

**First detection of carbapenemases-producing *Acinetobacter baumannii* and
Acinetobacter nosocomialis in Côte d'Ivoire**

ABSTRACT

Carbapenem resistant *Acinetobacter baumannii* are spreading worldwide and represent a major health threat for ~~severely ill~~immunocompromised patients. In this study, we describe for the first time NDM-producing *A. baumannii* and *Acinetobacter nosocomialis* isolates from Côte d'Ivoire, Africa. Four carbapenem-resistant *Acinetobacter* sp. isolates were isolated from urinary tract infections from three university hospital centers. The antimicrobial susceptibility testing and carbapenemase detection CarbAcinetoNP test were performed. The carbapenemases genes were ~~sought~~screened by PCR. Resistome, MLST and phylogeny were performed using whole genome sequencing. All isolates were resistant to β -lactams, aminoglycosides and fluoroquinolones. The *bla*_{NDM-1} and the *bla*_{OXA-58} genes were identified in the three *A. baumannii* isolates whereas *A. nosocomialis* carried only the *bla*_{NDM-1} gene. An amino acid change S83L was detected in GyrA in all *A. baumannii* isolates explaining fluoroquinolone resistance. MLST analysis revealed that all *A. baumannii* isolates belonged to the same Sequence type, ST-2. The *bla*_{NDM-1} and *bla*_{OXA-58} genes could not be transferred, suggesting a chromosomal location. This study highlights the emergence of NDM-1- and OXA-58-producing *A. baumannii* isolates belonging to the pandemic clone ST-2 in Côte d'Ivoire, Africa. Comparison of these three genomes revealed that they are highly related.

Key words: New Delhi metallo- β -lactamase, *Acinetobacter baumannii*, *Acinetobacter nosocomialis*

INTRODUCTION

~~Spread~~ ~~Emergence and spread~~ of carbapenemases in clinical Gram-negative organisms is an ~~important~~ ~~major~~ threat to public health. The most common carbapenemases in *Acinetobacter* are the class D oxacillinases (OXA), including OXA-23-like, OXA-40-like, OXA-51-like, and OXA-58-like enzymes [1]. In recent years, carbapenemases from classes B have also been involved. New Delhi metallo- β -lactamase 1 (NDM-1), a novel metallo- β -lactamase, was first reported in 2009 in a *Klebsiella pneumoniae* isolate from a Swedish patient repatriated to Sweden from India [2]. Since then, the emergence and global dissemination of carbapenemase-producing isolates, particularly NDM-1, has been reported in many countries [3]. These metallo- β -lactamases (MBLs) require divalent cations (usually zinc ions) as metal cofactors for enzymatic activity to hydrolyze all β -lactams, including carbapenems (except aztreonam) [4]. Until now, 18 variants of *bla*_{NDM} (<http://www.bldb.eu/>) have been described ~~so far~~ [5]. Moreover, NDM was described in Enterobacteriaceae as well as in non-fermenters especially in *A. baumannii* and *Pseudomonas aeruginosa* [6]. The first case of NDM-1-producing *Acinetobacter* spp. was reported in India in 2010 [6].

In Africa, NDM was also identified in Enterobacteriaceae in Morocco [7], Kenya [8], Algeria [9] and in *A. baumannii* in Egypt [10] and Algeria ([11],[12]). In West Africa, ~~there is~~ scarce data on the NDM-producers ~~remains available~~, even ~~though~~ Walktyet *al.* [13] reported the presence of NDM-1-producing *K. pneumoniae* in a patient who had been hospitalized in Nigeria. In the present study, we report the first description of three NDM-1- and OXA-58-producing *A. baumannii* isolates and of one NDM-1-producing *A. nosocomialis* from the Ivory Coast, West Africa.

MATERIALS AND METHODS

Bacterial strains and species identification

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Twenty randomly chosen *Acinetobacter* sp. isolates were selected at random from the biobank of the Pasteur Institute of Côte d'Ivoire, representing isolates from each of the hospitals served by this Institute. As the identification to the clinical bacteriology unit of the Institute was mainly based on biochemical tests, this collection was re-identified using Maldi TOF. Among this collection, four isolates (Aba1614, Ab12, Aba2114 and An2605) identified as carbapenem-resistant *Acinetobacter* sp. were all identified as *A. baumannii* by previously performed biochemical tests. However, in this study, these four isolates were re-identified using the Maldi-TOF technique. These four isolates were all isolated from urinary tract infections. The Aba1614 and Ab12 isolates were recovered from women at Yamoussoukro University Hospital and Abidjan-Cocody University Hospital, respectively. Aba2114 and An2605 were isolated from a male child hospitalized in the pediatric department of the Abidjan-Youpougon University Hospital. The identification of these isolates was carried out by standard laboratory methods and confirmed by the Bruker Daltonik Maldi TOF BioTyper software and also by searching for the *bla*OXA-51-like gene using PCR [14].

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Antimicrobial susceptibility testing and detection of carbapenemase phenotype.

Antimicrobial susceptibility testing was performed on isolates showing reduced susceptibility to carbapenems by disc diffusion method on Mueller–Hinton agar according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016) guidelines (<http://www.eucast.org>). The minimum inhibitory concentrations (MICs) of carbapenems, colistin, rifampin and tigecyclin were determined and interpreted as recommended by EUCAST guidelines. The presence of a carbapenem-hydrolyzing enzyme was confirmed using the CarbaAcineto NP test as previously described [15].

Molecular detection of carbapenemase genes

DNA extraction

Genomic DNA from each strain ~~that~~ tested positive for the combined imipenem/imipenem + EDTA disc test and imipenem/EDTA synergy test was extracted using the Gene JET Genomic DNA ~~p~~Purification ~~e~~Extraction Kit (Fermentas®) as recommended by the manufacturer.

Amplification of carbapenemase genes ~~using-pcr~~ PCR

The following primers ~~bla~~_{NDM}, ~~bla~~_{VIM}, ~~bla~~_{IMP}, ~~bla~~_{OXA-23}, ~~bla~~_{OXA-40} and ~~bla~~_{OXA-58} described by Poirel et al (2011) were used to amplify specific DNA sequences involved in resistance to carbapenem antibiotics by PCR (Table 1). Total reaction volume of 50 µl containing 2.5µl of each primer of 10 mM (Eurogentec, ~~Bel~~gium), 5 µl of MgCl₂ PCR buffer 10 x (Qiagen), 1µl of deoxyribonucleoside triphosphates (dNTPs, 10mM), 0.4µl of Taq polymerase (Qiagen), 36.6µl of ultrapure water and 2µl of bacterial genomic DNA was mixed. ~~This PCR mixture~~PCR was performed in a thermal cycler under the following conditions: initial denaturation for 3 min at 95°C followed by 35 cycles consisting of denaturation at 95°C for 30 s, hybridization at 55°C for 30 s and elongation at 72°C for 30 s followed by final elongation at 72°C for 7 min. The PCR products obtained were ~~migrated-run~~ on a 2% agarose gel ~~r~~Red Nucleic Acid Gel Stain for 40 minutes at 100 volts. DNA fragments were visualized with a gel viewer under UV light.

Whole Genome Sequencing and Bioinformatic analysis.

The Next Generation Sequencing (NGS) was carried out on these isolates at the Pasteur International Bioresources network (PIBnet) of the Institut Pasteur, Paris, France.

NGS data are ~~summa~~erized in Table 1.

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Table 1: Primers used for the screening of genes encoding for the production of carbapenemases (Poirel et al. 2011).

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Genes	PrimersSequences (5' - 3')	Amplicon size (bp)
<i>bla_{NDM}</i>	R : CGGAATGGCTCATCACGATC F : ACGATTCTCCCCTCTGCGC	800
<i>bla_{OXA-58}</i>	R : ACGATTCTCCCCTCTGCGC F : CGATCAGAATGTTCAAGCGC	800
<i>bla_{OXA-40}</i>	R : CGGGATCCCGTTAAATGATTCCAAGATTTCTAGCG F : GGAATTCCATGAAAAAATTTATACTTCC	186
<i>bla_{OXA-23}</i>	R : GCAAAAGCGACAATTTTCC F : TCTGGTTGTACGGTTCAGCA	501
<i>bla_{IMP}</i>	R : GGTTTAAAYAAAACAACCACC F : ACAYGGYTTGGTDGTTCTTG	500
<i>bla_{VIM}</i>	R : AATGCGCAGCACCAGGATAG F : GTTGGTCGCATATCGCAAC	500

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Whole Genome Sequencing and Bioinformatic analysis

The Next Generation Sequencing (NGS) was carried out on these isolates at the Pasteur International Bioresources network (PIBnet) of the Institut Pasteur, Paris, France.

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NGS data are summarized in Table 2. Short sequences (reads) of all isolates were assembled into more contigs by CLC genomics workbench version 9.5.3 (www.clcbio.com). Only contigs larger than 500-bp were retained for further analysis. Resistance was determined using the CGE web based Resfinder software [16] and Multilocus Sequence Typing (MLST) [17] based on contigs data was done using MLST CGE using both, the Pasteur institute MLST database (<http://www.pasteur.fr/mlst>). Annotations using the RAST (Rapid Annotation using Subsystem Technology) server (<http://rast.nmpdr.org/>) were performed to compare numbers of prophages and transposable elements ([18]; [19]). The Quinolone resistance-determining region (QRDR) analysis was conducted using the reference sequence for *gyrA* (AJF82744.1), *gyrB* (CU468230.2) and *parC* (X95816). The ATPase-encoding *comM* gene and the boundary junctions (J1 and J2) of the *A. baumannii* Resistance Island (RIs) and *uspA* gene were also searched in the genome of each isolate, using the reference sequences (LN865143.1), (KT828751.1), (KT828747.1) and (GQ268326.1) respectively. Similarly, the presence of an insertion sequence in front of *bla*_{ADC} and *bla*_{OXA-51} enzymes was also performed *in silico* on assembled genomes.

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Electroporation and conjugation experiments

To determine the genetic location of the *bla*_{NDM-1} and *bla*_{OXA-58} genes, plasmid DNA of the different isolates was extracted by using the Kieser extraction method (Kieser et al., 1984).

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The extracted plasmid DNA suspension was transferred by electroporation into *A. baumannii* CIP 70.10 as previously described [20]. Transformants were selected on ticarcillin (100mg/mL) containing Trypticase Soy Agar (TSA) plates. The bacterial conjugation was carried out according to the protocol described by Potron et al. (2014). Rifampicin-resistant

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strain *A. baumannii* BM 4547 was used as a recipient strain. Clones were selected by rifampicin (60mg/mL) and ticarcillin (100mg / mL) containing TSA plates.

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Nucleotide Sequence Accession Numbers

Genomes of Aba1614, Ab12, Aba2114 and An2605 were deposited at DDBJ/ENA/GenBank under these accession numbers NNSJ01000000, NJI01000000, NNSI01000000 and NNSH01000000 respectively.

RESULTS

Identification, antimicrobial susceptibility and carbapenem resistance detection.

In the isolates ~~collection~~ analyzed, four isolates were resistant to carbapenems. As the identification to the clinical bacteriology unit of the Institute was mainly based on biochemical tests, this collection was re-identified using MaldiTOF. These four isolates were all identified as *Acinetobacter baumannii* with biochemical tests but Maldi-TOF revealed that one of the isolates was actually *A. nosocomialis*. The presence of the *bla*_{OXA-51-like} gene in the three *A. baumannii* isolates and its absence in the *A. nosocomialis* isolate, confirmed the Maldi-TOF results. The three *A. baumannii* isolates were resistant to penicillins, cephalosporin, carbapenems, aminoglycosides, fluoroquinolone, tetracycline, sulphonamide and tigecyclin. They remained susceptible to colistin with an MIC of 1 µg/mL. *A. nosocomialis* was resistant to all antibiotics tested except to levofloxacin, ciprofloxacin and colistin with MIC of 0.25, 0.25 and 2 µg/mL respectively. All isolates were positive with the CarbaAcinetoNP test.

Profile of detected carbapenemase genes

The genes coding for resistance to Carbapenem, namely *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{OXA-23}, *bla*_{OXA-40} and *bla*_{OXA-58} were tested ~~in for~~ the strains with the Carbapenem-resistance phenotype. The electrophoretic profiles of the detected carbapenemase genes are shown in

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Figure 1. Genotyping revealed the presence of two types of genes; *bla*_{NDM} encoding class-B metalloenzyme-encoding gene *bla*_{NDM} and the *bla*_{OXA-58} gene encoding the class-D oxacillinase-encoding. These both genes are characterized by DNA fragments of 800 base pairs in size (Figure 1 and 2), but the other genes were not detected in this study.

Carbapenem-resistance and Resistome based on whole genome sequencing.

Initial PCR screening of these isolates revealed the presence of *bla*_{NDM-1} gene in all of them, and for the three *A. baumannii* isolates, the *bla*_{OXA-58} gene was additionally found. In order to dive deeper into the resistome of these isolates, we have sequenced their entire genomes using Illumina technology. Sequencing of the four isolates resulted in 1.9 to 2.3 10^6 reads with an average length of c.a. 150-bp, and resulted in 301 to 380 contigs as assembled using CLC workbench V.9.5.3. The G+C content was 39 %, which is compatible with *A. baumannii*. The results of NGS are displayed in table 2. These contigs were further annotated using the RAST server, which predicted 3650, 3612, 3601 and 3674 coding sequences (CDSs) in genome for Aba1614, Ab12, Aba2114 and An2605 respectively.

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Figure 1: Electrophoretic photographs of *bla*_{NDM} genes.

CN: negative control, *CP*: positive control, track 1, 8; 20 and 44 *Acinetobacter* strains, track 2 to 7 and 9;17;21 to 32 *P. aeruginosa* strains, *M*: molecular size marker of 1000 base pairs

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Figure 2: Electrophoretic photographs of *bla*OXA-58 genes.

cn: negative control, *cp*: positive control, track 1 to 4 *Acinetobacter* strains, track 1 to 4 *P. aeruginosa* strains, *M*: molecular size marker of 1000 base pairs.

These coding sequences include subsystems involved in essential metabolism of the bacteria, including cell wall and capsule, cell division and cell cycle, RNA, DNA and protein metabolisms these genomes. The *A. baumannii* and *A. nosocomialis* isolates had several transposable elements and phages/prophages. The isolates Aba1614, Ab12, Aba2114 and An2605 contained 13, 13, 13 and 11 (CDSs) transposable elements, respectively; 7, 7, 8 and 2 (CDSs) phages and prophages, respectively. The ATPase-encoding *comM* gene is known to be a hot spot for the integration of RIs in *A. baumannii*. Genomes of the three *A. baumannii* isolates harbored the interrupted *comM* gene by AbaR-type element with boundaries J1 and J2 of RIs and the intact *uspA* gene indicating a short version of the AbaR elements. The AbaR-type element inserted into the *comM* gene was aligned in the NCBI database (<http://www.ncbi.nlm.nih.gov/blast>). NCBI homology research indicates that the AbaR-type sequences (1 to 17992 bases) of the three *A. baumannii* isolates are closely related to AbaR4 sequences of *A. baumannii* strains LT-3 (accession no JN1299845) and *A. baumannii* AB210 (accession no HQ700358) which belonged to EC II and which were assigned to ST2 (MLST-IP) (<http://www.pasteur.fr/mlst>). The backbone of AbaR4 of these three *A. baumannii* isolates, was shown to be comprised of five open reading frames constituting the transposition module (*miC*, *miA*, *miB*, *orf1*, and *orf2*), together with two other genes encoding the universal stress protein (*uspA*) and sulfate permease (*sul*) and *orf1* and *orf2* encoding the hypothetical protein. The backbone of AbaR4 is fused to a DNA fragment carrying the insertion sequence *ISCR2* and the tetracycline and streptomycin resistance genes namely *tetB*, *tetR*, *strA*, and *strB*. The structure of the AbaR4-like element of the three *A. baumannii* isolates is illustrated in Figure 3.

The three *A. baumannii* isolates harbored *bla*_{NDM-1}, *bla*_{OXA-58}, *bla*_{TEM1-D}, *bla*_{ADC-25} genes and *bla*_{OXA-66} a variant of *bla*_{OXA-51-like}, while *A. nosocomialis* harbored *bla*_{NDM-1} and the *bla*_{CARB-8} genes. Genes encoding AMEs (Aminoglycoside modifying enzymes), MLS (Macrolide,

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Lincosamide and Streptogramin B), Sulphonamide, Tetracycline and Rifampicin resistance genes were detected in these isolates (Table 2). The Aba1614, Ab12 and Aba2114 strains harbored: AMEs (*aac(3)-IId*, *aac(3)-Ia*, *aph(3')-Ic*, *aadA1*, *strB*, *strA*), MLS (*mph (E)*, *msr (E)*), Sulphonamide (*sul1*, *sul2*) and Tetracycline(*tet(B)*, *tet(39)*). Whereas, the An2605 carried AMEs (*aac(3)-IId*, *aacA4*, *aph(3')-Via*, *aac(6')Ibc*), MLS (*mph (E)*, *msr (E)*) Sulphonamide (*sul1*, *sul2*), Tetracycline (*tet(X)* and *tet(39)*), phenicols (*floR*) and Rifampicin (*arr-3*). These results are in correlation with those obtained in antimicrobial susceptibility test.

The S83L substitution in the QRDR of *gyrA* in all *A. baumannii* isolates was detected explaining the resistance to fluoroquinolone. No mutations were observed in the QRDR of *gyrB* and *parC*.

Table 2: Genetic features and genotypic profiles of carbapenem-resistant *Acinetobacter* sp

Isolates	Reads	Nucleotides	Contigs	N50	Total	%GC	MLST	Resistome			
								β -lactamases	Carbapenemase	aminoglycoside	others
								nucleotide s (contigs)			
Aba1614	2360 670	354591912	301	25620	3 909 085	39.2	ST-2	<i>bla</i> _{TEM-1D} , <i>bla</i> _{ADC-25}	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-58} , <i>bla</i> _{OXA-66}	(<i>aac</i> (3)- <i>Ild</i> , <i>aac</i> (3)- <i>Ia</i> , <i>aph</i> (3')- <i>Ic</i> , <i>aadA1</i> , <i>strB</i> , <i>strA</i>)	<i>mph</i> (E), <i>msr</i> (E), <i>sul1</i> <i>sul2</i> , <i>tet</i> (B) <i>tet</i> (39)
Ab12	1933 388	290476740	375	21627	3 874 122	39.3	ST-2	<i>bla</i> _{TEM-1D} , <i>bla</i> _{ADC-25}	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-58} , <i>bla</i> _{OXA-66}	(<i>aac</i> (3)- <i>Ild</i> , <i>aac</i> (3)- <i>Ia</i> , <i>aph</i> (3')- <i>Ic</i> , <i>aadA1</i> , <i>strB</i> , <i>strA</i>)	<i>mph</i> (E), <i>msr</i> (E), <i>sul1</i> <i>sul2</i> , <i>tet</i> (B) <i>tet</i> (39)
Aba2114	1429 662	214641448	398	18600	3 857 648	39.3	ST-2	<i>bla</i> _{TEM-1D} , <i>bla</i> _{ADC-25}	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-58} , <i>bla</i> _{OXA-66}	(<i>aac</i> (3)- <i>Ild</i> , <i>aac</i> (3)- <i>Ia</i> , <i>aph</i> (3')- <i>Ic</i> , <i>aadA1</i> , <i>strB</i> , <i>strA</i>)	<i>mph</i> (E), <i>msr</i> (E), <i>sul1</i> <i>sul2</i> , <i>tet</i> (B) <i>tet</i> (39)
An2605	2739 496	411942767	278	28876	3 981 830	38.9		<i>bla</i> _{CARB-8}	<i>bla</i> _{NDM-1}	<i>aac</i> (3)- <i>Ild</i> , <i>aacA4</i> , <i>aph</i> (3')- <i>Via</i> , <i>aac</i> (6') <i>Ibc</i>	<i>floR</i> , <i>ARR-3</i> , <i>mph</i> (E), <i>msr</i> (E), <i>sul1</i> , <i>sul2</i> , <i>tet</i> (X), <i>tet</i> (39)

ST: Sequence Type

Figure 3: Structure of AbaR4 islands. The lengths of the target genes are not to scale. AbaR-type transposon backbone is shown by filled boxes bounded by inverted terminal repeats (IR_{AbaR}) shown as black bars. *comM* gene is represented by a black arrow interrupted by AbaR-type boxing. The white arrows represent the tetracyclin (*tetB* and *tetR*), streptomycin (*strA* and *strB*) resistance genes and the ISCR2 insertion sequence.

Genetic relatedness between *Acinetobacter* isolates:

MLST analysis established genetic relatedness between *Acinetobacter* isolates. This analysis showed that these isolates belonged to ST2, the global clonal line II (CC2, European Clone II).

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Genetic environments surrounding resistance gene:

The *bla*_{NDM-1} gene was localized on a Tn125(10099 bp) transposon with two copies of *ISAbal25* in the four isolates. This Tn125 as described previously. Sequences of the flanking regions of Tn125 have been aligned in the NCBI database. Analysis of these sequences revealed the presence of the *mfs* (Major facilitator superfamily) protein, encoding a metabolite of the putative facilitator / H⁺ symporter superfamily located on the chromosome of *A. baumannii*. In the *A. baumannii* isolates, *bla*_{OXA-58} gene was located between two copies of *ISAbal3*, one located at 20 bp upstream and the other at 28 bp downstream of the gene. Sequences of *ISAbal3* and *bla*_{OXA-58} gene were compared with that of the *A. baumannii* MAD strain. Repeated inverted sequences left of *ISAbal3* (IRL) were 24 bp each as previously described (Figure 4). Class 1 integrons were recovered from the four isolates of *Acinetobacter spp.* In the three isolates of *A. baumannii*, the integron carried the *aacC1* and *aadA1* gene cassettes conferring resistance to aminoglycosides and *A. nosocomialis*, it carried the gene cassettes *arr3* and *aadA4* conferring resistance to rifampicin and aminoglycosides, respectively (Figure 5). The presence of these genes confirms the aminoglycoside resistance

profile observed in isolates Ab12, Aba1614, Aba2114 and An2605 and to rifampicin only for An2605 (Table 3).

Genetic localization of the resistance genes.

Agarose gel analysis of Kieser extracted DNA revealed that Aba2114 isolate carried 154 kb plasmid and An2605 isolate carried three small plasmids, one of which was close to 10 kb and the other two less than 7-kb. No plasmids were detected in Aba1614 and Ab12 isolates. Their transfer by electroporation and conjugation experiments gave repeatedly negative results, suggesting that *bla*_{NDM-1} and *bla*_{OXA-58} genes were chromosomally-located in all isolates.

Figure 4: Schematic arrangement of *bla*_{OXA-58} structures identified in *A. baumannii* isolates from Côte d'Ivoire. (A) Structure identified in *A. baumannii* MAD isolate (accession no. AY665723) from France. (B) Structure identified in three isolates *A. baumannii* from Côte d'Ivoire. The lengths of the target genes are not to scale. The horizontal dotted line indicates the sequence separating the truncated *ISAb*₃ element from the Re27-1 repeat sequence. The two different transcription regulator genes (*araC1* and *araC2*), the threonine efflux protein gene (*lysE*) and the esterase gene (*Est*) are indicated. Vertical arrows are for the two Re27 sequences. The black boxers at the ends of *ISAb*₃ represent the left inverted repeats.

Figure 5: Features of class 1 integron carried aminoglycoside and rifampicin resistance genes in *A. baumannii* and *A. nosocomialis* isolates. (A) Class 1 integron of three *A. baumannii* isolates. (B) Class 1 integron of *A. nosocomialis* isolate. The lengths of the target genes and the exact location of the target site are not to scale. The black arrows indicated the target genes. Gene and site names are abbreviated according to their corresponding proteins: *intI1*: integrase, *attI1*: integron-associated recombination site, *aacC1*: AAC(3)-Ia aminoglycoside acetyltransferase, *attC*: IntI-type integrase recombination site, *aadA1*: ANT(3)-Ia aminoglycoside adenyltransferase, *orf1* and *orf2*: Hypothetical protein, *qacEdelta1*: Quaternary ammonium compounds resistance protein, *sul1*: sulfonamide reductase, *arr3*: rifampin ADP-ribosylating transferase and *aacA4*: aminoglycoside 60 -N-acetyltransferase.

Table 3: Antimicrobial susceptibility patterns of carbapenem-resistant *A. baumannii*-*A. nosocomialis*

Isolates	species	War/Hospital	Date of isolation	MICs (µg/ml)														
				TIC	TIM	PIP	TZP	CTX	CAZ	FEP	IMP	MER	GEN	AKN	TM	LEV	CIP	TE
Aba1614	<i>A.baumannii</i>	C/ UHYa	16-01-2014	>256	>256	>256	>256	>64	>64	>64	>32	>32	>4	128	4	8	>1	32
Ab12	<i>A.baumannii</i>	C/UHC	21-11-2013	>256	>256	>256	>256	>64	>64	>64	>32	>32	>4	128	>4	32	>1	32
Aba2114	<i>A.baumannii</i>	Pediatric/UHY	21-01-2014	>256	>256	>256	>256	>64	>64	>64	>32	>32	>4	128	>4	8		32
An2605	<i>A.nosocomialis</i>	Pediatric/UHY	26-05-2015	>256	16	>256	>256	>64	>64	>64	>32	>32	>4	12	>4	0.25	0.25	32

C: consultation, UHY: University hospital of Yopougnon, UHC: University hospital of Cocody, UHYa: University hospital of Yamoussoukro. PIP= piperacillin, TZP= piperacillin/tazobactam, TIC= ticarcillin, TIM= ticarcillin/clavulanic acid, CAZ= ceftazidime, CTX= cefotaxime, FEP= ceftazidime, IMP= imipenem, MER= meropenem, GEN= gentamicin, AKN= amikacin, TM= tobramycin, LVX= levofloxacin, TE= tetracycline

DISCUSSION:

Although most NDM genes are found in *Enterobacteriaceae* [21], they have also been detected in *Acinetobacter* species [22]–[25]. The first case of *bla*_{NDM-1} gene in *Acinetobacter* spp. was reported in India in 2010 [6]. Other reports from China, Egypt, France, Belgium and Brazil followed [22], [23], [26], [27]. In Africa, NDM-1-producing *A. baumannii* has already been reported in Algeria [11], Kenya [24] and Egypt [10]. However, in sub-Saharan Africa, data on carbapenemase-producers remain scarce. Here, we report four *Acinetobacter* sp. that harbor *bla*_{NDM-1} gene. The patients' history did not reveal a recent trip beyond the Ivorian borders. It is likely that the acquisition of NDM-1-producing *Acinetobacter* spp. occurred in the respective hospitals, but as no screening of carriers, not epidemiological surveys are undertaken in these hospitals it is difficult to evidence it. Alternatively, the patients may have acquired the isolates in the community, or through contact with patients that have travelled abroad. The strains Aba1614, Ab12 and Aba2114 carried *bla*_{NDM-1}, and the *bla*_{OXA-58} genes. This coexistence of *bla*_{NDM-1} with *bla*_{OXA-58} in *A. baumannii* has already been reported in Vietnam [28], India [29] but not yet in Africa. In Europe, *bla*_{OXA-58} gene carriers are declining in the profit of OXA-23 producers (<http://www.cnr-resistance-antibiotiques.fr>). Resistance to fluoroquinolone is mediated primarily through spontaneous mutation of genes in the QRDR, of DNA gyrase and topoisomerases IV. Alterations in the drug target due to modifications in the genes for DNA gyrase A (*gyrA*) or topoisomerase IV (*parC*) have been associated with high levels of resistance to fluoroquinolone [30], [31]. In this study, mutations in the *gyrA*, *gyrB* and *parC* genes were investigated in all isolates. Only the Ser-83-Leu mutation in the *gyrA* gene was observed in the three isolates of *A. baumannii*. Several studies have already reported this mutation of Ser-83 to Leu in *gyrA* to be involved in resistance to fluoroquinolone with MICs greater than 1 µg/mL [32], [33]. Our three *A. baumannii* isolates harbored the

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comM gene interrupted by an AbaR-type element. Resistance islands (AbaR), inserted into the target gene *comM* were reported recently in *A. baumannii* strains [34]–[36] and several studies have shown that the *uspA* gene in the AbaR backbone was intact (AbaR4) or interrupted by a large composite transposon bracketed by two copies of *IS_{Ppu12}* (Tn6018) [35], [37], [38]. The genetic environment of *bla_{NDM-1}* gene was identical to Tn125 (Poirel et al., 2012) was located on the chromosome, *A. baumannii* AB_NCGM321 (accession no. LC032101) belonging to the ST16 isolated in Vietnam, *A. baumannii* JH (accession no. JN872328) belonging to ST1, whose Tn125 was located on the chromosome. Interruption of the *mfs* protein by the transposon Tn125 has been described in strains *A. baumannii* 161/07 [39], *A. baumannii* JH [40]. The genetic environment of *bla_{OXA-58}* gene was identical to part of that described in the *A. baumannii* MAD strain (accession no. AY665723) isolated in France [41]. Sequences of the two copies of *IS_{Aba3}* had 98 % amino-acid identity with that described in *A. baumannii* MAD strain [41]. Resistance to β -lactams antibiotics including carbapenems is also attributed to alteration of porins, efflux pumps and alterations in the affinity or expression of penicillin-binding proteins [42], [43]. The mutations observed in the porins of our isolates compared to those of the *A. baumannii* ATCC 19606 reference genome can act on their functionality of these proteins and thus on the membrane permeability to several antibiotics. Analysis of the sequences of the efflux pump regulatory genes revealed mutations in the AdeR/AdeS, AdeB and AdeN system of AdeAB and AdeIJK pumps respectively [44]–[46], but none of these mutations have been functionally characterized. The three strains of *A. baumannii* belonged to clones ST2. This international clone has already been reported in Italy [47], [48], Spain [49], in Belgium [50], in Czech Republic [51], in France, in Saudi Arabia [23], Vietnam [52], in Kenya [24], in Algeria [12], [53], [54]. The *bla_{NDM-1}* gene is increasingly described in *A. nosocomialis* since its first description in China [55], Russia [56] and then in Algeria [54]. In addition to *bla_{NDM-1}*, and the *bla_{CARB-8}* genes have been detected in An2605. This carbapenem

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hydrolyzing β -lactamase gene has been described for the first time in the chromosome in *Oligellaurethralis* isolate; it is a variant of *bla*_{CARB-5} gene [57]. This gene has also been described in *Stenotrophomonas maltophilia*[58] and in *Salmonella sp.*[59]. The four isolates of *Acinetobacter spp.* are ~~the~~ multi-drug resistant (MDR); in addition to resistance to carbapenems, class 1 integrons have been found to have aminoglycoside resistance genes (*aacC1*, *aadA1* and *aadA4*) and rifampicin (*arr3*). This study showed that strains of *Acinetobacter spp.* producing *bla*_{NDM-1} gene have spread to these three hospitals, other more advanced studies taking into account a large number of isolates and hospitals in future can show-reveal the mapping of this diffusion.

CONCLUSION

This first description of *bla*_{NDM-1} gene in these four isolates should be of interest to biologists, clinicians and health authorities on the emergence of metallo- β -lactamases in Ivory Coast, an African country centrally located. Rapid detection methods for carbapenemases should be established in hospitals and university centers to investigate the extent of this diffusion and to limit their spread. This study showed that the international clone ST-2 has already diffused in two different districts of Côte d'Ivoire, that of lagoons (Abidjan) and lakes (Yamoussoukro).

Ethical approval:

Not required.

TRANSPARENCY DECLARATION

LD is co-inventor of the Carba NP Test, which patent has been licenced to bioMérieux (La Balme les Grottes, France).

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