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 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 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 by PCR. Resistome, MLST and phylogeny were performed using whole genome sequencing. All isolates were resistant to β-lactams, aminoglycosides and fluroquinolones. The $bla_{\text{NDM-1}}$ and the $bla_{\text{OXA-58}}$ genes were identified in the three *A. baumannii* isolates whereas *A. nosocomialis* carried only the $bla_{\text{NDM-1}}$ 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 $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-58}}$ 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. Comparison of these three genomes revealed that they are highly related.

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

INTRODUCTION

Spread of carbapenemases in clinical Gram-negative organisms is an important 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, scarce data on the NDM-producers remains available, even if Walkty *et 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.

Twenty randomly choosen 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 blaOXA-51-like gene using PCR [14].

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 Carba Acineto NP test as previously described [15].

Molecular detection of carbapenemase genes

DNA extraction

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

Amplification of carbapenemase genes par PCR

The following primers blaNDM, blaVIM, blaIMP, blaOXA-23, blaOXA-40 and blaOXA-58 described by Poirel et al (2011) were used to amplify specific DNA sequences involved in resistance carbapenem antibiotics by PCR (Table 1). Total reaction volume of 50 µl containing 2.5 µl of each primer of 10 mM (Eurogentec, Blegium), 5 µl of MgCl2 PCR buffer 10 x (Qiagen), 1 µl of deoxyribonucleoside triphosphates (dNTPs, 10 mM), 0.4 µl of Taq polymerase (Qiagen), 36.6 µl of ultrapure water and 2 µl of bacterial genomic DNA was mixed. This PCR mixture 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 on 2 % agarose gel 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 summerized in Table 1.

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

Genes	Primers Sequences (5'- 3')	Amplicon size (bp)
*bla*NDM	R : CGGAATGGCTCATCACGATC	800
	F : ACGATTCTCCCCTCTGCGC	
*bla*OXA-58	R : ACGATTCTCCCCTCTGCGC	800
	F : CGATCAGAATGTTCAAGCGC	
*bla*OXA-40	R : CGGGATCCCGTTAAATGATTCCAAGATTTTCTAGCG	186
	F : GGAATTCCATGAAAAAATTTATACTTCC	
*bla*OXA-23	R : GCAAAAGCGACAATTTTTCC	501
	F : TCTGGTTGTACGGTTCAGCA	
*bla*IMP	R : GGTTTAAYAAAACAACCACC	500
	F : ACAYGGYTTGGTDGTTCTTG	
*bla*VIM	R : AATGCGCAGCACCAGGATAG	500
	F : GTTGGTCGCATATCGCAAC	

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 summerized in Table 2. Shorts sequences (reads) of all isolates were assembled into more contigs by CLC genomics workbench version 9.5.3 (www.clcbio.com). Only contigs larger then 500-bp were retained for further analysis. Resistome 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 references 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 if an insertion sequence in front of blaADC and blaOXA-51 enzymes was also performed in silico on assembled genomes.

Electroporation and conjugation experiments

To determine the genetic location of the blaNDM-1- and blaOXA-58- genes, plasmid DNA of the different isolates was extracted by using the Kieser extraction method (Kieser et al., 1984). The extracted plasmid DNA suspention was transferred by electroporation into *A. baumannii* CIP 70.10 as previously described [20]. Transformants were selected on ticarcillin (100 mg/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

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

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 carbapenemase 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 Maldi TOF. These four isolates were all identified as *Acinetobacter baumannii* with biochemical tests but Maldi-TOF revealed that one of the isolates was actually *A. nososocomialis*. The presence of the $bla_{\text{OXA-51-like}}$ 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, sulphomanide 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 Carba AcinetoNP test.

Profile of detected carbapenemase genes

The genes coding for resistance to Carbapenem, namely blaNDM, blaVIM, blaIMP, blaOXA-23, blaOXA-40 and blaOXA-58 were tested for the strains with the Carbapenem-resistance phenotype. The electrophoretic profiles of the detected carbapenemase genes are shown in

Figure 1. Genotyping revealed the presence of two types of genes; blaNDM enconding class-b-metalloenzyme-enconding gene blaNDM and the blaOXA-58 gene the class-D-oxacillinase-encoding These both genes are characterized by DNA fragments of 800 base pairs (Figure 1 and 2), but the other gene was not detected in this study.

Carbapenem-resistance and Resistome.

Initial PCR screening of these isolates revealed the presence of $bla_{\text{NDM-1}}$ gene in all of them, and for the three *A. baumannii* isolates, the $bla_{\text{OXA-58}}$ 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 contig 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.

Figure 1: Electrophoretic photographs of blaNDM 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

Figure 2: Electrophoretic photographs of blaOXA-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 (tniC, tniA, tniB, 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 *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 $bla_{\text{NDM-1}}$, $bla_{\text{OXA-58,}}$ $bla_{\text{TEM1-D}}$, $bla_{\text{ADC-25}}$ genes and $bla_{\text{OXA-66}}$ a variant of $bla_{\text{OXA-51-like}}$, while *A. nosocomialis* harbored $bla_{\text{NDM-1}}$ and the $bla_{\text{CARB-8}}$ genes. Genes encoding AMEs (Aminoglycoside modifying enzymes), MLS (Macrolide,

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 fluroquinolone. 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**	**Contis**	**N50**	**Total nucleotides (contigs)**	**%GC**	**MLST**	**Resistome**			
								β-lactamases	**Carbapenemase**	**aminoglycoside**	**outhers**
Aba1614	2360670	354591912	301	25620	3 909 085	39.2	ST-2	*bla*$_{\text{TEM-1D}}$, *bla*$_{\text{ADC-25}}$	*bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{OXA-66}}$	(*aac(3)-IId, aac(3)-Ia,aph(3')-Ic, aadA1, strB, strA*)	*mph(E),msr(E),sul1 sul2,tet(B) tet(39)*
Ab12	1933388	290476740	375	21627	3 874 122	39.3	ST-2	*bla*$_{\text{TEM-1D}}$, *bla*$_{\text{ADC-25}}$	*bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{OXA-66}}$	(*aac(3)-IId, aac(3)-Ia,aph(3')-Ic, aadA1, strB, strA*)	*mph(E),msr(E),sul1 sul2,tet(B) tet(39)*
Aba2114	1429662	214641448	398	18600	3 857 648	39.3	ST-2	*bla*$_{\text{TEM-1D}}$, *bla*$_{\text{ADC-25}}$	*bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{OXA-66}}$	(*aac(3)-IId, aac(3)-Ia,aph(3')-Ic, aadA1, strB, strA*)	*mph(E),msr(E),sul1 sul2,tet(B) tet(39)*
An2605	2739496	411942767	278	28876	3 981 830	38.9		*bla*$_{\text{CARB-8}}$	*bla*$_{\text{NDM-1}}$	*aac(3)-IId, aacA4, aph(3')-Via, aac(6')Ibc*	*floR,ARR-3,mph(E), msr(E),sul1,sul2, tet(X), tet(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 ISCR*2* 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).

Genetic environments surrounding resistance gene:

The bla_{NDM-1} gene was localized on a Tn*125* (10099 bp) transposon with two copies of *ISAba125* in the four isolates. This Tn*125* as decsribed previously. Sequences of the flanking regions of Tn*125* 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 *ISAba3*, one located at 20 bp upstream and the other at 28 bp downstream of the gene. Sequences of *ISAba3* and bla_{OXA-58} gene were compared with that of the *A. baumannii* MAD strain. Repeated inverted sequences left of *ISAba 3* (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 $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-58}}$ genes were chromosomally-located in all isolates.

Figure 4: Schematic arrangement of $bla_{\text{OXA-58}}$ 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* isolates from Côte d'Ivoire. The lengths of the target genes are not to scale. The horizontal dotted line indicates the sequence separating the truncated *ISAba3* 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 *ISAba3* represent the left inverted repeats.

Figure 5: Features of class 1 integron carried aminogylcoside and rifampicin resitance 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: intl1: integrase, attI1: integron-associated recombination site, aacC1: AAC(3)-Ia aminoglycoside acetyltransferase, attC: IntI-type integrase recombination site, aadA1: ANT(3)-Ia aminoglycoside adenylyltransferase, orf1 and orf2: Hypothetical protein, qacEdeltal: 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	*A.baumannii*	C/ UHYa	16-01-2014	>256	>256	>256	>256	>64	>64	>64	>32	>32	>4	128	4	8	>1	32
Ab12	*A.baumannii*	C/UHC	21-11-2013	>256	>256	>256	>256	>64	>64	>64	>32	>32	>4	128	>4	32	>1	32
Aba2114	*A.baumannii*	Pediatric/UHY	21-01-2014	>256	>256	>256	>256	>64	>64	>64	>32	>32	>4	128	>4	8		32
An2605	*A.nosocomialis*	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= cefepime, 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 sp* 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 though 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 fluroquinolone is mediated primarily through spontaneous mutation of genes in the QRDR, of DNA gyrases 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 fluroquinolone [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 fluroquinolone with MICs greater than 1 µg/mL [32], [33]. Our three *A. baumannii* isolates harbored the *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* (Tn*6018*) [35], [37], [38].The genetic environment of $bla_{\text{NDM-1}}$ gene was identical to Tn*125* (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 Tn*125* was located on the chromosome. Interruption of the *mfs* protein by the transposon Tn*125* has been described in strains *A. baumannii* 161/07[39], *A. baumannii* JH [40]. The genetic environment of $bla_{\text{OXA-58}}$ 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 *ISAba3* 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 $bla_{\text{NDM-1}}$ gene is increasingly described in *A. nosocomialis* since its first description in China [55], Russia [56] and then in Algeria [54]. In addition to $bla_{\text{NDM-1}}$, and the $bla_{\text{CARB-8}}$ genes have been detected in An2605. This

carbenicillin-hydrolyzing β-lactamase gene has been described for the first time in the chromosome in *Oligella urethralis* isolate; it is a variant of $bla_{\text{CARB-5}}$ 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 $bla_{\text{NDM-1}}$ gene have spread to these three hospitals, other more advanced studies taking into account a large number of isolates and hospitals can show the mapping of this diffusion.

CONCLUSION

This first description of $bla_{\text{NDM-1}}$ 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 centally 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 Balmes les Grottes, France).

REFERENCES

[1] B. A. Evans et S. G. B. Amyes, « OXA β-Lactamases », *Clin. Microbiol. Rev.*, vol. 27, n° 2, p. 241‑263, avr. 2014, doi: 10.1128/CMR.00117-13.

[2] D. Yong *et al.*, « Characterization of a New Metallo-β-Lactamase Gene, blaNDM-1, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in Klebsiella pneumoniae Sequence Type 14 from India », *Antimicrob. Agents Chemother.*, vol. 53, n° 12, p. 5046‑5054, janv. 2009, doi: 10.1128/AAC.00774-09.

[3] J. M. Rolain, P. Parola, et G. Cornaglia, « New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? », *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.*, vol. 16, n° 12, p. 1699‑1701, déc. 2010, doi: 10.1111/j.1469-0691.2010.03385.x.

[4] T. R. Walsh, M. A. Toleman, L. Poirel, et P. Nordmann, « Metallo-β-Lactamases: the Quiet before the Storm? », *Clin. Microbiol. Rev.*, vol. 18, n° 2, p. 306‑325, avr. 2005, doi: 10.1128/CMR.18.2.306-325.2005.

[5] T. Naas *et al.*, « Beta-lactamase database (BLDB) – structure and function », *J. Enzyme Inhib. Med. Chem.*, vol. 32, n° 1, p. 917‑919, janv. 2017, doi: 10.1080/14756366.2017.1344235.

[6] K. Karthikeyan, M. A. Thirunarayan, et P. Krishnan, « Coexistence of blaOXA-23 with blaNDM-1 and armA in clinical isolates of Acinetobacter baumannii from India », *J. Antimicrob. Chemother.*, vol. 65, n° 10, p. 2253‑2254, oct. 2010, doi: 10.1093/jac/dkq273.

[7] A. Barguigua, K. Zerouali, K. Katfy, F. El Otmani, M. Timinouni, et N. Elmdaghri, « Occurrence of OXA-48 and NDM-1 carbapenemase-producing Klebsiella pneumoniae in a Moroccan university hospital in Casablanca, Morocco », *Infect. Genet. Evol.*, vol. 31, p. 142‑148, avr. 2015, doi: 10.1016/j.meegid.2015.01.010.

[8] L. Poirel, G. Revathi, S. Bernabeu, et P. Nordmann, « Detection of NDM-1-Producing Klebsiella pneumoniae in Kenya », *Antimicrob. Agents Chemother.*, vol. 55, n° 2, p. 934‑936, févr. 2011, doi: 10.1128/AAC.01247-10.

[9] A. Sassi, L. Loucif, S. K. Gupta, M. Dekhil, H. Chettibi, et J.-M. Rolain, « NDM-5 Carbapenemase-Encoding Gene in Multidrug-Resistant Clinical Isolates of Escherichia coli from Algeria », *Antimicrob. Agents Chemother.*, vol. 58, n° 9, p. 5606‑5608, sept. 2014, doi: 10.1128/AAC.02818-13.

[10] M. A. E.-G. El-Sayed-Ahmed, M. A. Amin, W. M. Tawakol, L. Loucif, S. Bakour, et J.-M. Rolain, « High Prevalence of blaNDM-1 Carbapenemase-Encoding Gene and 16S rRNA armA Methyltransferase Gene among Acinetobacter baumannii Clinical Isolates in Egypt », *Antimicrob. Agents Chemother.*, vol. 59, n° 6, p. 3602‑3605, janv. 2015, doi: 10.1128/AAC.04412-14.

[11] E. Mesli, M. Berrazeg, M. Drissi, S. N. Bekkhoucha, et J.-M. Rolain, « Prevalence of carbapenemase-encoding genes including New Delhi metallo-β-lactamase in Acinetobacter species, Algeria », *Int. J. Infect. Dis.*, vol. 17, n° 9, p. e739‑e743, sept. 2013, doi: 10.1016/j.ijid.2013.02.024.

[12] K. Zenati, A. Touati, S. Bakour, F. Sahli, et J. M. Rolain, « Characterization of NDM-1- and OXA-23-producing Acinetobacter baumannii isolates from inanimate surfaces in a hospital environment in Algeria », *J. Hosp. Infect.*, vol. 92, n° 1, p. 19‑26, janv. 2016, doi: 10.1016/j.jhin.2015.09.020.

[13] A. Walkty *et al.*, « Isolation of multiple carbapenemase-producing Gram-negative bacilli from a patient recently hospitalized in Nigeria », *Diagn. Microbiol. Infect. Dis.*, vol. 81, n° 4, p. 296‑298, avr. 2015, doi: 10.1016/j.diagmicrobio.2015.01.005.

[14] J. F. Turton, N. Woodford, J. Glover, S. Yarde, M. E. Kaufmann, et T. L. Pitt, « Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species », *J. Clin. Microbiol.*, vol. 44, n° 8, p. 2974‑2976, août 2006, doi: 10.1128/JCM.01021-06.

[15] L. Poirel et P. Nordmann, « Rapidec Carba NP Test for Rapid Detection of Carbapenemase Producers », *J. Clin. Microbiol.*, vol. 53, n° 9, p. 3003‑3008, sept. 2015, doi: 10.1128/JCM.00977-15.

[16] E. Zankari *et al.*, « Identification of acquired antimicrobial resistance genes », *J. Antimicrob. Chemother.*, vol. 67, n° 11, p. 2640‑2644, nov. 2012, doi: 10.1093/jac/dks261.

[17] M. V. Larsen *et al.*, « Multilocus sequence typing of total-genome-sequenced bacteria », *J. Clin. Microbiol.*, vol. 50, n° 4, p. 1355‑1361, avr. 2012, doi: 10.1128/JCM.06094-11.
[18] T. Brettin *et al.*, « RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes », *Sci. Rep.*, vol. 5, p. 8365, févr. 2015, doi: 10.1038/srep08365.
[19] R. Overbeek *et al.*, « The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST) », *Nucleic Acids Res.*, vol. 42, n° Database issue, p. D206-214, janv. 2014, doi: 10.1093/nar/gkt1226.
[20] L. Poirel et P. Nordmann, « Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-58 in Acinetobacter baumannii », *Antimicrob. Agents Chemother.*, vol. 50, n° 4, p. 1442‑1448, avr. 2006, doi: 10.1128/AAC.50.4.1442-1448.2006.
[21] P. Nordmann, T. Naas, et L. Poirel, « Global Spread of Carbapenemase-producing Enterobacteriaceae », *Emerg. Infect. Dis.*, vol. 17, n° 10, p. 1791‑1798, oct. 2011, doi: 10.3201/eid1710.110655.
[22] L. Dortet, L. Poirel, et P. Nordmann, « Worldwide Dissemination of the NDM-Type Carbapenemases in Gram-Negative Bacteria », *BioMed Research International*, 2014. https://www.hindawi.com/journals/bmri/2014/249856/
[23] R. A. Bonnin *et al.*, « Dissemination of New Delhi metallo-β-lactamase-1-producing Acinetobacter baumannii in Europe », *Clin. Microbiol. Infect.*, vol. 18, n° 9, p. E362‑E365, sept. 2012, doi: 10.1111/j.1469-0691.2012.03928.x.
[24] G. Revathi, L. K. Siu, P.-L. Lu, et L.-Y. Huang, « First report of NDM-1-producing Acinetobacter baumannii in East Africa », *Int. J. Infect. Dis.*, vol. 17, n° 12, p. e1255‑e1258, déc. 2013, doi: 10.1016/j.ijid.2013.07.016.
[25] Y. Pfeifer *et al.*, « Molecular characterization of blaNDM-1 in an Acinetobacter baumannii strain isolated in Germany in 2007 », *J. Antimicrob. Chemother.*, vol. 66, n° 9, p. 1998‑2001, sept. 2011, doi: 10.1093/jac/dkr256.
[26] M. Pillonetto *et al.*, « First Report of NDM-1-Producing Acinetobacter baumannii Sequence Type 25 in Brazil », *Antimicrob. Agents Chemother.*, vol. 58, n° 12, p. 7592‑7594, déc. 2014, doi: 10.1128/AAC.03444-14.
[27] R. A. Bonnin, L. Poirel, et Nordmann, Patrice, « New Delhi metallo-β-lactamase-producing Acinetobacter baumannii: a novel paradigm for spreading antibiotic resistance genes », *Future Microbiol.*, vol. 9, n° 1, p. 33‑41, déc. 2013, doi: 10.2217/fmb.13.69.
[28] D. N. Tran *et al.*, « Emergence of New Delhi metallo-beta-lactamase 1 and other carbapenemase-producing Acinetobacter calcoaceticus-baumannii complex among patients in hospitals in Ha Noi, Viet Nam », *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 36, n° 2, p. 219‑225, févr. 2017, doi: 10.1007/s10096-016-2784-8.
[29] S. Chatterjee *et al.*, « Carbapenem Resistance in Acinetobacter baumannii and Other Acinetobacter spp. Causing Neonatal Sepsis: Focus on NDM-1 and Its Linkage to ISAba125 », *Front. Microbiol.*, vol. 7, août 2016, doi: 10.3389/fmicb.2016.01126.
[30] A. Hamouda et S. G. B. Amyes, « Novel gyrA and parC point mutations in two strains of Acinetobacter baumannii resistant to ciprofloxacin », *J. Antimicrob. Chemother.*, vol. 54, n° 3, p. 695‑696, sept. 2004, doi: 10.1093/jac/dkh368.
[31] S. Bruchmann, A. Dötsch, B. Nouri, I. F. Chaberny, et S. Häussler, « Quantitative contributions of target alteration and decreased drug accumulation to Pseudomonas aeruginosa fluoroquinolone resistance », *Antimicrob. Agents Chemother.*, vol. 57, n° 3, p. 1361‑1368, mars 2013, doi: 10.1128/AAC.01581-12.
[32] J. Vila, J. Ruiz, P. Goñi, A. Marcos, et T. Jimenez de Anta, « Mutation in the gyrA gene of quinolone-resistant clinical isolates of Acinetobacter baumannii. », *Antimicrob. Agents Chemother.*, vol. 39, n° 5, p. 1201‑1203, mai 1995.

[33] S. Park *et al.*, « Alterations of gyrA, gyrB, and parC and Activity of Efflux Pump in Fluoroquinolone-resistant Acinetobacter baumannii », *Osong Public Health Res. Perspect.*, vol. 2, n° 3, p. 164‑170, déc. 2011, doi: 10.1016/j.phrp.2011.11.040.
[34] M. D. Adams, E. R. Chan, N. D. Molyneaux, et R. A. Bonomo, « Genomewide Analysis of Divergence of Antibiotic Resistance Determinants in Closely Related Isolates of Acinetobacter baumannii », *Antimicrob. Agents Chemother.*, vol. 54, n° 9, p. 3569‑3577, sept. 2010, doi: 10.1128/AAC.00057-10.
[35] V. Post, P. A. White, et R. M. Hall, « Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant Acinetobacter baumannii », *J. Antimicrob. Chemother.*, vol. 65, n° 6, p. 1162‑1170, juin 2010, doi: 10.1093/jac/dkq095.
[36] P.-E. Fournier *et al.*, « Comparative Genomics of Multidrug Resistance in Acinetobacter baumannii », *PLOS Genet.*, vol. 2, n° 1, p. e7, janv. 2006, doi: 10.1371/journal.pgen.0020007.
[37] R. A. Bonnin, L. Poirel, et P. Nordmann, « AbaR-type transposon structures in Acinetobacter baumannii », *J. Antimicrob. Chemother.*, vol. 67, n° 1, p. 234‑236, janv. 2012, doi: 10.1093/jac/dkr413.
[38] L. Krizova, L. Dijkshoorn, et A. Nemec, « Diversity and Evolution of AbaR Genomic Resistance Islands in Acinetobacter baumannii Strains of European Clone I », *Antimicrob. Agents Chemother.*, vol. 55, n° 7, p. 3201‑3206, janv. 2011, doi: 10.1128/AAC.00221-11.
[39] Y. Pfeifer *et al.*, « Molecular characterization of blaNDM-1 in an Acinetobacter baumannii strain isolated in Germany in 2007 », *J. Antimicrob. Chemother.*, vol. 66, n° 9, p. 1998‑2001, sept. 2011, doi: 10.1093/jac/dkr256.
[40] L. Poirel, R. A. Bonnin, A. Boulanger, J. Schrenzel, M. Kaase, et P. Nordmann, « Tn125-Related Acquisition of blaNDM-Like Genes in Acinetobacter baumannii », *Antimicrob. Agents Chemother.*, vol. 56, n° 2, p. 1087‑1089, janv. 2012, doi: 10.1128/AAC.05620-11.
[41] L. Poirel et P. Nordmann, « Genetic Structures at the Origin of Acquisition and Expression of the Carbapenem-Hydrolyzing Oxacillinase Gene blaOXA-58 in Acinetobacter baumannii », *Antimicrob. Agents Chemother.*, vol. 50, n° 4, p. 1442‑1448, janv. 2006, doi: 10.1128/AAC.50.4.1442-1448.2006.
[42] A. Siroy *et al.*, « Global Comparison of the Membrane Subproteomes between a Multidrug-Resistant Acinetobacter baumannii Strain and a Reference Strain », *J. Proteome Res.*, vol. 5, n° 12, p. 3385‑3398, déc. 2006, doi: 10.1021/pr060372s.
[43] C. Héritier, L. Poirel, T. Lambert, et P. Nordmann, « Contribution of Acquired Carbapenem-Hydrolyzing Oxacillinases to Carbapenem Resistance in Acinetobacter baumannii », *Antimicrob. Agents Chemother.*, vol. 49, n° 8, p. 3198‑3202, août 2005, doi: 10.1128/AAC.49.8.3198-3202.2005.
[44] J.-R. Sun, M.-C. Chan, T.-Y. Chang, W.-Y. Wang, et T.-S. Chiueh, « Overexpression of the adeB Gene in Clinical Isolates of Tigecycline-Nonsusceptible Acinetobacter baumannii without Insertion Mutations in adeRS », *Antimicrob. Agents Chemother.*, vol. 54, n° 11, p. 4934‑4938, nov. 2010, doi: 10.1128/AAC.00414-10.
[45] N. Rosenfeld, C. Bouchier, P. Courvalin, et B. Périchon, « Expression of the Resistance-Nodulation-Cell Division Pump AdeIJK in Acinetobacter baumannii Is Regulated by AdeN, a TetR-Type Regulator », *Antimicrob. Agents Chemother.*, vol. 56, n° 5, p. 2504‑2510, mai 2012, doi: 10.1128/AAC.06422-11.
[46] E.-J. Yoon, P. Courvalin, et C. Grillot-Courvalin, « RND-Type Efflux Pumps in Multidrug-Resistant Clinical Isolates of Acinetobacter baumannii: Major Role for AdeABC Overexpression and AdeRS Mutations », *Antimicrob. Agents Chemother.*, vol. 57, n° 7, p. 2989‑2995, juill. 2013, doi: 10.1128/AAC.02556-12.
[47] C. Mammina *et al.*, « Epidemiology and clonality of carbapenem-resistant Acinetobacter baumannii from an intensive care unit in Palermo, Italy », *BMC Res. Notes*, vol. 5, p. 365, juill. 2012, doi: 10.1186/1756-0500-5-365.

[48] M. Iacono *et al.*, « Whole-Genome Pyrosequencing of an Epidemic Multidrug-Resistant Acinetobacter baumannii Strain Belonging to the European Clone II Group », *Antimicrob. Agents Chemother.*, vol. 52, n° 7, p. 2616‑2625, janv. 2008, doi: 10.1128/AAC.01643-07.
[49] P. Villalón, S. Valdezate, M. J. Medina-Pascual, G. Carrasco, A. Vindel, et J. A. Saez-Nieto, « Epidemiology of the Acinetobacter-derived cephalosporinase, carbapenem-hydrolysing oxacillinase and metallo-β-lactamase genes, and of common insertion sequences, in epidemic clones of Acinetobacter baumannii from Spain », *J. Antimicrob. Chemother.*, vol. 68, n° 3, p. 550‑553, mars 2013, doi: 10.1093/jac/dks448.
[50] P. Bogaerts *et al.*, « Emergence of NDM-1-producing Acinetobacter baumannii in Belgium », *J. Antimicrob. Chemother.*, vol. 67, n° 6, p. 1552‑1553, juin 2012, doi: 10.1093/jac/dks041.
[51] A. Nemec *et al.*, « Emergence of carbapenem resistance in Acinetobacter baumannii in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II », *J. Antimicrob. Chemother.*, vol. 62, n° 3, p. 484‑489, sept. 2008, doi: 10.1093/jac/dkn205.
[52] T. Tada *et al.*, « Dissemination of clonal complex 2 Acinetobacter baumannii strains co-producing carbapenemases and 16S rRNA methylase ArmA in Vietnam », *BMC Infect. Dis.*, vol. 15, oct. 2015, doi: 10.1186/s12879-015-1171-x.
[53] S. Bakour *et al.*, « First report of 16S rRNA methylase ArmA-producing Acinetobacter baumannii and rapid spread of metallo-β-lactamase NDM-1 in Algerian hospitals », *J. Infect. Chemother.*, vol. 20, n° 11, p. 696‑701, nov. 2014, doi: 10.1016/j.jiac.2014.07.010.
[54] M. Yagoubat, A. Ould El-Hadj-Khelil, A. Malki, S. Bakour, A. Touati, et J.-M. Rolain, « Genetic characterisation of carbapenem-resistant Gram-negative bacteria isolated from the University Hospital Mohamed Boudiaf in Ouargla, southern Algeria », *J. Glob. Antimicrob. Resist.*, vol. 8, p. 55‑59, mars 2017, doi: 10.1016/j.jgar.2016.10.008.
[55] R. Zhang *et al.*, « Emergence of NDM-producing non-baumannii Acinetobacter spp. isolated from China », *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 33, n° 5, p. 853‑860, mai 2014, doi: 10.1007/s10096-013-2024-4.
[56] V. A. Ageevets *et al.*, « Emergence of carbapenemase-producing Gram-negative bacteria in Saint Petersburg, Russia », *Int. J. Antimicrob. Agents*, vol. 44, p. 152‑155, mai 2014.
[57] H. Mammeri, L. Poirel, N. Mangeney, et P. Nordmann, « Chromosomal Integration of a Cephalosporinase Gene from Acinetobacter baumannii into Oligella urethralis as a Source of Acquired Resistance to β-Lactams », *Antimicrob. Agents Chemother.*, vol. 47, n° 5, p. 1536‑1542, mai 2003, doi: 10.1128/AAC.47.5.1536-1542.2003.
[58] L.-F. Hu *et al.*, « Increase in the Prevalence of Resistance Determinants to Trimethoprim/Sulfamethoxazole in Clinical Stenotrophomonas maltophilia Isolates in China », *PLoS ONE*, vol. 11, n° 6, juin 2016, doi: 10.1371/journal.pone.0157693.
[59] X. Meng *et al.*, « Antibiotic Susceptibility and molecular screening of Class I Integron in Salmonella isolates recovered from retail raw chicken carcasses in China », mars 2017. http://online.liebertpub.com/doi/pdfplus/10.1089/mdr.2015.0359 (consulté le 16 août 2017).