Outbreaks of multidrug-resistant *Acinetobacter baumannii* strains in a Kenyan teaching hospital

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ABSTRACT

*Acinetobacter baumannii* is a serious nosocomial pathogen with a high propensity to cause outbreaks. Whilst outbreaks of *A. baumannii* have been reported in many regions worldwide, few data are available from East Africa. In this study, 25 *A. baumannii* isolates derived from a single institution located in Nairobi, Kenya, between September 2010 and September 2011 were examined. Antimicrobial susceptibility testing was performed by the disk diffusion method and the relatedness among the isolates was examined by pulsed-field gel electrophoresis, repetitive sequence-based PCR (rep-PCR) and multilocus sequence typing. The examined isolates clustered into three distinct groups. The most prevalent sequence type (ST) was ST110 (17 isolates), followed by ST92 (5 isolates) and ST109 (3 isolates). All isolates exhibited resistance to cefepime, ceftazidime, ticarcillin/clavulanic acid, cefotaxime/clavulanic acid, piperacillin/tazobactam, cefoxitin, ciprofloxacin, gentamicin, nitrofurantoin, fosfomycin trometamol, trimethoprim/sulfamethoxazole, amikacin, meropenem and imipenem, with the exception of four isolates. Two isolates belonging to ST92 and two isolates belonging to ST109 were susceptible to amikacin; one of these amikacin-susceptible ST109 isolates was also susceptible to meropenem and imipenem. All isolates were positive for OXA 51-like and all carbapenem-resistant isolates were OXA-23 positive.
1. Introduction

*Acinetobacter baumannii* is a Gram-negative coccobacillus that has emerged as a serious healthcare-associated pathogen in recent years. *Acinetobacter baumannii* infection may result in pneumonia, bloodstream infection, urinary tract infection, meningitis and wound infection as well as other manifestations of infection [1].

*Acinetobacter baumannii* has a high propensity to cause nosocomial outbreaks, possibly due to its persistence in the hospital environment and its remarkable ability to acquire drug resistance. Several studies indicate significantly increased resistance rates in epidemic *A. baumannii* strains compared with sporadic strains [1].

To distinguish outbreak strains from epidemiologically unrelated *A. baumannii* strains, typing methods such as pulsed-field gel electrophoresis (PFGE), repetitive sequence-based PCR (rep-PCR) and multilocus sequence typing (MLST) are employed [1]. PFGE and rep-PCR have been demonstrated to be more discriminatory than MLST, which is routinely used to put epidemiological information in a global context [2].

Whilst outbreaks of *A. baumannii* have been reported in many regions worldwide, including Europe, North America, Latin America, Australia, Asia, the Middle East and South Africa [1], few data are available for East African *A. baumannii* isolates. In the current study, antibiotic susceptibility testing, MLST, rep-PCR and PFGE were performed for 25 *A. baumannii* strains isolated at Aga Khan University Hospital, Nairobi (AKUH, N), Kenya.
2. Materials and methods

2.1. Collection and identification of Acinetobacter baumannii isolates

The 25 non-repetitive A. baumannii isolates investigated in this study were cultivated at the Division of Microbiology of AKUH, N from the following specimen types: 9 A. baumannii isolates were derived from tracheal aspirates, 5 isolates were from pus swabs, 3 were from nasal swabs, 2 isolates each were from sputum, cerebrospinal fluid (CSF) and urine, and 1 isolate each was derived from blood and unspecified tissue. The two CSF isolates and the tissue sample isolate were considered clinically significant and the patients were specifically treated with colistin. Both CSF samples came from patients with ventricular shunts. All isolates were collected from September 2010 to September 2011. Although a previous outbreak of multiresistant Acinetobacter in the intensive care unit (ICU) [3] had been stopped with highly stringent infection control measures, multidrug-resistant Acinetobacter continued to be isolated from patients admitted to the hospital. Fourteen of the patients had a history of prior admission to the hospital, whilst eight had a history of admission to various other hospitals in Nairobi. Thirteen of the patients were in the ICU at the time of specimen collection. However, their hospital stay was at different time periods.

Identification of the isolates as A. baumannii was reconfirmed by 16S rDNA sequencing in addition to PCR detection of the bla\textsubscript{OXA-51-like} oxacillinase [4,5].
2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the clinical isolates was determined by the disk diffusion method on Mueller–Hinton agar (Oxoid Ltd., Basingstoke, UK) according to Clinical and Laboratory Standard Institute (CLSI) recommendations [6]. Antibiotic disks were supplied by Becton, Dickinson & Co. (Franklin Lakes, NJ) and the following antibiotics were tested: amikacin; cefepime; meropenem; imipenem; ceftazidime; ticarcillin/clavulanic acid; cefotaxime/clavulanic acid; piperacillin/tazobactam; cefoxitin; ciprofloxacin; gentamicin; nitrofurantoin; fosfomycin trometamol; and trimethoprim/sulfamethoxazole.

2.3. Bacterial genotyping

Genomic DNA was extracted using an UltraClean® Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) as recommended by the manufacturer and was used for MLST and rep-PCR analysis. MLST was performed as previously described [7], making use of the A. baumannii MLST website (http://pubmlst.org/abaumannii/) developed by Keith Jolley and located at the University of Oxford (Oxford, UK). rep-PCR and PFGE typing were used for the increased resolution required for investigation of clonality in outbreak analysis.

rep-PCR typing was performed using a DiversiLab Acinetobacter Kit (bioMérieux, Marcy l’Étoile, France). DiversiLab fingerprints were analysed with DiversiLab software using the Pearson correlation statistical method to determine clonal relationships. Isolates were considered as clonal when the rep-PCR patterns showed ≥95% similarity.
PFGE was conducted with the restriction enzyme *Apa*I using a CHEF-DR® III system (Bio-Rad, Hercules, CA) as previously described [8].

2.4. Resistance gene analysis

Presence of the metallo-β-lactamase gene *bla*IMP as well as the extended-spectrum β-lactamase (ESBL) gene *bla*PER-1 and the AmpC β-lactamase gene *bla*CMY-2 was determined by PCR [9–11]. The occurrence of carbapenem-hydrolysing class D β-lactamase (oxacillinase) (CHDL) genes, namely *bla*OXA-51-like, *bla*OXA-23-like and *bla*OXA-58-like, was determined by PCR [12]. Detection of insertion sequence IS*Aba*1 upstream of *bla*OXA-51-like was performed using PCR [13].

3. Results and discussion

All isolates were resistant to cefepime, ceftazidime, ticarcillin/clavulanic acid, cefotaxime/clavulanic acid, piperacillin/tazobactam, cefoxitin, ciprofloxacin, gentamicin, nitrofurantoin, fosfomycin trometamol and trimethoprim/sulfamethoxazole. In addition, all isolates except one (AKUH AB008) showed resistance to meropenem and imipenem. In addition to being susceptible to meropenem and imipenem, isolate AKUH AB008 was also susceptible to amikacin, as were three other isolates. Resistance profiles of meropenem, imipenem and amikacin are illustrated in Fig. 1.

These investigations showed that three clones of *A. baumannii* were found within this Kenyan institution. The 25 isolates investigated in this study grouped into three different sequence types (STs) belonging to three different clonal complexes (CCs).
The most prevalent sequence type was ST110 (17 isolates), followed by ST92 (5 isolates) and ST109 (3 isolates). Worldwide, ST92 and the associated CC92, also known as European clone 2 or worldwide clonal lineage 2, represent the most widespread sequence type(s). ST109 is part of clonal cluster CC1, which is also found worldwide (http://pubmlst.org/abaumannii/).

In this study, the strains of each sequence type shared similar PFGE and rep-PCR profiles. It is worth noting that all of the ST110 isolates were resistant to all of the antibiotics investigated, whilst two isolates of ST92 and two isolates of ST109 were susceptible to amikacin; one of the amikacin-susceptible ST109 isolates was also susceptible to meropenem and imipenem. Isolates of the same sequence type clustered together when analysed by the DiversiLab rep-PCR typing method and PFGE (Fig. 1).

The naturally occurring \textit{bla}^{\text{OXA-51-like}} gene was detected in all 25 \textit{A. baumannii} isolates and the acquired CHDL \textit{bla}^{\text{OXA-23}} gene was present in all meropenem-resistant isolates, whilst the meropenem-susceptible isolate was negative for the \textit{bla}^{\text{OXA-23-like}} gene. The \textit{bla}^{\text{OXA-58-like}} gene was not detected, and all isolates tested negative for \textit{bla}_{\text{PER-1}} and \textit{bla}_{\text{CMY-2}}. Two isolates (AKUH 012 and 015), both ST109, were positive for the element IS\textit{Aba1}–\textit{bla}^{\text{OXA-51-like}}, whilst the remaining 23 isolates were negative for the presence of this element.

ST110 \textit{A. baumannii} have been previously found in India [14], South Korea [15] and Australia [16]; however, in these settings ST110 did not account for the majority of strains. Although in the current study ST110 isolates were resistant to all of the
antibiotics investigated, invariably producing OXA-23, antibiotic susceptibility and resistance mechanisms of ST110 were less uniform in other settings [14,15].

Whilst in the current study $\text{bla}_{\text{OXA-23}}$ was the major carbapenem resistance mechanism detected, a previous report from AKUH, N has shown the antibiotic resistance gene structure IS$\text{aba}_1–\text{bla}_{\text{OXA-23}}$ to be the main mechanism of carbapenemase resistance among 10 Kenyan $A. \text{baumannii}$ isolates [3]. In the current study, two strains of ST109 $A. \text{baumannii}$ harboured the IS$\text{aba}_1–\text{bla}_{\text{OXA-51-like}}$ element. However, the carbapenemase-producing genes $\text{bla}_{\text{IMP}}$ and $\text{bla}_{\text{OXA-58}}$ as well as the ESBL $\text{bla}_{\text{PER-1}}$ and AmpC-producing gene $\text{bla}_{\text{CMY-2}}$ were not detected.

Thus, the molecular epidemiology of carbapenem-resistant $A. \text{baumannii}$ isolates from a single hospital in Kenya reveals the presence of organisms associated with infection elsewhere in the world. The time course of the outbreaks is illustrated in Fig. 2. It is noteworthy that the first isolate of each sequence type was in a patient transferred from another hospital. Unfortunately, as has occurred elsewhere in the world, introduction of strains from other hospitals and person-to-person spread of hospital-adapted strains of $A. \text{baumannii}$ appears to be occurring in Kenyan healthcare facilities.

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References


**Fig. 1.** Molecular typing, antimicrobial resistance profile and source of isolation of 25 *Acinetobacter baumannii* clinical isolates. Of note, all isolates were resistant to all antibiotics tested, including meropenem (MEM), imipenem (IMP) and amikacin (AN), with the exception of four isolates; isolates 19, 21 and 23 were susceptible to AN and isolate 24 was susceptible to MEM, IMP and AN. R, resistant; S, susceptible. ICU, intensive care unit; HDU, haemodialysis unit.

**Fig. 2.** Time course of *Acinetobacter baumannii* outbreaks according to sequence type (ST). Isolates are depicted with diamonds; isolates within circles were derived from patients staying in the intensive care unit or haemodialysis unit, and isolates outside circles were derived from patients hospitalised in other wards.