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The transferability of bla_{OXA-23} gene in multidrug-resistant *Acinetobacter baumannii* isolates from Saudi Arabia and Egypt

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Carbapenem-resistant *Acinetobacter* spp. have been increasingly reported worldwide including Saudi Arabia and Egypt. We examined 64, non-repetitive, *Acinetobacter baumannii* isolates collected in 2013 and 2014 from four different medical centres (two from Saudi Arabia and two from Egypt). All the isolates were resistant to ceftazidime and ciprofloxacin. The *intI1* harbouring bla_{GES-11} and *aac-6′-1b* was detected in 19% (n = 12) of the isolates. IS*Aba1* over-expression of bla_{ADC} gene was observed in 65% (n = 42) of isolates. Of all the isolates 19% (n = 12) had IS*Aba1* upstream of the $bla_{OXA-51-like}$ gene, 69% (n = 44) carried the bla_{OXA-23} gene within the Tn*2006* structure, 8% (n = 5) had $bla_{OXA-24-like}$ gene and 9% (n = 6) harboured either bla_{VIM-2} or bla_{NDM-1} gene. Eighty nine percent (n = 57) of isolates were resistant to imipenem and had an MIC of ≥8 mg/L. Pulsed-field gel electrophoresis (PFGE) typing revealed the presence of 23 different PFGE. Three PFGE types were very widespread, ST236 (CC104) (PFGE type 1, n = 15), ST208 (CC92) (PFGE type 2, n = 10), ST884 (CC unassigned) (PFGE type 3, n = 7) in and across all four medical centres. The bla_{OXA-23} gene was found to be present on a 60 kb transferable plasmid in both PFGE type 1 and 2 but was absent in PFGE type 3. This is the first study to report on the emergence of ST236 in Saudi Arabia and Egypt, and spread of distinct carbapenem resistant *A. baumannii* clones belonging to ST884, ST945 and ST1096 in Saudi Arabia.

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1. Introduction

Acinetobacter baumannii has gained a lot of interest in the past decade due to its increasing prevalence in the hospital environment in addition to its ability to resist antimicrobial compounds. There have been various reports worldwide that have identified this organism as a major threat especially in immunocompromised patients (Dijkshoorn et al., 2007). Carbapenem resistance in *A. baumannii* has been associated with the production of class A, B, C and class D β-lactamases. Class B β-lactamases are metallo-β-lactamases (MBL) whereas class D β-lactamases are the OXA-like enzymes that are also known as carbapenem-hydrolysing class D β-lactamases (CHDL) (reviewed by Karah et al., 2012). Furthermore, carbapenem resistance in *Acinetobacter* sp. is due to mechanisms such as alterations in outer membrane permeability, increase in drug efflux and modification of the affinity of penicillin-binding proteins (PBPs) (del Mar Tomás et al., 2005; Mussi et al., 2005; Vila et al., 2007). Insertion sequences (IS), which are usually tightly regulated, are capable of independent transposition in the microbial genome and are important in providing genetic variability to bacteria (Mugnier et al., 2009a). IS*Aba1*, belonging to the IS4 family, has been associated with increased expression of several antibiotic resistance genes in *A. baumannii* including $bla_{OXA-51-like}$, bla_{ADC}, $bla_{OXA-23-like}$, $bla_{OXA-58-like}$ and $bla_{OXA-235-like}$ (Corvec et al., 2003, 2007; Higgins et al., 2013; Lopes et al., 2013; Mugnier et al., 2009b). A recent study described the genetic diversity of carbapenem resistant *A. baumannii* strains isolated from paediatric cancer patients during the years 2010–2011, from two different medical centres in Cairo, Egypt. It was reported that 73% of the isolates were resistant to carbapenems. This was mainly due to the presence of bla_{OXA-23}, bla_{OXA-58} and bla_{OXA-40} genes and IS located upstream of the $bla_{OXA-51-like}$ genes (Al-Hassan et al., 2013). The prevalence

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of carbapenem-resistant *A. baumannii* (CRAB) has been found to be extremely high in the Middle Eastern countries (Mugnier et al., 2009a; Zowawi et al., 2015). There have been reports of CRAB in patients with diabetes mellitus in Saudi Arabia. They have become a serious problem in immunocompromised patients and the scenario is particularly worrying in Middle-Eastern countries due to the increasing number of infections that are difficult to treat (Alsultan et al., 2009, 2013). The aim of this study was to characterise and understand the molecular epidemiology of clinical isolates of *A. baumannii* from two medical centres in Saudi Arabia and two from Egypt, collected in 2013 and 2014.

2. Materials and methods

2.1. Bacterial isolates

Sixty four clinical isolates of *A. baumannii*, isolated from patients visiting the out-patient department, were obtained between 2013 and 2014 from four medical centres; King Salman Hospital (KSH, $n=42$) and King Saud Medical Centre (KSMC, $n=4$) located in Riyadh, Saudi Arabia and Kasr Al-Ainy (KAA, $n=16$) & Dar Al-Fouad (DAF, $n=2$) located in Cairo, Egypt to test the antibiotic susceptibilities and establish the cause of infection. The samples processed included endotracheal tube aspirates (ETT), wound swabs (WS), bedsore swabs (BSS), tracheal swabs (TS), urine (U), blood (B), sputum (S), pus (P), tissue (T) and respiratory secretions (RS). KSH is located at distance of 19.3 km from KSMC and KAA is at a distance of 38.5 km from DAF.

2.2. Species identification and antimicrobial susceptibility testing

The samples collected from various sources were processed by the microbiology staff at the microbiology laboratory at the respective centres in Saudi Arabia and Egypt. Preliminary identification was performed using the Vitek 2 compact automated system (BioMérieux, Marcy L'Etoile) and were stored in brain heart infusion broth with 10% glycerol. Preliminary identification was performed by streaking a loopful of the stored isolate onto MacConkey agar (Oxoid Ltd., Basingstoke, UK) plates and incubation at 37 °C for 24 h. Following incubation a single colony was streaked onto 5% horse blood agar plate (E&O, Bonnybridge, UK) and was incubated at 37 °C for 24 h. DNA extraction was performed according to the manufacturer's instructions using the Promega Wizard Genomic DNA purification kit (Promega, Madison, WI). The isolates were confirmed as *A. baumannii* by PCR and sequencing of the *rpoB* gene using the primers Ac696F and Ac1093R described previously (La Scola et al., 2006) and by the PCR amplification of the intrinsic $bla_{\text{OXA-51-like}}$ gene (Héritier et al., 2005). The MICs of imipenem, ceftazidime and ciprofloxacin were determined using the E-test (AB BIODISK, Solana, Sweden) as described by the manufacturer. The results were interpreted using the BSAC guidelines for susceptibility testing (BSAC, 2015).

2.3. Screening for presence of antibiotic resistance genes

All isolates were screened for the presence of carbapenemase genes ($bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-143-like}}$ and $bla_{\text{OXA-235-like}}$) by multiplex PCR as described previously (Higgins et al., 2013). The primer ISF (5′-GTT GCA CTT GGT CGA TAG AAA A-3′) and was used in conjunction to 23R (Higgins et al., 2013) and 23F (Higgins et al., 2013) with ISR (5′-CACTGCTCACCGATAAA C-3′) for the identification of any insertion elements upstream or downstream of the $bla_{\text{OXA-23-like}}$ gene respectively. The upstream region of $bla_{\text{OXA-51-like}}$ was identified using the 69A and 69B primers (Héritier et al., 2005). The *intI1* gene was amplified and sequenced using the primers Int1A (5′-CCT GTT CGG TTG GTA AGC TG-3′) and Int1B (5′-TCC CGA CCA GAC TGC ATA AG-3′). The PCR conditions used to generate the amplicons for sequencing were: 94 °C for 5 min, followed by thirty five cycles of 94 °C for 15 s, 57 °C for 15 s, and 72 °C for 30 s, followed by final extension at 72 °C for 6 min. Multiplex PCR was performed for the identification of bla_{GIM}, bla_{IMP}, bla_{SIM}, bla_{SPM}, bla_{VIM} classes of metallo-β-lactamases (Ellington et al., 2007). The whole gene amplification and sequencing of bla_{VIM} was performed with primers VIM-F1, VIM-R2 (Frasson et al., 2013). The bla_{NDM} amplification and sequencing was completed using the primers NDM-Full F and NDM-Full R (Hornsey et al., 2011). PCR was performed on all isolates as described earlier on all isolates to identify insertion elements causing disruption of the *carO* gene (29 kDa outer membrane protein) which leads to carbapenem resistance (Mussi et al., 2005). Additionally, primers FU and RU were used for amplification of the intergenic region upstream of the bla_{ADC} gene and primers FD and RD were used to amplify the intergenic region downstream of the bla_{ADC} gene (Lopes et al., 2013). The quinolone resistance mediating genes *gyrA* and *parC* were analysed for presence of mutations by PCR and sequencing as described previously (Lopes et al., 2013). The sequencing of the amplicons was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

2.4. Conjugation and plasmid extraction

Transconjugation by filter mating assay was performed as described earlier (Neela et al., 2008) using the clinical *A. baumannii* strain A2 harbouring $bla_{\text{OXA-23}}$ as donor and the carbapenem-susceptible clinical *A. baumannii* A88 as recipient. S1 nuclease digestion was performed followed by PFGE, to determine the location of $bla_{\text{OXA-23}}$ gene (Lopes et al., 2012).

2.5. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)

*Apa*I macrorestriction followed by PFGE was used to characterise the epidemiological relatedness of the isolates by technique described elsewhere (Lopes et al., 2012). The gels were stained with 0.1% SafeView solution (NBS Biologicals Ltd., Cambridgeshire, UK) and viewed using the Diversity Database software image capturing system (Bio-Rad Laboratories Ltd., UK). Cluster analysis was performed using the unweighted pair group method with mathematical averaging (UPGMA), and relatedness was calculated using the band-based Dice coefficient with a 1.5% band tolerance and 1.5% optimisation setting for the whole profile. BIONUMERICS v2.5 software (Applied Maths, Sint-Martens-Latem, Belgium) was used for gel analysis and a value of 85% was chosen as the threshold for the establishment of clonal relatedness of the strains (Lopes et al., 2012). Furthermore, bacterial strain typing by MLST was performed according to the Oxford scheme (Bartual et al., 2005).

3. Results and discussion

All 64 isolates were confirmed as *A. baumannii* by $bla_{\text{OXA-51-like}}$ and *rpoB* gene PCR and sequencing. Eighty nine percent ($n=57$) of isolates were found to be resistant to imipenem with MIC of ≥8 mg/L. All the isolates were resistant to ceftazidime and ciprofloxacin with MICs of ≥32 mg/L and ≥4 mg/L respectively (Table 1). The $bla_{\text{OXA-69}}$, $bla_{\text{OXA-66}}$ and $bla_{\text{OXA-71}}$ have been associated with Worldwide Clones I (CC2), II (CC1) and III (CC3), respectively (Al-Hassan et al., 2013). Genotyping of the $bla_{\text{OXA-51-like}}$ genes revealed genotypic diversity between the strains with $bla_{\text{OXA-66}}$ being predominant (39%, $n=25$) followed by $bla_{\text{OXA-51}}$ (25%, $n=16$), $bla_{\text{OXA-69}}$ (14%, $n=9$) and $bla_{\text{OXA-131}}$ (11%, $n=7$). Other $bla_{\text{OXA-51-like}}$ genes (11%, $n=7$) included OXA-65, -64, -68, -100, -403, -404. Enzymes from strains A37 and A5 were novel and

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