Characteristics of epidemic and sporadic strains of Acinetobacter baumannii isolated in Abu Dhabi hospitals

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We compared the antibiotic susceptibility, clonal lineages and resistance genes of singleton Acinetobacter baumannii strains to those of isolates representing repeatedly encountered molecular types in five Abu Dhabi hospitals. One hundred and ten clinically relevant, non-repeat strains were typed by blaOXA-51-like allele sequencing and by PFGE, and selected isolates also by MLST. Resistance was assessed by MIC determinations and by disc diffusion. Genotyping was carried out by PCR, targeting 28 genes. The 80 epidemic strains belonged to worldwide lineages 1, 2 and 7, representing 11 pulsotypes and 9 genotypes, while the 30 sporadic isolates exhibited a high level of genetic variability and, with the exception of a small subgroup, were not associated with any recognized epidemic lineages. All epidemic subtypes carried the ISAb1-linked blaOXA-23 gene, and harboured the int, the blaPER and the armA genes significantly more frequently than their sporadic counterparts. They were all multi-drug resistant, including non-susceptibility to carbapenems, and were often extensively drug resistant, a phenomenon rarely seen among sporadic strains. Epidemic strains represented 78.8% of intensive care unit isolates, causing more respiratory infections, while sporadic strains were more frequently isolated from wound and soft tissue infections. The study showed that among strains collected at the same time and from the same region, the very heterogeneous, sensitive sporadic strains, with the exception of a few non-susceptible singleton isolates, clearly differed from the highly resistant epidemic ones, which belonged to multiple pulsotypes and genotypes clustered into three worldwide clonal lineages carrying blaOXA-64, blaOXA-66 and blaOXA-69, respectively.

INTRODUCTION

In a remarkably short period of time, from relative obscurity, Acinetobacter baumannii has emerged to be one of the most important opportunistic pathogens of our days (Peleg et al., 2008). Its widespread antibiotic resistance, particularly its decreasing susceptibility to broad-spectrum β-lactams, such as third-generation cephalosporins and carbapenems, as well as to aminoglycosides is alarming (Peleg et al., 2008). The main mechanism of resistance against β-lactams is the production of various extended-spectrum β-lactamases, AmpC-type enzymes and carbapenemases of the metallo-β-lactamase and oxacillinase groups (Gordon & Wareham, 2010). Their respective genes are often transcribed from surrogate promoters located on IS elements (Gordon &
Wareham, 2010). Resistance to aminoglycosides is also mainly due to degrading enzymes with varying specificities. Lately, ribosomal target-modifying enzymes, such as ArmA, leading to broad-spectrum, high-level resistance, have also been encountered with increasing frequency. Several of the resistance genes are located on mobile genetic elements, facilitating their spread. These trends leave very few drugs (e.g. colistin, tigecycline) as therapeutic options, and strains non-susceptible even to these suboptimal agents have been reported (Gordon & Wareham, 2010).

A. baumannii often causes nosocomial outbreaks. However, this capacity is apparently not a universal feature of all strains of the species (Dijkshoorn et al., 2007). Epidemic strains generally belong to a few clonal lineages, lately called worldwide (WW) lineages 1 to 8, showing good correlation with the allelic type of the species-specific blaOXA-51-like gene (Evans et al., 2008; Zander et al., 2012).

Despite the considerable attempts made to reveal factors associated with the capacity to survive in hospitals, so far no specific traits of A. baumannii have been linked to the epidemic character of strains beyond the fact that epidemic isolates are typically more resistant to antibiotics and are often multi-drug resistant (MDR) (Dijkshoorn et al., 2010; Dijkshoorn et al., 1996; Jawad et al., 1998). However, these comparative studies either did not analyse the resistance genes carried, or often investigated strains collected at different times from different geographical areas. Therefore, to minimize the influence of various environments, in the current study we compared the antibiotic susceptibility of and the resistance genes carried by strains repeatedly encountered or isolated only once during the same study period, at five hospitals in the Abu Dhabi Emirate, UAE.

**METHODS**

**Strains.** Between March and November 2008, five hospitals in two cities of Abu Dhabi Emirate (Tawam and Al Ain hospitals from Al Ain and Mafraq, Sheikh Khalifa and Al Rabha Hospitals from Abu Dhabi) submitted all clinically relevant, non-repeat A. baumannii strains isolated. Altogether 110 strains were collected. The distribution of the isolates received from Tawam, Sheikh Khalifa, Mafraq, Al Ain and Rahba Hospitals was 36.4 %, 23.6 %, 23.6 %, 13.6 % and 2.7 %, respectively. Of the strains, 35.5 % were recovered from respiratory infections, 30.0 % from wound and soft tissue, 14.5 % from blood or catheters, 11.8 % from urine and 8.2 % from other samples. Thirty per cent of the strains were received from the intensive care units (ICUs) of the hospitals. Species identification was confirmed by a PCR targeting the blaOXA-51-like gene (Turton et al., 2006). Strains were preserved in Tryptic Soy Broth (TSB) (Oxoid) containing 10 % glycerol at −80 °C.

**Susceptibility testing.** Susceptibility to representative of the antimicrobial categories recommended recently to assess the extent of resistance in Acinetobacter (i.e. aminoglycosides, antipseudomonal carbapenems, antipseudomonal fluoroquinolones, antipseudomonal penicillins + β-lactamase inhibitors, extended-spectrum cephalosporins, folate pathway inhibitors, penicillins + β-lactamase inhibitors, polymyxins and tetracyclines) (Magiorakos et al., 2012) was tested. The quantitative antibiotic susceptibility to ceftazidime, imipenem, meropenem, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, colistin and tigecycline was assessed by Etests (bioMérieux), while susceptibility to ampicillin-sulbactam, piperacillin-tazobactam, doxycycline and trimethoprimer-sulfamethoxazole was established by disc diffusion. For all tests Escherichia coli ATCC 25922 was used as control. Data were interpreted according to the CLSI criteria (CLSI, 2012), when available. Isolates were considered MDR if they were non-susceptible to at least one agent in three or more of the above antimicrobial categories; or extensively drug resistant (XDR) if non-susceptible to at least one agent in all but two or fewer antimicrobial categories (Magiorakos et al., 2012).

**Detection of antibiotic resistance and resistance-related genes.** IMP, VIM, NDM, OXA-23, OXA-24, OXA-48-like and OXA-58 carbapenemase genes were detected as described by Ghazawi et al. (2012). The presence of the integron (int) gene and the positioning of ISAb1 upstream of blaOXA-51-like, blaoxa-23 or blaoxa-amp: genes were determined by PCR (Segal et al., 2005; Turton et al., 2006). blaoxa-9, blaoxa-85 and aminoglycoside-resistance genes aadA, aac(3)-Ia, aac(3)-Iia, aac(6’)-Ib, aac(3’)-Ia, aph(3’)-IIa, armA, strA, strB, rmtA, rmtB, rmtC, rmtD and npmA were also tested by PCR (Akers et al., 2010; Cao et al., 2002; Clark et al., 1999; Fritsche et al., 2008; Han et al., 2004; Libisch et al., 2008). The core genotype of a clonal subtype was defined as the set of genes present in ≥ 50 % of its members.

**Molecular typing.** All strains were subjected to PFGE (Seifert et al., 1994). The macrorestriction patterns were compared according to the Dice similarity index (1–1 % tolerance interval) using the GelCompare II software (Applied Maths). Strains exhibiting at least 85 % similarity were clustered into a pulsotype. The blaOXA-51-like allele of all strains was determined by sequencing (Evans et al., 2008). Randomly selected isolates from groups of strains exhibiting similar pulsotypes and blaOXA-51-like allele combinations were subjected to multi-locus sequence typing (MLST; Bartual et al., 2005). Sequencing was performed using the Big Dye Cycle Terminator V.3.1 (Applied Biosystems) in two directions using the 3130X Genetic Analyzer (Applied Biosystems). Sequences were analysed using the program MEGA V4 (Tamura et al., 2007).

**Statistical analysis.** The association of specific genes and antibiotic resistances with specific groups of isolates was assessed by cross-tabulation and by Fisher’s exact test. In order to avoid the bias due to the similarity of strains within a clone, when frequencies of genes or of antibiotic non-susceptibilities were analysed, data of strain clusters were reweighted inversely to their respective clone sizes. The calculations were done using the SPSS 19.0 program (IBM Corporation).

**RESULTS**

**Establishing the sporadic and epidemic nature of the strains**

Strains were assigned to clones based on their molecular types, i.e. on the allelic type of their blaOXA-51-like gene and on their pulsotypes. A strain was considered ‘epidemic’ if it shared the same combination of the blaOXA-51-like allele and pulsotype with at least one other isolate recovered during the study period. On the other hand, strains with unique blaOXA-51-like gene and pulsotype combinations were marked as sporadic. Based on these criteria, of the 110 strains 80 (72.7 %) were considered epidemic while 30 were regarded sporadic.

Approximately a third (32.5 %) of the epidemic and 23.3 % of the sporadic strains were isolated from samples from the...
ICUs of the hospitals. Despite the fact that the difference was not statistically significant ($P=0.484$), 78.8% of the strains causing infections in ICUs were of the epidemic type. Forty per cent of the epidemic strains were isolated from respiratory samples, compared to 23.3% of the sporadic isolates ($P=0.121$). The ratio was almost the opposite regarding wound and soft tissue samples: 40% of the sporadic strains and 26.3% of the epidemic strains ($P=0.098$) were recovered from these specimens. Somewhat higher percentages of the epidemic strains were cultured from the circulation (blood and catheters) and from urine (16.3 and 12.5%, respectively) than their sporadic counterparts (10–10%).

**Clonal distribution of the epidemic strains**

The 80 epidemic strains represented three major clones. Members of clone AD1 ($n=38$) carried $bla_{OXA-69}$, and contained five subtypes (pulsotypes), A–E. Clone AD2 clustered 14 strains with $bla_{OXA-64}$ and had two subtypes (F and G), while $bla_{OXA-66}$-containing strains ($n=28$) formed epidemic clone AD3 with four pulsotypes (H–K) (Fig. 1).

Randomly selected representatives of each subtype were submitted to MLST (Bartual et al., 2005). Members of clone AD1 belonged to clonal complex (CC) 109 represented by ST109 (subtypes B and C), or by its single (ST95, subtypes D and E) or double locus variants (ST434, subtype A). In clone AD2 both subtypes belonged to ST110. Most subtypes in epidemic clone AD3 belonged to CC92; i.e. subtypes J and K were ST189 and subtype H was ST92. The fourth subtype of this clone (I) exhibited a different sequence type, ST254, a sequence type sharing $gdhB$ and $recA$ alleles with ST92 only (Table 1).

Representatives of all three clones were isolated from multiple hospitals. AD1 isolates were recovered from all five participating hospitals; members of clone AD3 were present in four hospitals, while those of clone AD2 were limited to the two hospitals in the city of Al Ain. The presence in multiple hospitals was even true if subtypes within the clones were considered: while in most cases a ‘host-hospital’ (i.e. where a representative of the particular subtype was mostly isolated from) could clearly be identified, all subtypes with more than two members were recovered from more than one institution (Fig. 1).

**Heterogeneity of the sporadic isolates**

The 30 singleton strains carried unique $bla_{OXA-51}$-like allele and pulsotype combinations. Based on their $bla_{OXA-51}$-like genes, sporadic isolates were further divided into two

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**Fig. 1.** Distribution of epidemic clones and sporadic strains. Boxes A–K represent subtypes within clones AD1–3. Asterisks mark sporadic, subgroup A isolates which carry $bla_{OXA-69}$ (*), $bla_{OXA-64}$ (**) or $bla_{OXA-66}$ (**), but with unique pulsotypes different from those of the respective epidemic clones. Black boxes indicate the hospitals (T, Tawam; A, Al Ain; M, Mafraq; S, Sheikh Khalifa; R, Rahba) where the strains were isolated.
subgroups. Subgroup A contained eight strains carrying one of the blaOXA-51-like alleles also present among the epidemic isolates (two strains having blaOXA-64 and blaOXA-69 and four harbouring blaOXA-66, respectively) and differing from them only by their unique PFGE patterns (Fig. 1). On the other hand, 22 strains (subgroup B) carried 15 different alleles of the blaOXA-51-like genes. Three strains contained blaOXA-98, two had blaOXA-65 and blaOXA-83, while one each carried blaOXA67, -68, -78, -89, -91, -94, -95 and -208, respectively. In three strains sequence variants of the gene already deposited in GenBank (AJ309734, EU255289, AJ309734) without assigned OXA numbers were encountered. Furthermore, in the remaining four members of sporadic subgroup B, variants of the blaOXA-51-like gene that have not been described before were identified (Table 2).

**Antibiotic susceptibility**

Overall, a high level of resistance was observed among the strains investigated. With the exception of colistin, against any single antibiotic tested, at least 40 % of the strains exhibited non-susceptibility. Nevertheless, non-susceptibility was significantly more common among isolates belonging to epidemic clones, reaching (ciprofloxacin) or approaching (ceftazidime, meropenem, imipenem, gentamicin, trimethoprim-sulfamethoxazole) 100 % (Table 3). As a consequence, all epidemic strains qualified as MDR, compared to 36.5 % of all sporadic, and 22.7 % of the subgroup B sporadic strains (Table 3). Actually, 48 of the 80 epidemic strains (60.0 %) even qualified as XDR, while only four sporadic strains (13.3 %, three in subgroup A and one in subgroup B, respectively) showed such broad-spectrum resistance (data not shown). The XDR feature was characteristic of subtypes A, B and C (but not of D and E) of clone AD1, both subtypes (F and G) of clone AD2 and subtypes H and I of AD3 (Table 1).

Only one epidemic strain exhibited an elevated MIC value (i.e. 3 mg l\(^{-1}\)) for colistin. Regarding tigecycline, for which no break-point figures were available, the MIC\(_{90}\) value for both groups of strains was 4 mg l\(^{-1}\). However, the MIC\(_{50}\) of the epidemic strains was 3 mg l\(^{-1}\) compared to 0.75 mg l\(^{-1}\) for sporadic isolates.

**Detection of antibiotic resistance and resistance-related genes**

Epidemic strains carried twice as many of the 28 β-lactam and aminoglycoside-resistance genes tested (an average of 7.9 genes per strain) as the sporadic isolates (3.8 per strain).

No blaOXA-24, blaVIM, blaIMP, blaGES, blaNDM, rmt or npm genes were detected in the collection and blaOXA-58 was found only in three sporadic isolates. Of the β-lactam resistance genes, blaOXA-23, blaPER and the int gene were significantly more characteristic of epidemic isolates. Of the β-lactam-resistance genes only ISAba1-linked blaAmpC was found in approximately equal ratios among the
epidemic and sporadic isolates (P = 0.445). While some of the aminoglycoside-resistance genes also exhibited higher frequencies in the epidemic group, this reached the level of statistical significance only in the case of armA (Table 4).

Although the number of isolates was too low for statistical analysis, sporadic subgroup A isolates carried more frequently most of the resistance genes tested than the members of the more heterogeneous subgroup B (Table 4).

Sporadic isolates showed a high variability in their genes: the 30 strains exhibited 21 different genotypes (data not shown). On the other hand, the 11 clonal subtypes of the three epidemic clones exhibited nine core genotypes (Table 1). The ISAbal-linked blaOXA-23 gene was part of the core genotype of all clones, just as was, with the exception of subtypes D and E, the int gene. The blaPER and ISAbal-blaAmPC genes were characteristic of certain subtypes (F, G, J for the former, and H, I, J, K for the latter gene) while absent from the core genotype of others.

No aminoglycoside-resistance genes were present in the core genotype of all the subtypes. Nevertheless, some, e.g. aadA (A, B, C, H, I), armA (F, G, H, I) and strAB (F, G, H, I, J, K), were predominantly associated with certain subtypes, although being present in some of the sporadic isolates as well (Table 1). The subtypes carrying armA uniformly exhibited high-level resistance to all aminoglycosides tested (amikacin and netilmicin > 256 mg l⁻¹, gentamicin and tobramycin > 1024 mg l⁻¹) (data not shown). The presence of the armA among the XDR isolates significantly correlated with susceptibility restricted to colistin only (P < 0.0001) (Table 1).

**DISCUSSION**

We compared the genotype and antibiotic susceptibility of two sets of A. baumannii strains: the first group represented molecular types isolated repeatedly while members of the second one were encountered only once during the study period, in five Abu Dhabi hospitals. All three major clusters of epidemic strains belonged to known lineages (Fig. 1, Table 1). Members of cluster AD3 represented the globally most widely distributed lineage, i.e. WW2 carrying blaOXA-66 (Evans et al., 2008; Higgins et al., 2010; Karah et al., 2012), with three of its four subtypes belonging to CC92. This lineage is not uncommon in the Middle East either and has earlier been reported from Kuwait (Bonnin et al., 2012) and Iran (Peymani et al., 2012). The other major clone (AD1) with blaOXA-69, belonging to CC109, is also broadly distributed (WW1) (Evans et al., 2008; Higgins et al., 2010; Karah et al., 2012), with isolates also being reported from this region, including Pakistan (Hamouda et al., 2010), Kuwait (Al-Sweih et al., 2012), and earlier also the UAE (Mugnier et al., 2008). Members of AD2 with blaOXA-66 have recently been linked to WW7 (Peymani et al., 2012; Zander et al., 2012). Previously this allele has been found in an isolate from Singapore (Evans et al., 2008) and in a blaNDM-1-carrying strain in Germany, in a patient repatriated from Serbia (Pfeifer et al., 2011). Our findings, together with recent reports from Kuwait (Bonnin et al., 2012), suggest that members of this lineage are strongly established in the Middle East. It is of interest that while the Kuwaiti strains carried blaGES-11, we failed to detect this gene, while our isolates carried blaPER and armA, not reported for the Kuwaiti isolates (Bonnin et al., 2012).

All subtypes of the three epidemic clones carried the blaOXA-23 gene downstream from an ISAbal element (Tables 1 and 4). Globally, this gene is the most widely distributed acquired oxacillinase in Acinetobacter (Mugnier et al., 2010) and it has also been described previously in the UAE (Mugnier et al., 2008). It was noteworthy, however, that while in some countries of the region, i.e. Pakistan and Iran (Evans et al., 2011; Peymani et al., 2012), it is similarly widespread as in Abu Dhabi, it is nearly not as common in Kuwait (Al-Sweih et al., 2012). These findings emphasize the possibility of considerable variations in a larger region within the same clonal lineages.

The blaPER, the int and the armA genes, although significantly more common among the epidemic strains, were unevenly distributed among their subtypes (Table 1). This may suggest that some of these genes could have been acquired by horizontal gene transfer. Although we were unable to confirm its transfer in vitro, previously we showed that one member of subtype F of AD2 carried a blaPER-gene on a 200 kb plasmid (Opazo et al., 2012). Recently, the plasmid-encoded nature of armA was also demonstrated in an MDR clone widespread in China (Zhou et al., 2011). All epidemic subtypes were MDR, including resistance to carbapenems – a striking difference from sporadic isolates

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**Table 2. New blaOXA-51-like genes found in sporadic isolates**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Closest match*</th>
<th>Deduced amino acid change†</th>
<th>GenBank no. of new sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM114</td>
<td>OXA70</td>
<td>237 Gln→Arg</td>
<td>JX865391</td>
</tr>
<tr>
<td>NM61</td>
<td>OXA88</td>
<td>36 Lys→Glu</td>
<td>JX865392</td>
</tr>
<tr>
<td>NM69</td>
<td>OXA64</td>
<td>38 Gly→Ala</td>
<td>JX865393</td>
</tr>
<tr>
<td>NM83</td>
<td>OXA217</td>
<td>188 Lys→Asn</td>
<td>JX865394</td>
</tr>
</tbody>
</table>

*The most similar known blaOXA-51-like sequence.
†Amino acid numbers are those of the closest match.
### Table 3. Antibiotic non-susceptibility of epidemic and sporadic isolates

<table>
<thead>
<tr>
<th>Strains</th>
<th>n</th>
<th>Antibiotic non-susceptibility (%)</th>
<th>Etest</th>
<th>Disc diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CAZ</td>
<td>MER</td>
</tr>
<tr>
<td>All</td>
<td>110</td>
<td>77.3</td>
<td>76.4</td>
<td>76.4</td>
</tr>
<tr>
<td>Epidemic</td>
<td>80</td>
<td>96.25</td>
<td>97.5</td>
<td>97.5</td>
</tr>
<tr>
<td>Sporadic</td>
<td>30</td>
<td>26.7</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>P</strong> &lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sporadic A</td>
<td>8</td>
<td>75.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Sporadic B</td>
<td>22</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
</tr>
</tbody>
</table>

### Table 4. Distribution of resistance and resistance-related genes among epidemic and sporadic isolates

<table>
<thead>
<tr>
<th>Strains</th>
<th>n</th>
<th>Presence of gene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>int</td>
</tr>
<tr>
<td>All</td>
<td>110</td>
<td>62.7</td>
</tr>
<tr>
<td>Epidemic</td>
<td>80</td>
<td>75.0</td>
</tr>
<tr>
<td>Sporadic</td>
<td>30</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>P</strong> &lt; 0.001</td>
</tr>
<tr>
<td>Sporadic A</td>
<td>8</td>
<td>75.0</td>
</tr>
<tr>
<td>Sporadic B</td>
<td>22</td>
<td>13.6</td>
</tr>
</tbody>
</table>
The respective molecular type fall beyond the time frame of could, in fact, be epidemic if repeated infections caused by isolates. Strains considered sporadic by the current study strains was more specific to identify epidemic than sporadic.

It should be noted, however, that the approach used to group subtypes carrying and several isolates of clone AD3, were XDR, with the entire AD2 clone, as well as several subtypes of clone AD1 ArmA being susceptible to colistin only. Although four XDR isolates were also found among the singleton isolates, as well, three of them belonged to subgroup A, sharing several features with the epidemic group (Table 1).

Global and regional data vary regarding the variety of clones co-existing in hospitals and the extent of possible inter-hospital transfer (Adams-Haduch et al., 2011; Al-Sweih et al., 2012; Landman et al., 2002; Lee et al., 2009; Rodriguez-Baño et al., 2004; Weisenberg et al., 2011). Nevertheless, it was surprising that in this study none of the epidemic clones, actually not even the subtypes recovered from more than two cases were restricted only to a single hospital (Fig. 1). The most likely explanation for this finding is an extensive inter-hospital transfer of strains. These data emphasize the need to introduce routine molecular typing when following MDR pathogens.

The fact that epidemic strains were more common in respiratory samples could be explained by the fact that $48.7\%$ of these samples were collected from ICUs, i.e. units of high antibiotic use (data not shown). In fact, epidemic strains represented $89.5\%$ of respiratory isolates from ICUs, and $78.8\%$ of the isolates from all ICU samples showed the epidemic, i.e. highly resistant, character (data not shown). On the other hand, the opposite trend was seen with isolates recovered from wound and soft tissue samples: $40\%$ of the sporadic strains derived from this specimen type compared to $26.3\%$ of the epidemic strains. Not surprisingly, $89.9\%$ of these samples were submitted from non-ICU departments (data not shown), likely to have a more variable intensity of antibiotic use offering a better chance of survival for the more susceptible sporadic isolates. Although our data showed a significant difference in antibiotic susceptibility between sporadic and resistant strains, an important limitation of the study should be kept in mind, namely that data regarding the time of hospitalization before isolation of A. baumannii were not available to us. Therefore, while we assume that most of the repeatedly encountered epidemic strains were likely hospital-acquired, such an assumption cannot be made for the sporadic isolates. Nevertheless, the likely susceptibility to antibiotics anticipated among community-acquired strains could explain why they, once imported, cannot be maintained in the more selective hospital environment to cause multiple infections.

Compared to the relative uniformity of epidemic isolates, the significantly more susceptible sporadic strains exhibited remarkable variety, carrying altogether 18 different bla<sub>OXA-51-like</sub> alleles and exhibiting 21 different genotypes. It should be noted, however, that the approach used to group strains was more specific to identify epidemic than sporadic isolates. Strains considered sporadic by the current study could, in fact, be epidemic if repeated infections caused by the respective molecular type fall beyond the time frame of the study. We surmise that members of sporadic subgroup A, i.e. isolates with bla<sub>OXA-51-like</sub> alleles characteristic of epidemic clones but exhibiting different pulsotypes, could be such candidates. This assumption is supported by the fact that these strains carried genes and expressed antibiotic susceptibility patterns closer to those of the epidemic isolates than to those of the subgroup B sporadic strains (Table 3 and 4). Isolates of this latter subgroup carried a large variety (15 types) of bla<sub>OXA-51-like</sub> alleles, the overwhelming majority, with the exception of bla<sub>OXA-68</sub>, bla<sub>OXA-83</sub> and bla<sub>OXA-83h</sub> not being associated with any of the worldwide lineages (Zander et al., 2012). Nevertheless, our data do not exclude the alternative explanation either, i.e. subgroup A strains may indeed represent isolates with limited survival capacity in hospitals while otherwise resembling epidemic strains. Although we consider this scenario less likely, if further studies proved the existence of such sporadic, but resistant strains within some of the worldwide lineages, they might be particularly useful to study non-drug-resistance-related differences between sporadic and epidemic strains, if any exist.

This study, conducted in a region of high endemcity, resistance and inter-hospital transfer showed that the overwhelming majority of A. baumannii isolates recovered from sporadic infections represent a highly heterogeneous group, with the majority of the isolates not being associated with any worldwide lineages. They carry fewer antibiotic-resistance genes and are consequently less resistant to antibiotics than their MDR, or often XDR, epidemic counterparts. In the region studied, members of all epidemic clones carried ISAb1-linked bla<sub>OXA-23</sub> and were significantly more likely to have the int, blaper, and ArmA genes. Epidemic strains dominate in the ICUs, frequently causing respiratory infections, while sporadic isolates were more likely to infect non-ICU patients, with wound and soft tissue infections being the most common presentation. Further studies should clarify whether the few resistant singleton isolates carrying the genetic make-up of a major worldwide lineage in fact represent the epidemic type or, alternatively, they are indeed sporadic strains lacking some fitness factors, yet to be identified.

ACKNOWLEDGEMENTS

This work was supported by the Faculty of Medicine and Health Sciences, UAE University grants NP/09/16, NP 10/07 and NP-10-11/1018 and by UAE University grants 01-10-8-11/09 and 1439-08-02-10. The dedication of personnel at the hospitals’ microbiology laboratories is highly appreciated.

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Epidemic and sporadic Acinetobacter baumannii


