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SHORT REPORT
Acinetobacter calcoaceticus–Acinetobacter baumannii complex species in clinical specimens in Singapore

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SUMMARY

This study was performed to determine the prevalence, distribution of specimen sources, and antimicrobial susceptibility of the *Acinetobacter calcoaceticus–Acinetobacter baumannii* (Acb) species complex in Singapore. One hundred and ninety-three non-replicate Acb species complex clinical isolates were collected from six hospitals over a 1-month period in 2006. Of these, 152 (78·7%) were identified as *A. baumannii*, 18 (9·3%) as '*Acinetobacter pittii*' [genomic species (gen. sp.) 3], and 23 (11·9%) as '*Acinetobacter nosocomialis*' (gen. sp. 13TU). Carbapenem resistance was highest in *A. baumannii* (72·4%), followed by *A. pittii* (38·9%), and *A. nosocomialis* (34·8%). Most carbapenem-resistant *A. baumannii* and *A. nosocomialis* possessed the $bla_{\text{OXA-23-like}}$ gene whereas carbapenem-resistant *A. pittii* possessed the $bla_{\text{OXA-58-like}}$ gene. Two imipenem-resistant strains (*A. baumannii* and *A. pittii*) had the $bla_{\text{IMP-like}}$ gene. Representatives of carbapenem-resistant *A. baumannii* were related to European clones I and II.

Key words: Antibiotic resistance, bacterial infections, bacterial typing, hospital microbiology, molecular epidemiology.

The *Acinetobacter calcoaceticus–Acinetobacter baumannii* (Acb) species complex comprises four species. *A. calcoaceticus* is clinically unimportant whereas *A. baumannii* is a well-established pathogen. The significance of *Acinetobacter* genomic species (gen. sp.) 3 and *Acinetobacter* gen. sp. 13TU is uncertain because identification to species level is not routine. A recent paper has proposed the names '*Acinetobacter pittii*' and '*Acinetobacter nosocomialis*' for these respective species [1] and these names will be validly published by citation in Validation list 140 of the July 2011 issue of the *International Journal of Systematic and Evolutionary Microbiology* (J. Euzeby, personal communication), and are used here.

This study was performed to determine the prevalence, distribution of specimen sources, and antimicrobial susceptibility of the Acb species complex in Singapore. One hundred and ninety-three non-replicate Acb species complex clinical isolates were collected from six hospitals over a 1-month period in 2006. Identification of *A. baumannii* was carried out by a one-tube multiplex PCR [2]. Intergenic spacer

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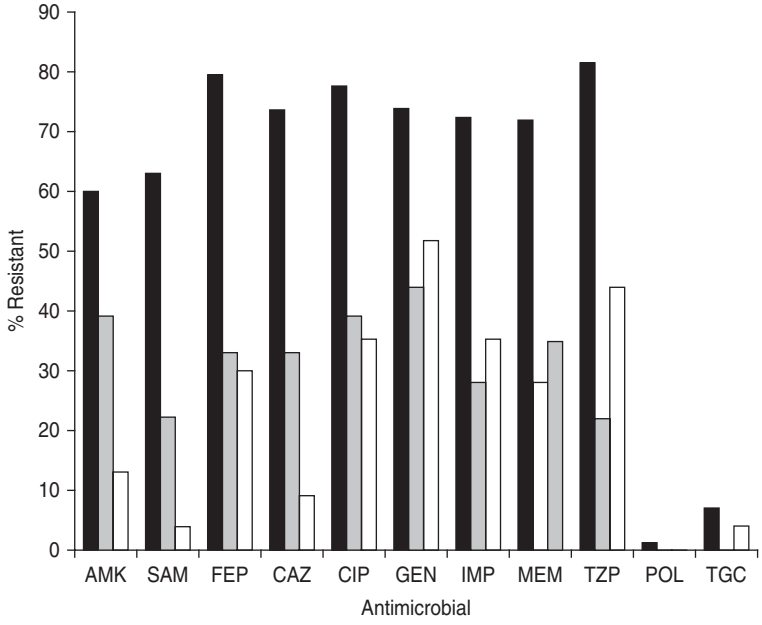

Fig. 1. Antimicrobial resistance profiles of *A. baumannii* (■), *A. pittii* (▨), and *A. nosocomialis* (□). For abbreviations of antimicrobials see main text.

(ITS) sequencing and genomic fingerprint analysis based on selective amplification of restriction fragments (AFLP™) were used for identification of other members of the Acb species complex [3, 4]. AFLP analysis was also used to type nine isolates of carbapenem-resistant *A. baumannii* in the present study, representing clusters defined by RAPD–PCR using the primers DAF4 and M13 (data not shown), and six archived isolates from Hospital S that had previously been characterized [5]. AFLP profiles generated were compared with each other and to a library of >2000 reference strains of all *Acinetobacter* spp. including taxonomically and epidemiologically defined strains. Isolates were identified as the same species, European clone or strain based on percentage similarities of ⩾50%, ⩾80% or ⩾90%, respectively. Multilocus sequence typing (MLST) using the Institut Pasteur scheme was performed on five isolates representative of the main AFLP defined clusters at 80% similarity (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html).

Minimal inhibitory concentrations (MICs) of sulbactam-ampicillin (SAM), piperacillin-tazobactam (TZP), ceftazidime (CAZ), cefepime (FEP), imipenem (IPM), meropenem (MEM), amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), polymixin B (POL), and tigecycline (TGC) were determined by microbroth dilution using custom Sensititre plates (Trek Diagnostic Systems Ltd, UK). Antimicrobial susceptibilities were interpreted in accordance with the guidelines of the Clinical Laboratory Standards Institute [6], except for tigecycline where the manufacturer's breakpoint for Enterobacteriaceae was used.

Genes encoding $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, and $bla_{\text{OXA-143}}$ carbapenemases were detected by multiplex PCR [7]. The presence of insertion sequences preceding the bla_{OXA} genes in carbapenem-resistant (meropenem or imipenem MIC ⩾8 mg/l) isolates was detected using the forward primers ISAba1B, ISAba2A, ISAba3C, and ISAba4B in combination with reverse primers for bla_{OXA} genes [8, 9]. Metallo-β-lactamase genes were sought using a multiplex method [10].

One hundred and fifty-two (78·7%) isolates were identified as *A. baumannii*, 18 (9·3%) as *A. pittii*, and 23 (11·9%) as *A. nosocomialis*. The distribution of isolates according to the species and type of specimen showed that most (63·2% *A. baumannii*, 55·6% *A. pittii*, 60·9% *A. nosocomialis*) were recovered from respiratory and wound specimens and the respective proportions from blood for these species were 7·2%, 16·7% and 26·1%; *A. baumannii* and *A. pittii* were of similar frequency from urine specimens (25·7% and 27·8%, respectively).

The antimicrobial resistance profiles of the different species are shown in Figure 1. A high proportion of *A. baumannii* (110 isolates, 72·4%), but also five

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(27·8%) *A. pittii* and eight (34·8%) *A. nosocomialis* isolates were resistant to carbapenems. Overall, 150 (77·7%) isolates were multidrug resistant, defined as resistant to three or more antimicrobial agents. This comprised 127 (83·6%) of *A. baumannii* isolates, 11 (61·1%) of *A. pittii*, and 12 (52·2%) of *A. nosocomialis*.

One hundred and sixteen isolates (108 *A. baumannii*, eight *A. nosocomialis*) were positive for $bla_{\text{OXA-23-like}}$. Of the isolates that were resistant to imipenem, IS*Aba1* was located upstream of this OXA gene (IS*Aba1*-$bla_{\text{OXA-23-like}}$) in 70 *A. baumannii* and seven *A. nosocomialis*. Only two imipenem-susceptible *A. baumannii* had $bla_{\text{OXA-23-like}}$. In both cases, there was no IS element upstream of the $bla_{\text{OXA-23-like}}$ gene.

All *A. baumanii* isolates and one *A. nosocomialis* were positive for the $bla_{\text{OXA-51-like}}$ gene. Of the imipenem-resistant *A. baumannii*, IS*Aba1* was upstream of the OXA-51-like gene in only 12 isolates (IS*AbaI*-$bla_{\text{OXA-51-like}}$) and in only three of these was IS*AbaI*-$bla_{\text{OXA-51-like}}$ likely to be the major contributor to imipenem resistance as the remainder also possessed IS*AbaI*-$bla_{\text{OXA-23-like}}$ concurrently. This is in contrast to Taiwan where *A. baumannii* carbapenem resistance was mostly associated with IS*AbaI*-$bla_{\text{OXA-51-like}}$ [11]. It has been suggested that the presence of $bla_{\text{OXA-51-like}}$ genes can serve to identify *A. baumannii* [12]. The presence of $bla_{\text{OXA-51-like}}$ in *A. nosocomialis* in this study, and as recently described in Taiwan [13], suggests that this may not be a sufficiently specific marker.

Thirteen isolates were positive for $bla_{\text{OXA-58-like}}$ (one *A. baumannii*, four *A. nosocomialis*, eight *A. pittii*). Of the imipenem-resistant isolates, this gene was preceded by IS*Aba3* (IS*Aba3*-$bla_{\text{OXA-58-like}}$) in an isolate of *A. nosocomialis* and three *A. pittii* isolates. Only one imipenem-resistant *A. baumannii* isolate (positive for $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$. both not with IS*AbaI* located upstream) and one imipenem-resistant *A. pittii* (positive for IS*Aba3*-$bla_{\text{OXA-58-like}}$) had the $bla_{\text{IMP-like}}$ gene. None of the isolates tested was positive with primers for $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-143}}$, ISAba2A, or ISAba4B.

The AFLP profiles of two isolates including a $bla_{\text{OXA-69}}$ containing outbreak strain isolated from Hospital S in 2001 [5], clustered with clone I profiles at 80%. Both had MLST sequence type (ST)1, clonal complex (CC)1. Seven isolates were linked with clone II isolates at 78%; of these, four were isolated from hospital S in 2001 and 2006, and included the predominant outbreak strain in 2001 that contained $bla_{\text{OXA-66}}$. One of these isolates was identified by MLST to ST2, CC2 [5]. No isolate belonged to clone III. With one exception, all isolates that clustered with clones I and II were positive for $bla_{\text{OXA-23-like}}$. Altogether, assignment of isolates by AFLP to clones I and II correlated with assignment by MLST to ST1 and ST2, respectively, emphasizing the global spread of these two clones which appear to be associated with $bla_{\text{OXA-23-like}}$ genes [14].

Six isolates, including the predominant outbreak strains from Hospital S in 1996 that contained $bla_{\text{OXA-64}}$ had AFLP profiles that were unrelated to European clones I–III. Two isolates from 1996 with $bla_{\text{OXA-64}}$ and $bla_{\text{OXA-88}}$ were found to have the MLST ST25 and the novel type ST111, respectively. ST25 has been associated with *A. baumannii* in Greece, Italy and Turkey [15].

In our survey, the relative prevalence of *A. nosocomialis* seems to be greater than that reported in other studies. In Ireland, clinical isolates of *A. pittii* exceeded that of *A. baumannii* by a factor of 1·8, while carbapenem resistance in these *A. pittii* isolates (22%) also exceeded that of *A. baumannii* (4%) [16]. *A. nosocomialis* made up only 5·4% of isolates in the Czech Republic whereas *A. baumannii* (mostly clone II but also clone I) and *A. pittii* accounted for 73·5% and 20·4%, respectively [17]. In that study, *A. pittii* and *A. nosocomialis* isolates were susceptible to most antimicrobials tested including the carbapenems. Further, an 8-year survey in a university hospital in The Netherlands found *A. pittii* (40·3% of strains belonging to the Acb complex) was second to *A. baumannii* (55·8%) whereas *A. nosocomialis* (3·9%) was much less common [4]. The prevalence of multidrug resistance in *A. pittii* ranged from 0% to 22% over the course of the study and no carbapenem-resistant isolates were detected.

The present situation in Singapore is therefore similar to that in China and Korea where spread of international clones with $bla_{\text{OXA-23-like}}$ is responsible for most of the carbapenem-resistant *A. baumannii* [18, 19]. The relatively high rates of occurrence of *A. nosocomialis* and *A. pittii*, their presence in bloodstream infections, and the multidrug and carbapenem resistance in these species underscore their potential clinical significance.

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DECLARATION OF INTEREST

None.

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