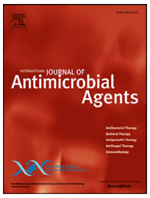
International Journal of Antimicrobial Agents 53 (2019) 530–532

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag



Letter to the Editor

Occurrence of carbapenemase-producing Enterobacteriaceae in Togo, West Africa

Sir,

Carbapenems are considered a last-resort therapy for infections caused by multidrug-resistant (MDR) Gram-negative bacteria. Resistance to carbapenems emerged during the last decade mostly due to dissemination of carbapenemase-producing Enterobacteriaceae (CPE). In Enterobacteriaceae, carbapenemases belong to: (i) Ambler class A enzymes, mostly *Klebsiella pneumoniae* carbapenemase (KPC); (ii) metallo-β-lactamases of NDM-, VIM- and IMP-types, and (iii) Amber Class D carbapenem-hydrolysing β-lactamases of the OXA-48 family.

The alarming spread of CPE is reported worldwide but data from Africa, particularly West Africa, are scarce [1]. Here, we characterized several carbapenem non-susceptible Enterobacteriaceae isolated in Togo.

From 15 April to 31 August 2016, 152 Enterobacteriaceae were isolated from clinical samples of patients hospitalized in Sylvanus Olympio University Hospital, Lome, Togo. Eight (5.2%) isolates had reduced susceptibility to carbapenems according to EUCAST breakpoints from 2018 (i.e. minimum inhibitory concentration [MIC] to imipenem or meropenem $>$ 2mg/L, or MIC to ertapenem $>$ 0.5 mg/L) (Table 1). Most of these isolates (6/8) were from urine; two *Enterobacter* spp. were from pus. Results of antimicrobial susceptibility testing performed by disk diffusion, as recommended by EUCAST, are summarized in Table 1. All isolates except *E. cloacae* subsp. *cloacae* 768 were resistant to aminoglycosides, fluoroquinolones and sulfamethoxazole-trimethoprim. Most of the isolates (7/8) were susceptible to amikacin, and fosfomycin remained systematically susceptible.

Carbapenemase activity was detected using the Carba NP test in four isolates. Whole genome sequencing was performed by Pasteur International Bioresources Networking (Paris, France). Total raw data sequences of each isolate (accession numbers NRQN01000000, NRQJ01000000, NSIX01000000, NSIY01000000, NRJI01000000, NRJJ01000000, NSJG01000000, NSJH01000000) were subjected to the ResFinder-2.1 Server (http://www.genomicepidemiology.org/) for identification of acquired antimicrobial resistance genes, to the MLST 1.8 server (http://www.genomicepidemiology.org/) to determine the sequence-type (ST), and to PlasmidFinder to identify plasmid replicases. For all isolates with a positive Carba NP test, carbapenemase-encoding genes ($bla_{\text{NDM-5}}$ or $bla_{\text{OXA-181}}$) were detected. Genomic features are summarized in Table 1.

For all CPE, transconjugants were obtained by the matting-out assay in azide-resistant *E. coli* J53. In all cases, the carbapenemase gene-carrying plasmids belonged to IncX3 type (Table 1).

In OXA-181 producers (n=3), the $bla_{\text{OXA-181}}$ was introduced onto an IncX3 plasmid from a ColE2-type plasmid, and linked to the quinolone resistance gene *qnr*S1, as described in the first report of an OXA-181-producing *E. coli* isolate from China (Accession number KP400525) [2]. This plasmid was also detected in an OXA-181-producing *E. coli* isolate from Burkina Faso [3], a border country of Togo. Although the two OXA-181-producing *E. cloacae* subsp. *cloacae* of ST456 were found to be clonally-related (6 single-nucleotide polymorphisms [SNPs] along the whole genome), indicating patient-to-patient transmission, another level of carbapenemase dissemination through plasmid transfer was indicated because the same $bla_{\text{OXA-181}}$ gene-carrying IncX3 plasmid was found in the OXA-181-producing *K. pneumoniae* isolate. Both OXA-181-producing *E. cloacae* subsp. *cloacae* isolates were also found to be resistant to colistin with MIC at 4 μg/mL determined by broth microdilution (Table 1). None of the acquired colistin resistance genes (*mcr-1* to *-8*) were identified and transformation experiments failed to obtain any colistin-resistant clone. Of note, *E. cloacae* subsp. *cloacae* corresponds to the cluster XI of *Enterobacter cloacae* complex according to the Hoffmann classification [4]. The majority of strains belonging to this cluster XI harbour intrinsic heteroresistance to colistin [5]. Although *E. cloacae* subsp. *cloacae* (= Hoffmann cluster XI) isolates were reported to be rare among carbapenemase-producing *Enterobacter cloacae* complex isolates [6, 7], this subspecies, which is prone to colistin resistance [5], may be prevalent in Togo. Indeed, one of these two colistin-resistant OXA-181-producing *E. cloacae* subsp. *cloacae* was recovered from an outpatient, indicating a possible dissemination in the community in Togo.

The NDM-5 producer was *Enterobacter xiangfangensis,* a newly described species among *Enterobacter cloacae* complex. This species was recently reported to be the most predominant species among carbapenemase-producing *Enterobacter cloacae* complex [6, 7]. The IncX3 $bla_{\text{NDM-5}}$-carrying plasmid was found to be identical to the previously described plasmid pNDM_MGR194 (Accession number KF220657) that has disseminated in India [8] and China [9]. As the same putative link with China was observed with the $bla_{\text{NDM-5}}$- and the $bla_{\text{OXA-181}}$-carrying plasmids, this may indicate the possible dissemination from China to West Africa of CPE on top of the close sino-Africa relationships that started at the beginning of the 21st century.

Decreased susceptibility to carbapenems was caused by expression of CTX-M-15 associated with decreased permeability of the outer membrane (mutated OmpB [D182E, A619T]) for ST131 *E. coli* 751, and overexpression of the chromosome-encoded ACT-16 associated with decreased permeability of the outer membrane for the *Enterobacter hormaechei* subsp. *oharae* 768 (Table 1).

Finally, three isolates belong to known international, multidrug-resistant, high-risk clones: *E. coli* ST131, *K. pneumoniae* clonal

https://doi.org/10.1016/j.ijantimicag.2018.11.019

Table 1

Characteristics of patients and samples, and antimicrobial susceptibilities and genomic characteristics of carbapenem-resistant Enterobacteriaceae isolates.

Species	*Enterobacter xiangfangensis*	*Enterobacter xiangfangensis*	Enterobacter cloacae subsp. cloacae	*Enterobacter cloacae subsp. cloacae*	*Enterobacter cloacae* subsp. *cloacae*	*Enterobacter hormaechei* subsp. *oharae*	*Escherichia coli*	*Klebsiella pneumoniae*
Isolate name	616294	1179	749	716754	898	768	751	787
Sample	Pus[$]	Pus	Urine	Urine	Urine	Urine	Urine	Urine
Patient characteristics								
age	30	41	5	64	37	35	47	62
sex	M	M	M	M	M	F	M	M
hosp. unit	General Surgery	Outpatient	Paediatrics	Outpatient	Outpatient	Gynaecology	Visceral Surgery	Cardiology
Carba NP Test	+	-	+	+	-	-	-	+
Carbapenemase	NDM-5	None	OXA-181	OXA-181	None	None	None	OXA-181
Antimicrobial susceptibility testing: categorisation (MIC)[a]								
Amoxicillin	R	R	R	R	R	R	R	R
Amoxicillin + CLA	R	R	R	R	R	R	R	R
Ticarcillin	R	R	R	R	R	R	R	R
Ticarcillin + CLA	R	R	R	R	R	R	R	R
Piperacillin	R	R	R	R	R	R	R	R
Piperacillin + TZB	R	S	R	R	R	I	R	R
Temocillin	R	S	R	R	R	S	R	R
Cefotaxime	R	R	R	R	R	R	R	R
Ceftazidime	R	R	R	R	R	R	R	R
Cefepime	R	R	R	R	R	s	R	R
Aztreonam	R	R	R	R	R	R	R	R
Meropenem[b]	I (4)	S (0.12)	I (3)	I (3)	S (0.03)	S (0.03)	S (0.03)	I (3)
Imipenem[b]	S (2)	S (0.19)	S (0.25)	S (0.25)	S (0.12)	S (0.12)	S (0.12)	S (0.25)
Ertapenem[b]	R (> 32)	R (3)	R (> 32)	R (> 32)	R (1.5)	I (1)	R (> 32)	R (> 32)
Gentamicin	R	R	R	R	R	S	R	R
Amikacin	S	S	S	S	R	S	S	S
Tobramycin	R	R	R	R	R	S	R	R
Colistin	S (0.5)	S (0.5)	R (4)	4 (R)	S (0.5)	S (0.25)	S (0.5)	S (0.5)
SXT	R	R	R	R	R	S	R	R
Levofloxacine	R	R	R	R	R	S	R	R
Fosfomycin	S	S	S	S	S	S	S	S
Sequence type	ST182	ST66[*]	ST456	ST456	ST156	ST266	ST131[*]	ST11[*]
Acquired antimicrobial resistance genes								
β-lactams	$\underline{\textit{bla}}_{\text{NDM-5}}$, $\textit{bla}_{\text{CTX-M-15}}$, $\textit{bla}_{\text{TEM-1B}}$, $\textit{bla}_{\text{ACT-16}}$	$\textit{bla}_{\text{OXA-1}}$, $\textit{bla}_{\text{ACT-7}}$	$\underline{\textit{bla}}_{\text{OXA-181}}$, $\textit{bla}_{\text{CTX-M-15}}$, $\textit{bla}_{\text{CMH-3}}$	$\underline{\textit{bla}}_{\text{OXA-181}}$, $\textit{bla}_{\text{CTX-M-15}}$, $\textit{bla}_{\text{CMH-3}}$	$\textit{bla}_{\text{CTX-M-15}}$, $\textit{bla}_{\text{OXA-1}}$, $\textit{bla}_{\text{OXA-9}}$, $\textit{bla}_{\text{CMH-3}}$, $\textit{bla}_{\text{TEM-1A}}$	$\textit{bla}_{\text{ACT-16}}$	$\textit{bla}_{\text{CTX-M-15}}$	$\underline{\textit{bla}}_{\text{OXA-181}}$, $\textit{bla}_{\text{CTX-M-15}}$, $\textit{bla}_{\text{OXA-1}}$
Aminoglycosides	*aac(6′)Ib-cr, strA, strB, aac(3)-Iia*	*aac(6′)Ib-cr*	*aac(6′)Ib-cr, strA, strB, aac(3)-Iia*	*aac(6′)Ib-cr, strA, strB, aac(3)-Iia*	*aadA1, strA, strB, aacA4, aac(3)Iia*	-	*aac(3)Iia, aac(6′)Ib-cr, aadA5*	*aac(6′)Ib-cr, aac(3)-Iia, aadA2, aph(3′)Ia*
Quinolones	*qnrB1, aac(6′)Ib-cr*	*qnrB1, aac(6′)Ib-cr*	$\underline{\textit{qnrS1}}$, *aac(6′)Ib-cr*	$\underline{\textit{qnrS1}}$, *aac(6′)Ib-cr*	-	-	*aac(6′)Ib-cr,*	$\underline{\textit{qnrS1}}$, *oqxA, oqxB, aac(6′)Ib-cr*
Other antibiotics	*sul2, fosA, tet(A), tet(B), dfrA14, catB3*	*fosA, dfrA14, catA2, catB3*	*sul2, fosA, tet(A), dfrA1*	*sul2, fosA, tet(A), dfrA1*	-	*fosA*	*mph(A), sul1, dfrA17, catB3*	*mph(A), sul1, fosA, dfrA12, catB3*
Carbapenemase gene-carrying plasmid								
Type	IncX3	-	IncX3, ColKP3	IncX3, ColKP3	-	-	-	IncX3, ColKP3
Size	50 kb	-	50 kb	50 kb	-	-	-	50 kb
Other plasmids	IncFIB, IncFII	IncFII, IncFIB	ColRNAI	ColRNAI	IncFII(Yp), IncHI2, ColpVC, IncFIA(HI1), IncR	-	Col156, IncFIA, IncFIB(AP001918), IncFII, ColpVC	IncFII, IncFIB, IncR

[$] Necrosis of skin flap on the knee

[a] SXT, sulfamethoxazole + trimethoprim; CLA, clavulanate; TZB, tazobactam; R, resistant; I, intermediate; S, susceptible

[b] MIC were determined by E-test

[c] MIC were determined by broth microdilution

[*] International multidrug-resistant high-risk clone

Underlined genes are localised on the carbapenemase gene-carrying plasmid complex 11 and *E. xiangfangensis* (already known as *E. cloacae* complex of ST66), increasing the risk of occurrence. Further investigations are needed to confirm the dissemination of these high-risk clones in Togo and to boost the urgent implementation of an active national surveillance program.

Declarations

Funding

This work was supported by the Assistance Publique – Hôpitaux de Paris, by a grant from the Université Paris Sud (EA 7361), and by the LabEx LERMIT supported by a grant from the French National Research Agency (ANR-10-LABX-33)

Competing Interests

None to declare.

Ethical Approval

Not applicable.

References

[1] Manenzhe RI, Zar HJ, Nicol MP, Kaba M. The spread of carbapenemase-producing bacteria in Africa: a systematic review. J Antimicrob Chemother 2015;70:23–40.

[2] Liu Y, Feng Y, Wu W, Xie Y, Wang X, Zhang X, et al. First Report of OXA-181-producing *Escherichia coli* in China and characterization of the isolate using whole-genome sequencing. Antimicrob Agents Chemother 2015;59:5022–5.

[3] Ouedraogo AS, Compain F, Sanou M, Aberkane S, Bouzinbi N, Hide M, et al. First description of IncX3 plasmids carrying $\mathit{bla}_{\text{OXA-181}}$ in *Escherichia coli* clinical isolates in Burkina Faso. Antimicrob Agents Chemother 2016;60:3240–2.

[4] Hoffmann H, Roggenkamp A. Population genetics of the nomenspecies *Enterobacter cloacae*. Appl Environ Microbiol 2003;69:5306–18.

[5] Guerin F, Isnard C, Sinel C, Morand P, Dhalluin A, Cattoir V, et al. Cluster-dependent colistin hetero-resistance in *Enterobacter cloacae* complex. J Antimicrob Chemother 2016;71:3058–61.

[6] Beyrouthy R, Barets M, Marion E, Dananche C, Dauwalder O, Robin F, et al. Novel *Enterobacter* lineage as leading cause of nosocomial outbreak involving carbapenemase-producing strains. Emerg Infect Dis 2018;24:1505–15.

[7] Peirano G, Matsumura Y, Adams MD, Bradford P, Motyl M, Chen L, et al. Genomic epidemiology of global carbapenemase-producing *Enterobacter* spp., 2008-2014. Emerg Infect Dis 2018;24:1010–19.

[8] Krishnaraju M, Kamatchi C, Jha AK, Devasena N, Vennila R, Sumathi G, et al. Complete sequencing of an IncX3 plasmid carrying $\mathit{bla}_{\text{NDM-5}}$ allele reveals an early stage in the dissemination of the $\mathit{bla}_{\text{NDM}}$ gene. Indian J Med Microbiol 2015;33:30–8.

[9] Zhang F, Xie L, Wang X, Han L, Guo X, Ni Y, et al. Further spread of $\mathit{bla}_{\text{NDM-5}}$ in *Enterobacteriaceae via* IncX3 plasmids in Shanghai. China. Front Microbiol 2016;7:424.

Sika Dossim
Laboratoire de Microbiologie, Centre Hospitalier Universitaire Sylvanus Olympio de Lome
Departement des Sciences Pharmaceutiques, Faculte des Sciences de la Sante de l'Universite de Lome
Centre de Biologie moleculaire et d'immunologie de la Faculte des Sciences de la Sante de l'Universite de Lome

Rémy A. Bonnin
Unite EA7361 "Structure, dynamic, function and expression of broad spectrum β-lactamases", Universite Paris Sud, Universite Paris Saclay, LabEx Lermit, Faculty of Medicine, Le Kremlin-Bicêtre, France
Evolution and Ecology of Resistance to Antibiotics Unit, Institut Pasteur – APHP -Universite Paris Sud, Paris, France
Bacteriology-Hygiene unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France
Associated French National Reference Center for Antibiotic Resistance: Carbapenemase-producing Enterobacteriaceae, Le Kremlin-Bicêtre, France

Mounerou Salou
Laboratoire de Microbiologie, Centre Hospitalier Universitaire Sylvanus Olympio de Lome
Departement des Sciences Pharmaceutiques, Faculte des Sciences de la Sante de l'Universite de Lome
Centre de Biologie moleculaire et d'immunologie de la Faculte des Sciences de la Sante de l'Universite de Lome

Kpatcha Tanga, Virginie Godonou
Laboratoire de Microbiologie, Centre Hospitalier Universitaire Sylvanus Olympio de Lome

Anoumou Yaotsè Dagnra, Mireille Prince David
Laboratoire de Microbiologie, Centre Hospitalier Universitaire Sylvanus Olympio de Lome
Centre de Biologie moleculaire et d'immunologie de la Faculte des Sciences de la Sante de l'Universite de Lome
Departement des Sciences fondamentales et biologiques, Faculte des Sciences de la Sante de l'Universite de Lome

Thierry Naas, Laurent Dortet*
Unite EA7361 "Structure, dynamic, function and expression of broad spectrum β-lactamases", Universite Paris Sud, Universite Paris Saclay, LabEx Lermit, Faculty of Medicine, Le Kremlin-Bicêtre, France
Evolution and Ecology of Resistance to Antibiotics Unit, Institut Pasteur – APHP -Universite Paris Sud, Paris, France
Bacteriology-Hygiene unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France
Associated French National Reference Center for Antibiotic Resistance: Carbapenemase-producing Enterobacteriaceae, Le Kremlin-Bicêtre, France

*Corresponding Author: Dr. Laurent Dortet, Service de Bactériologie-Hygiène, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France,
Tel: +33 1 45 21 36 24 / Fax: +33 1 45 21 63 40.
E-mail addresses: laurent.dortet@aphp.fr, laurent.dortet@bct.aphp.fr (L. Dortet)

Received 26 September 2018
Accepted 15 November 2018