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Short Communication

First report of NDM-1-producing Acinetobacter baumannii in East Africa

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S U M M A R Y

Background: The emergence of carbapenem-resistant Acinetobacter baumannii (CRAB) was observed in a Kenyan hospital from 2009 to 2010. Further investigation of the dissemination of CRAB isolates and the molecular characterization of associated resistance determinants were therefore performed.

Methods: Antibiotic susceptibilities were determined by broth microdilution and Etest. Metallo-β-lactamases were detected by Etest method. Clonal relationships were studied by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). β-Lactam and aminoglycoside resistance determinants and the clonal relatedness to widespread European clones were studied by PCR and sequencing.

Results: Sixteen CRAB isolates from 10 patients possessed six pulsortypes; half of the isolates belonged to the European clone II (ECII) lineage. ECII strains were typed as MLST sequence type 2 (ST2) and ST109, and non-ECII strains as ST25 and ST113. All isolates harbored ISaba1-blaOXA-23, blaOXA-51-like, blaADC, and class 1 integron, including one that also harbored blaNDM-1. ADC-57 and two integron cassettes (arr-2-cmlA5 and aadB-aadA2-cmlA6-aadA15) were newly-identified. Non-ECII isolates, designated non-ECII clone, carried arrM and integron cassette arr-2-cmlA5.

Conclusions: Two distinct clones of CRAB – ECII and non-ECII epidemic clones – were disseminated in Kenya. The concomitance of ISaba1-blaOXA-23 was the major mechanism contributing to CRAB. The first identification of ECII CRAB and New Delhi metallo-β-lactamase 1 (NDM-1) extensively drug-resistant A. baumannii in East Africa is of concern.

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1. Introduction

The global spread of carbapenem-resistant Acinetobacter baumannii (CRAB) has been observed and is considered a sentinel event of emerging antimicrobial resistance.¹ In an extensive survey, the European clone II (ECII) clonal complex was found to be the most widespread clone of clinical CRAB isolates on five continents.¹ Reports describing CRAB dissemination in the African continent are scarce and the studies have been limited to northern and southern regions of Africa.²

Carbapenem resistance mechanisms in A. baumannii are more commonly mediated by carbapenem-hydrolyzing class D β-lactamases (CHDLs) and less often by class B metallo-β-lactamases (MBLs).² To our knowledge, several CHDLs (OXA-23, OXA-24, OXA-58, and OXA-97) and a few MBLs (GES and NDM-1) have been identified so far in clinical A. baumannii isolates in Africa.³ OXA-23-producing CRAB has been reported from African countries including Algeria, Tunisia, Libya, Egypt, Senegal, Nigeria, South Africa, and Madagascar. The acquisition of the blaOXA-23 gene has been associated with four genetic structures that consist of diverse conjunctions with insertion sequences ISaba1 and ISaba4, and all of these structures have been identified in Tunisia, Algeria, Libya, and South Africa.³

Before 2008, A. baumannii was uncommon among pathogens recovered at Aga Khan University Hospital, Kenya, and it had remained susceptible to several antibiotics including extended-spectrum cephalosporins, carbapenems, and aminoglycosides. CRAB appeared in 2009 and caused an outbreak in an intensive care unit that lasted for 5 months before being controlled by strict and sustained infection control measures. During the same period, clonally-related New Delhi metallo-β-lactamase 1 (NDM-1)-producing Klebsiella pneumoniae isolates were identified in our hospital and were found to carry various genes encoding β-lactam and aminoglycoside resistance.³ In the present study, we
investigated clonal dissemination and mechanisms of resistance to carbapenem and aminoglycosides in CRAB isolates.

2. Methods

2.1. Isolates and antimicrobial susceptibility testing

CRAB isolates were collected in Aga Khan University Hospital, a 250-bed tertiary care facility, from January 2009 to August 2010. Among the 16 isolates, 10 were from seven Kenyan patients, three were from two Rwandan patients, and three were from a Tanzanian patient. The isolates were initially identified by API 20E and confirmed using the Vitek system (Vitek AMS, bioMérieux Vitek Systems Inc., Hazelwood, MO, USA); further species identification was done by multiplex PCR and recA sequencing. The minimum inhibitory concentrations (MICs) were determined by broth microdilution method with Sensititre plates (TREK Diagnostic Systems, West Sussex, UK), except the MICs of imipenem, which were determined with MBL Etest strips (AB Biodisk, Solna, Sweden), and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antimicrobial agents tested using broth microdilution included ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, ceftazolin, ceftazidime, ceftriaxone, cefepime, cefmetazole, meropenem, ciprofloxacin, levofloxacin, gentamicin, amikacin, tigecycline, and trimethoprim/sulfamethoxazole. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as quality control strains.

2.2. Molecular typing and PCR assays

All isolates were typed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). 

- β-Lactamase genes (blaKPC, blaSHV, blaNDM, blavim, blaproA, blarbA, blatoXA-23, blatoXA-51, blatoXXXXX, blablaADC), amikacin resistance genes (armA, armB, armC, armD, aac(6’)-I, aac(6’)-II, aac(6’)-III, and aph(3’)-VI), the relative genetic mobile elements (intI1, intI2, intI3, ISAba1, and ISAba4), the variable regions of class I integrons, and clonality (European clones I, II, III) were investigated by PCR, and the amplicons were sequenced as described previously. 

<table>
<thead>
<tr>
<th>Isolate</th>
<th>IPM MIC</th>
<th>MBL</th>
<th>ISAbA1 + blaoXa-23</th>
<th>ISAbA1 + blaoXa-51-like</th>
<th>AMK MIC</th>
<th>16S-rRNA methylase</th>
<th>AME</th>
<th>CAZ MIC</th>
<th>ISAbA1 + blaoXa-Abr</th>
<th>European clone</th>
<th>ST</th>
<th>Pulotype</th>
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<td>-</td>
<td>(OXA-64) ≥64</td>
<td>+</td>
<td>(ArmA) + (Aac(6’)-I, Apf(3’)-VI) ≥64</td>
<td>-</td>
<td>(ADC-26)</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>I</td>
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<td>2</td>
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<td>+</td>
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MIC, minimum inhibitory concentration; IPM, imipenem; AMK, amikacin; CAZ, ceftazidime; MBL, metallo-β-lactamase; AME, aminoglycoside-modifying enzyme; ST, sequence type.

The sequences of the variable regions of the integrons of representative isolates 5 and 14 have been deposited in GenBank under the accession numbers H6141279 and H6148722, respectively. The nucleotide sequence of the novel ADC-57 enzyme has been assigned the accession number H6258925.

3. Results

3.1. Isolation and clonal relatedness of collected isolates

Among 16 isolates, 10 (55.6%) were obtained from tracheal aspirate; the others were from bone marrow aspirate, cerebrospinal fluid, catheter tip, axillary swab, nasal swab, urine, blood, and debrided tissue samples. Six pulsotypes were identified among these isolates and were determined to be non-clonally related. Eight isolates (50%) belonging to the pulsotypes III, V, VI, were found to be affiliated with ECLI lineages. 

3.2. Antimicrobial susceptibility

All CRAB isolates were resistant to β-lactams, with the exception of one cefepime-intermediate isolate. With respect to fluoroquinolone antibiotics, there was a higher resistance rate to ciprofloxacin (100%) than to levofloxacin (43.8%). With regard to
Figure 1. Relationships between integron cassette genes, encoded resistance, and clonal lineages among carbapenem-resistant Acinetobacter baumannii isolates. Cassette types (1–4) are arranged in order of integron PCR amplicon size. The open reading frames of the identified cassette gene are shown as arrows, with the direction of transcription indicated by the arrowheads. Aminoglycoside resistance genes are shown as hatched arrows, rifampin resistance genes as filled arrows, chloramphenicol resistance genes as gray arrows, and other identified genes as clear arrows. Clear rectangles indicate 5′- and 3′-conserved segments (CSs). The 5′-CS and 3′-CS amplification primers are indicated by filled arrowheads. GEN, gentamicin; SPT, spectinomycin; STR, streptomycin; RIF, rifampin; CHL, chloramphenicol.

![Figure 1](image-url)

3.4. Associations among clonality, antimicrobial susceptibility profiles, and detected resistance determinants

Half of the study isolates were associated with the ECII lineage (ECII isolates), while the other half were not (non-ECII isolates). ECII isolates harbored the blaOXA-66 gene, which was carried with a specific integron cassette arrangement (aacC1-orfX-orfX-orfX-aadA1). Non-ECII isolates carried the same cassette and arrA (Figure 1), indicating their clonality, and were designated non-ECII clone. The blaOXA-66-positive isolate (isolate 1) belonging to non-ECII clone and ST25, exhibited high-level resistance to imipenem (MIC ≥ 512 μg/ml) and retained its susceptibility to ampicillin/subbacram, tigecycline, and colistin. All non-ECII strains carrying arrA were resistant to amikacin, except two ST113 strains (isolate 12 and 13), which were susceptible to this antimicrobial drug (Table 1).

4. Discussion

Different pulsotypes of our ECII CRAB isolates with dissimilar antibiograms have been reported from South Africa, suggesting that the ECII clone has been disseminated to and has perhaps acquired carbapenem resistance in East Africa. Apart from three European clonal complexes, the relationship with some other novel genotypes in the global dissemination of CRAB has only recently been addressed; only the ST25 clone has been suggested to be well-established and highly associated with carbapenem resistance. Three ST25 isolates (isolates 4, 5, and 6) from different patients, belonging to the non-ECII clone and sharing an identical PFGE pattern, were presumed to comprise a small-sized outbreak.

CRAB isolates in this study were susceptible to amikacin (50%) and levofloxacin (56.3%); these susceptibility rates are higher than those reported in the global surveillance study (35.7% and 5.6%, respectively). Although tigecycline has so far never been used in East Africa, one ECII isolate exhibited resistance to tigecycline, which is of concern.

Three carbapenem resistance gene structures were observed: ISAb1-blaOXA-23, ISAb1-blaOXA-51-like, and blaNDM-1. ISAb1-blaOXA-23 was the major carbapenem resistance mechanism found, as isolates from other African countries. An intriguing finding was the identification of an OXA-23 and NDM-1 co-producing extensively drug-resistant A. baumannii (XDR-AB) isolate (isolate 1). This is the first report of NDM-1-encoded XDR-AB in East Africa. Previous reports have shown NDM-1-producing bacteria mainly of the Enterobacteriaceae species. In Kenya, we reported the first isolations of NDM-1 in K. pneumoniae. Whether the XDR-AB carrying NDM-1 was due to the horizontal transfer of the resistance gene by broad-host plasmid needs further investigation. Three isolates (isolates 1, 2, and 3) from the same patient shared an identical pulsotype. Isolate 1 was from the axilla and not from a respiratory-related sample, indicating the nosocomial acquisition of NDM-1 from another source or skin contact. As well as arrA, isolate 1 also carried aac(6′)-Ih and aph(3′)-VI, leading to high-level amikacin resistance (Table 1).

In conclusion, this study shows the emergence of the genetic structure ISAb1-blaOXA-23, conferring carbapenem resistance in A. baumannii in Kenya, in two distinct clonal lineages. One lineage is linked to international clone ECII and the other is a newly identified epidemic non-ECII clone carrying a novel integron cassette and the ArrA gene. Remarkably, one ST25 CRAB isolate belonging to non-ECII clone had acquired MBL NDM-1 and amikacin resistance determinants, including arrA, aac(6′)-Ih, and aph(3′)-VI. Highly stringent control measures should be applied to prevent these resistance genes spreading further into other bacterial species.

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Ethical approval: The study was approved by the Aga Khan University Hospital Research and Ethics Committee.

Conflict of interest: The authors declare that no conflicts of interest exist.

References