the subspecies level, as reported for other species such as *Salmonella* species or *Escherichia* species, for example (Seng et al., 2010). This could be a rapid and alternative method to better survey epidemiology of these bacteria, especially for a suspected outbreak and/or emergence of specific clones, in order to implement rapid infection control measures. In our series of isolates, the main molecular support explaining the resistance to carbapenems was the presence of plasmidic *bla*OXA-23 carbapenemase-encoding genes along with the coexistence of *bla*OXA-72 for some strains. In Africa, *A. baumannii* strains producing *bla*OXA-23 have been isolated from South Africa, Libya and Egypt (Zarrilli et al., 2009), and to the best of our knowledge, only one *A. baumannii* isolate carrying a *bla*OXA-23 gene from an unknown origin was obtained from a patient in Algeria in 2004 (Mugnier et al., 2010). Five strains isolated from Sétif between September 2010 and May 2011 contained a *bla*OXA-72 gene, including two strains that contained both *bla*OXA-23 and *bla*OXA-72 carbapenemase-encoding genes, which is reported for what we believe to be the first time in Algeria. OXA-72 carbapenemase enzyme has been previously reported in the Asia-Pacific region (Lu et al., 2009), in Europe.
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Fig. 1. Cross-wise minimum spanning dendrogram generated by the Biotyper 2.0 program for the 71 A. baumannii clinical isolates from Sétif and Tizi-Ouzou. The arbitrary distance level at 300, 400 or 500 is indicated as vertical black lines. Clustering of the strains according to these three cut-off values significantly associates imipenem-sensitive strains from Tizi-Ouzou in one cluster and imipenem-resistant strains from Sétif in a second cluster. An example of the clustering is shown at a cut-off value set at 300: the red cluster contains significantly more imipenem-sensitive strains from Tizi-Ouzou as compared to imipenem-resistant strains from Sétif, which are represented by the blue cluster (P<10^{-3}). ND, Not determined.

(Barnaud et al., 2010; Franolić-Kukina et al., 2011; and in South America (Werneck et al., 2011) but not in North Africa. Association of multiple blaOXA genes has been observed in A. baumannii strains isolated in other countries including A. baumannii carrying blaOXA-23 and blaOXA-58 in China and A. baumannii carrying blaOXA-23, blaOXA-24 and blaOXA-58 in Thailand (Mendes et al., 2009). Finally, although 12 clinical isolates containing blaOXA-58 were isolated in Tlemcen, Algeria, in 2008 (Drissi et al., 2010) and one clinical isolate containing blaOXA-DM-1 was isolated from a patient hospitalized in Paris, France, and repatriated from Oran, Algeria, in July 2011 (Boulanger et al., 2012), none of our clinical isolates from Sétif and Tizi-Ouzou contained these genes. These results likely suggest that the epidemiology of carbapenem resistance in A. baumannii has changed in Algeria, with blaOXA-23 being the most prevalent carbapenemase-encoding gene circulating in Algeria and is now endemic.

Acknowledgements

We thank Linda Hadjadj for technical assistance. This work was partly funded by the CNRS.

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