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Short Communication

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

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SUMMARY

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 pulsotypes; 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 IS*Aba1*–bla_{OXA-23}, $bla_{OXA-51-like}$, bla_{ADC}, and class 1 integron, including one that also harbored bla_{NDM-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 *armA* 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 IS*Aba1*–bla_{OXA-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.

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.[1,2]

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 bla_{OXA-23} gene has been associated with four genetic structures that consist of diverse conjunctions with insertion sequences IS*Aba1* and IS*Aba4*, and all of these structures have been identified in Tunisia, Algeria, Libya, and South Africa.[2,3]

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.[4] In the present study, we

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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.[5]

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.[6] The antimicrobial agents tested using broth microdilution included ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefazolin, 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).[5,7]

β-Lactamase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM}, bla_{SIM}, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24/OXA\text{-}40\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$ and bla_{ADC}), amikacin resistance genes (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *aac(6′)-Iad*, *aac(6′)-Ib*, *aac(6′)-Ih* and *aph(3′)-VI*), the relative genetic mobile elements (*intI1*, *intI2*, *intI3*, IS*Aba1*, and IS*Aba4*), the variable regions of class 1 integrons, and clonality (European clones I, II, III) were investigated by PCR, and the amplicons were sequenced as described previously.[5,8–10] bla_{NDM} was detected with the primers ATGGAATTGCCCAATATTATGC and CGAAAGTCAGGCTGTGTTG. The full-length of the bla_{NDM}, bla_{ADC}, and $bla_{OXA\text{-}23}$ genes were sought with primer pairs NDM ATGGAATTGCCCAATATTATGC and TCAGCGCAGCTTGTCGG, ADC ATGCGATTTAAAAAAATTTCTTGTC and TTATTTCTTTATTGCATTCAGCAC, and OXA23 TTATTTTCTATTGATCTGGTGTTT and TAGAGTTTCTGTCAAGC, respectively.

2.3. Nucleotide accession numbers

The sequences of the variable regions of the integrons of representative isolates 5 and 14 have been deposited in GenBank under the accession numbers **HQ141279** and **HQ148722**, respectively. The nucleotide sequence of the novel ADC-57 enzyme has been assigned the accession number **HQ258925**.

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 ECII lineages. MLST analysis revealed four sequence types (ST). Among eight ECII strains, seven belonged to ST2 (2-2-2-2-2-2-2) and one to ST109 (26-4-2-2-9-1-5). In the other eight non-ECII strains, six belonged to ST25 (3-3-2-4-7-2-4) and two to ST113 (3-3-3-4-7-4-4) (Table 1).

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

Table 1
Characteristics of carbapenem-resistant *Acinetobacter baumannii* isolates

		Relative carbapenem resistance				Relative aminoglycoside resistance			Relative cephalosporin resistance	Clonality		
Isolate No.	IPM MIC	MBL[a]	IS*Aba1* + $bla_{OXA\text{-}23}$	IS*Aba1* + $bla_{OXA\text{-}51\text{-}like}$	AMK MIC	16S-rRNA methylase	AME[b]	CAZ MIC	IS*Aba1* + $bla_{ADC\text{-}like}$	European clone[c]	ST	Pulsotype
1	≥512	+ (NDM-1)	+	– (OXA-64)	≥64	+ (ArmA)	+ (Aac(6′)-Ih, Aph(3′)-VI)	≥64	– (ADC-26)	-	25	I
2	16	-	+	– (OXA-64)	≥64	+ (ArmA)	–	≥64	– (ADC-26)	-	25	I
3	16	-	+	– (OXA-64)	≥64	+ (ArmA)	–	≥64	– (ADC-26)	-	25	I
4	16	-	+	– (OXA-64)	≥64	+ (ArmA)	–	≥64	– (ADC-26)	-	25	II
5	32	-	+	– (OXA-64)	≥64	+ (ArmA)	–	≥64	– (ADC-26)	-	25	II
6	32	-	+	– (OXA-64)	≥64	+ (ArmA)	–	≥64	– (ADC-26)	-	25	II
7	32	-	+	+ (OXA-66)	≤2	–	–	≥64	+ (ADC-30)	II	2	III
8	64	-	+	+ (OXA-66)	≤2	–	–	≥64	+ (ADC-30)	II	2	III
9	16	-	+	+ (OXA-66)	≤2	–	–	≥64	+ (ADC-30)	II	2	IIIa
10	32	-	+	+ (OXA-66)	≤2	–	–	≥64	+ (ADC-30)	II	2	IIIb
11	32	-	+	+ (OXA-66)	≤2	–	–	≥64	+ (ADC-30)	II	2	IIIb
12	64	-	+	– (OXA-64)	4	+ (ArmA)	–	≥64	– (ADC-57)	-	113	IV
13	64	-	+	– (OXA-64)	4	+ (ArmA)	–	≥64	– (ADC-57)	-	113	IVa
14	16	-	+	– (OXA-67)	≤2	–	–	≥64	– (ADC-26)	II	109	V
15	16	-	+	– (OXA-66)	16	–	+ (Aph(3′)-VI)	≥64	+ (ADC-30)	II	2	VI
16	32	-	+	– (OXA-66)	32	–	+ (Aph(3′)-VI)	≥64	+ (ADC-30)	II	2	IIIa

MIC, minimum inhibitory concentration; IPM, imipenem; AMK, amikacin; CAZ, ceftazidime; MBL, metallo-β-lactamase; AME, aminoglycoside-modifying enzyme; ST, sequence type.

[a] The screened MBL genes included bla_{IMP}-like, bla_{VIM}-like, bla_{SIM}, bla_{GIM}, bla_{SPM}, and bla_{NDM}.

[b] The screened AME genes included amikacin resistance genes *aac(6′)-Iad*, *aac(6′)-Ib*, *aac(6′)-Ih*, and *aph(3′)-VI*.

[c] Determined using the multiplex PCR assay developed by Turton et al.[10]

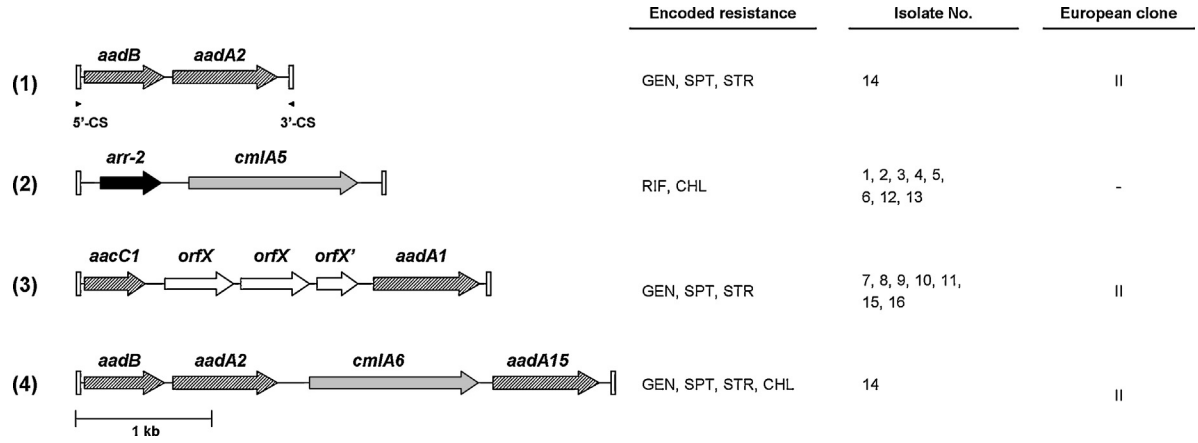

Figure 1. Relationships between integron cassette genes, encoded resistance, and clonal lineages among carbapenem-resistant *Acinetobacter baumannii* isolates. Cassette types (1–4) are arranged in order of integron PCR amplicon size. The open reading frames of the identified cassette gene are shown as 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.

aminoglycosides, the resistance rates were 68.8% for gentamicin and 37.5% for amikacin. All isolates were susceptible to colistin. One isolate (isolate 14) was resistant to tigecycline.

3.3. Detection of resistance genes and resistance determinants

All isolates harbored $bla_{\text{OXA-23}}$, $bla_{\text{OXA-51-like}}$, and bla_{ADC} genes, with one isolate also harboring $bla_{\text{NDM-1}}$. One novel ADC, $bla_{\text{ADC-57}}$, was designated with five amino acid substitutions (K150Q, S167P, G242D, L253F, and R342G) in comparison to the ADC-1 protein. The IS*Aba1* element was detected upstream of all $bla_{\text{OXA-23}}$, $bla_{\text{ADC-30}}$, and $bla_{\text{OXA-66}}$ gene-harboring isolates. Eight isolates harbored *armA*, two harbored *aph(3′)-VI*, and one harbored both *aac(6′)-Ih* and *aph(3′)-VI* (Table 1). Four different types of gene cassette were identified, including two novel ones (*arr-2-cmlA5* and *aadB-aadA2-cmlA6-aadA15*). Each isolate had one integron gene cassette, except isolate 14 which had two cassettes. These integron cassette genes encoded resistance to rifampin, chloramphenicol, and gentamicin (Figure 1).

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 $bla_{\text{OXA-66}}$ gene, which was carried with a specific integron cassette arrangement (*aacC1-orfX-orfX-orfX′-aadA1*). Non-ECII isolates all carried the *arr-2-cmlA5* integron cassette and *armA* (Figure 1), exhibited their clonality, and were designated non-ECII clone. The $bla_{\text{NDM-1}}$-positive isolate (isolate 1) belonging to non-ECII clone and ST25, exhibited high-level resistance to imipenem (MIC $\geq$ 512 μg/ml) and retained its susceptibility to ampicillin/sulbactam, tigecycline, and colistin. All non-ECII strains carrying *armA* 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,[1] 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.[7] 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).[1] 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: IS*Aba1*-$bla_{\text{OXA-23}}$, IS*Aba1*-$bla_{\text{OXA-51-like}}$, and $bla_{\text{NDM-1}}$. IS*Aba1*-$bla_{\text{OXA-23}}$ was the major carbapenem resistance mechanism found, as in reports from other African countries.[2,3] 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.[4] In Kenya, we reported the first isolations of NDM-1 in *K. pneumoniae*.[4] 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 *armA*, 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 IS*Aba1*-$bla_{\text{OXA-23}}$, 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 ArmA gene. Remarkably, one ST25 CRAB isolate belonging to non-ECII clone had acquired MBL NDM-1 and amikacin resistance determinants, including *armA*, *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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