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# GES-type and OXA-23 carbapenemase-producing *Acinetobacter baumannii* in Turkey

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Sir,

*Acinetobacter baumannii* is one of the most common organisms causing healthcare-associated infections in hospitals, especially within intensive care units (ICUs). Carbapenem resistance in *A. baumannii* is most often mediated by acquired class D carbapenem-hydrolysing β-lactamases and in particular OXA-23.[1] Recently, the emergence of Ambler class A GES-type carbapenemases in *A. baumannii* has been demonstrated, being identified in France, Belgium and Kuwait.[2–4]

The aim of our study was to analyse the molecular epidemiology and clinical features of carbapenem-resistant *A. baumannii* (CRAB) clinical isolates recovered in a Turkish hospital. Isolates were recovered from patients hospitalized in two ICUs (respiratory and anaesthesiology) at the 2000 bed Ege University Medical School Hospital located in İzmir, western Turkey, from 1 March to 1 September 2012 (a 6 month period). The total number of patients in the two ICUs over the study period was 318. The total number of patient days (length of stay×patient number) was 5935.

The CRAB isolates were recovered from clinical specimens of patients diagnosed with ventilator-associated pneumonia (VAP) and/or bacteraemia. Patients who were colonized with *A. baumannii*, but without any sign of infection, were excluded from the study.

A total of 60 patients (24 females and 36 males; mean age 64±2 years) were included in the study. The 60 CRAB isolates were identified by incubation at 44°C and by using the Vitek 2 identification system (bioMérieux, La Balme-les-Grottes, France). They were then further confirmed by specific PCR targeting the $bla_{OXA-51}$-like genes intrinsic to *A. baumannii*.[2] The mean APACHE II score of the patients was 19±7.2. Forty-five patients (75%) had VAP and 15 (25%) had bacteraemia. None of these patients had received an appropriate empirical antimicrobial therapy. Fifteen (10 bacteraemia and 5 VAP) patients died before having being treated with an appropriate antimicrobial therapy within the first 48 h (mortality rate 100%). The overall crude mortality rate for the 45 patients for whom the antimicrobial treatment was appropriate was 55.6% ($P<0.001$). The antibiotic regimens were mostly combinations, being either colistin/tigecycline ($n=20$), colistin/netilmicin ($n=12$), colistin/meropenem ($n=3$), tigecycline/meropenem ($n=6$) or colistin monotherapy ($n=4$) (Table 1).

Antibiotic susceptibility was determined by disc diffusion and MICs of tigecycline, colistin, imipenem and meropenem were determined by using Etest strips (AB bioMérieux, La Balme-les-Grottes, France) and interpreted according to the updated CLSI guidelines when applicable.[5] All of the 60 isolates were resistant to all β-lactams, including broad-spectrum cephalosporins and carbapenems, and to fluoroquinolones. Resistance rates to amikacin, gentamicin and netilmicin were 73%, 65% and 36%, respectively. In addition, 33% of the isolates were resistant to all aminoglycosides tested. Four isolates showed reduced susceptibility to tigecycline (MICs of 1 mg/L) and five isolates were highly resistant (MICs of 3 mg/L). All of the isolates remained susceptible to colistin.

Identification of carbapenemase and class A extended-spectrum β-lactamase (ESBL) genes was performed by PCR and sequencing,[2–4] showing that the 60 isolates were all positive for the $bla_{OXA-23}$ carbapenemase gene. In addition, five isolates were positive for the $bla_{GES-11}$ gene, encoding an ESBL possessing a weak carbapenemase activity.[6] PCR mapping identified the *aacA4* gene cassette encoding the AAC(6′)-Ib aminoglycoside acetyltransferase together with the *dfrA7* gene cassette encoding resistance to trimethoprim downstream of $bla_{GES-11}$.

In order to determine the genetic location of carbapenemase- or ESBL-encoding genes, conjugation assays were performed using *A. baumannii* BM4547 (rifampicin resistant) as the recipient strain, as described previously.[2] Mating-out assays allowed *A. baumannii* BM4547 transconjugants harbouring the $bla_{GES-11}$ gene to be obtained. Those *A. baumannii* transconjugants expressed both OXA-23 and GES-11 and the corresponding $bla_{GES-11}$ and $bla_{OXA-23}$ genes were identified on a single 100 kb plasmid. The transconjugants showed resistance to all β-lactams, including carbapenems and aminoglycosides. Plasmid typing performed by *A. baumannii* PCR-based replicon typing[7] identified the 100 kb plasmid as belonging to group 6, carrying the two carbapenemase genes in all transconjugants.

Genotypic analysis of the 60 clinical isolates was performed by PFGE and allowed them to be grouped into five distinct pulsotypes

**Table 1.** Clinical characteristics of patients infected by the GES-producing *A. baumannii* isolates

| Age (years) | Gender | Comorbidity | Infection | APACHE II score | Length of stay in ICU[a] | Empirical treatment | Treatment after culture | Outcome |
|---|---|---|---|---|---|---|---|---|
| 48 | female | connective tissue disorder | VAP | 21 | 9 | meropenem | tigecycline + colistin | death |
| 67 | male | solid organ tumour + abdominal surgery | VAP | 32 | 14 | meropenem | netilmicin + colistin | death |
| 83 | male | multiple trauma | VAP + bacteraemia | 11 | 5 | meropenem | — | death |
| 79 | male | solid organ tumour | bacteraemia | 10 | 5 | levofloxacin | tigecycline + meropenem | survival |
| 49 | female | solid organ tumour + abdominal surgery | bacteraemia | 15 | 14 | ceftriaxone | tigecycline + netilmicin | survival |

[a]Number of days before isolation of *A. baumannii*.

(data not shown). This grouping was further confirmed by using the Diversilab technique (bioMérieux), which indicated that the five GES-11-positive isolates belonged to three different clonal lineages, with three isolates belonging to a single clone (data not shown). Multilocus sequence typing performed as described previously[8] on the five GES-11-positive *A. baumannii* isolates revealed that they all belonged to ST2, being part of the worldwide-distributed clone II group.

The current emergence of CRAB is a significant source of concern worldwide. Strains producing the carbapenemases OXA-58 and OXA-23 have been previously identified in Turkey. Here, only OXA-23 producers were identified, despite the fact that five clones were concomitantly identified in this hospital. The clonal diversity identified here revealed a polyclonal outbreak situation in the ICUs during that period of time. As previously reported, the overall mortality of the patients infected with those CRABs was very high, especially when they did not receive adequate empirical therapy. According to the resistance profile of the studied isolates, colistin was the most effective antimicrobial.

While our work was in progress, another study reported the occurrence of GES-11 and GES-12 enzymes among *A. baumannii* isolates in Turkey.[9] This highlights the need for implementation of adequate screening techniques to identify patients colonized by those multidrug-resistant strains.

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## Transparency declarations

None to declare.

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# pACICU2 is a conjugative plasmid of *Acinetobacter* carrying the aminoglycoside resistance transposon Tn*aphA6*

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