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OXA-244-Producing *Escherichia coli* Isolates, a Challenge for Clinical Microbiology Laboratories

Yannick Hoyos-Mallecot,[a,b,c] Thierry Naas,[a,b,c,d] Rémy A. Bonnin,[a,b,c,d] Rafael Patino,[d] Philippe Glaser,[d] Nicolas Fortineau,[a,b,c,d] Laurent Dortet[a,b,c,d]

Bacteriology-Hygiene Unit, Assistance Publique-Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France[a]; EA7361, Université Paris Sud, Université Paris Saclay, LabEx Lermit, Faculty of Medicine, Le Kremlin-Bicêtre, France[b]; Associated French National Reference Center for Antibiotic Resistance, Le Kremlin-Bicêtre, France[c]; Evolution and Ecology of Resistance to Antibiotics Unit, Institut Pasteur-Assistance Publique-Hôpitaux de Paris-Université Paris Sud, Paris, France[d]

ABSTRACT OXA-244 is a single-point-mutant derivative of OXA-48 displaying reduced carbapenemase activity. Here, we report the microbiological features of seven OXA-244-producing *Escherichia coli* isolates. Only one isolate grew on ChromID Carba Smart medium (bioMérieux), but six of the seven isolates grew on ChromID extended-spectrum-β-lactamase (ESBL) medium (bioMérieux), as they coproduced an ESBL and/or a plasmid-encoded cephalosporinase. The production of a carbapenemase was detected in 57.1%, 71.4%, 71.4%, and 100% of the *E. coli* isolates using the Carba NP test, the Rapidec Carba NP test (bioMérieux), a matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) hydrolysis assay (Bruker), and the OXA-48 K-SeT assay (Coris BioConcept), respectively. Our results indicate that OXA-244-producing *E. coli* isolates are difficult to detect, which may lead to their silent spread.

KEYWORDS OXA-244, detection, screening, tests, carbapenemase activity, OXA-48-like

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Address correspondence to Thierry Naas, thierry.naas@aphp.fr.

The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) is becoming a major clinical issue (1). In this context, expert committees have set up guidelines to prevent the spread of CPEs (2). Thus, it is recommended to screen individuals at risk of being colonized, especially patients who were hospitalized previously in countries with high CPE prevalence, in order to isolate colonized patients as soon as possible, to implement contact precautions, and to strongly recommend cohorting with dedicated nursing staff. Screening procedures involving plating of rectal swabs on screening medium to detect all carbapenem-resistant isolates, with high sensitivity and sufficient specificity to rule out CPE carriage, should be performed as soon as possible (2, 3).

OXA-244, a single-point-mutant derivative of OXA-48 with reduced carbapenemase activity, was initially observed in a Spanish *Klebsiella pneumoniae* isolate (4). Subsequently, it was fortuitously found in a CTX-M-producing *Escherichia coli* isolate in Germany (5), in four *Enterobacter aerogenes* isolates in Russia (6), in *E. coli* VAL from France (7), and in an *E. coli* isolate from Southeast Asia that coproduced CTX-M-14 (8). While the $bla_{OXA-244}$ gene was chromosomally encoded in the *E. coli* VAL isolate, it was plasmid located in *K. pneumoniae* and *E. aerogenes* (4, 6, 7).

The aim of this study was to evaluate different screening approaches and confirmatory tests useful for detecting OXA-244-producing *E. coli* (OXA-244-*Ec*) isolates. In addition, we investigated the genetic relatedness of seven OXA-244-*Ec* isolates from different geographical origins that were received at the French National Reference Center (F-NRC) for CPEs.

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In August 2015, a 44-year-old Egyptian man was admitted to the Bicêtre Hospital (Le Kremlin-Bicêtre, France) for an episode of erysipelas of his right leg, which was treated with intravenous amoxicillin. After 2 weeks, the patient was discharged with a favorable outcome. Because this patient (as a repatriated patient) was considered to be at risk for multidrug-resistant (MDR) bacterial carriage according to French CPE guidelines, rectal swabs were plated on ChromID Carba Smart medium (bioMérieux, La Balme-les-Grottes, France), a selective chromogenic biplate for screening for CPE, and ChromID ESBL medium (bioMérieux), a chromogenic plate for screening for extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae*. The ChromID Carba Smart medium remained sterile, while *E. coli* 85H4 grew on the ChromID ESBL plate (Table 1). Antimicrobial susceptibilities, as determined by the disk diffusion technique on Mueller-Hinton agar (Bio-Rad, Marnes-La-Coquette, France) and interpreted according to the EUCAST breakpoints, as updated in 2016 (http://www.eucast.org/clinical_breakpoints/), revealed that the *E. coli* 85H4 isolate was highly resistant to temocillin (absence of an inhibition zone) and displayed reduced susceptibility to ertapenem (zone diameter of 19 mm), thus requiring confirmatory testing for carbapenemase production, as recommended by EUCAST (9). The results of the Rapidec Carba NP test (bioMérieux) were positive for *E. coli* 85H4, although no colony grew on the ChromID Carba Smart plate (10). In-house PCR sequencing, as described previously (11), revealed the presence of a gene coding for OXA-244, a R214G OXA-48 variant (Table 1) (4). Since OXA-244-*Ec* 85H4 also produced an ESBL, the ChromID ESBL medium was used to screen 34 contact patients for the Egyptian patient; for 4 patients, positive *E. coli* cultures on ChromID ESBL medium were obtained. Antibiotic susceptibility testing and in-house PCR testing of five independent colonies revealed that none carried the $bla_{\text{OXA-244}}$ gene (data not shown).

Phenotypic characterization of OXA-244-*Ec*. The ability to reliably detect OXA-244-*Ec* using ChromID Carba Smart and ChromID ESBL plates and to confirm the presence of a carbapenemase was further investigated with *E. coli* VAL (7) and five other OXA-244-*Ec* isolates referred to the F-NRC for CPEs. The susceptibilities to different antibiotics of the seven OXA-244-*Ec* isolates are shown in Table 2. For all OXA-244-*Ec* isolates, 100 μl of a 0.5 McFarland solution was plated on ChromID ESBL and ChromID Carba Smart media. Only one of the seven isolates did not grow on the ChromID ESBL medium (Table 1); that isolate was susceptible to cephalosporins, which explained the absence of growth on the ChromID ESBL medium (Table 2). In contrast, only one isolate grew on the OXA-48 side of the ChromID Carba Smart plate (Table 1) and none grew on the Carba side; that strain displayed the highest MICs for temocillin (>1,024 mg/liter) and for moxalactam, a β-lactam classically used for testing impermeability problems (Table 1) (12). All of the OXA-244-*Ec* isolates exhibited only slightly decreased susceptibility to carbapenems, which explained the absence of growth on the Carba side of the ChromID Carba Smart plate (Table 1). The biochemical confirmation tests used for carbapenemase detection were positive for 57.1% (4/7 isolates), 71.4% (5/7 isolates), 71.4% (5/7 isolates), and 100% (7/7 isolates) of the isolates using the Carba NP test (13), the Rapidec Carba NP test (bioMérieux) (10), the MBT STAR-BL test, a commercial matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based assay (Maldi-Biotyper; Bruker, Illkirch, France), and a lateral flow immunoassay (LFIA) called the OXA-48 K-SeT assay (Coris BioConcept, Gembloux, Belgium) (14) respectively (Table 1).

Molecular detection of resistance genes. Using the commercially available Xpert Carba-R v2 assay, as recommended by the manufacturer (Cepheid, Toulouse, France) (15, 16), $bla_{\text{OXA-48-like}}$ genes were detected in all seven OXA-244-*Ec* isolates. Whole-genome sequencing (WGS) was performed to determine the resistome of these OXA-244-*Ec* isolates using the ResFinder server (http://cge.cbs.dtu.dk/services/ResFinder-2.1) (17) (Table 2). A good correlation between the genetic profile and the phenotypic resistance profile for routinely tested β-lactams, colistin, fosfomycin, phenicol, sulfonamide-trimethoprim, and tetracycline antibiotics was found (Table 2). For amin-

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TABLE 1 Clinical and phenotypic characteristics of the OXA-244-*Ec* isolates

Isolate	ST[a]	Clone[b]	Approximate plasmid size (kb)[c]	Year of isolation	Source of isolation	Origin	MIC (mg/liter) and susceptibility[d]				Inhibition zone (mm) for MOX[e]	Test results[f]					
							IMP	MEM	ETP	TEM		OXA-48 K-SeT	Carba NP	Rapidec Carba NP	MALDI-TOF MS	ChromID ESBL	ChromID Carba Smart[g]
86J1	ST-361	1	160, 110, 70	2015	Rectal	Egypt	0.5 (S)	0.5 (S)	2 (R)	>1,024	7	+	+	+	+	+	+/−
62D3	ST-1722	2	Abs	2014	Urine	Unknown	0.38 (S)	0.38 (S)	1 (I)	128	21	+	+	+	+	+	−/−
69E6	ST-38	3	Abs	2014	Rectal	Unknown	0.25 (S)	0.38 (S)	3 (R)	128	20	+	+/−	+	+	+	−/−
78B5	ST-38	3	Abs	2015	Rectal	Unknown	0.38 (S)	0.5 (S)	3 (R)	256	21	+	+	+	+	+	−/−
VAL (4 isolates)	ST-38	3	120, 60, 10	2013	Urine	France	0.5 (S)	0.75 (S)	2 (R)	96	21	+	−	+/−	−	−	−/−
73G4	ST-3541	4	115	2015	Unknown	Egypt	0.25 (S)	0.19 (S)	0.75 (I)	128	20	+	+	+	+	+	−/−
85H4	ST-3541	4	115	2015	Rectal	Egypt	0.38 (S)	0.25 (S)	2 (R)	384	20	+	+/−	+/−	−	+	−/−

[a]Overview of STs identified by the MLST 1.8 server (19).

[b]Rep-PCR analysis was performed using the DiversiLab technique.

[c]Abs, absent.

[d]Susceptible (S), intermediate (I), and resistant (R) interpretations were according to the 2016 EUCAST guidelines (http://www.eucast.org/clinical_breakpoints/). IMP, imipenem; MEM, meropenem; ETP, ertapenem; TEM, temocillin.

[e]MOX, moxalactam.

[f]+, positive test or culture result; −, negative test or culture result; +/−, equivocal test result.

[g]Bacterial growth was checked on both sides of the biplate (ChromID OXA-48/ChromID Carba).

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TABLE 2 Resistance genes and phenotypic susceptibility of the OXA-244-*Ec* isolates

Isolate	Acquired resistance genes[a]									Observed phenotype[b]									
	β-Lac	AMG	CST	FOS	C	FQ	SUL	TR	TET	AMX	AMC	CTX	AMG[c]	CST	FOS	C	FQ	SXT	TET
86J1	$bla_{OXA-244}$, bla_{TEM-1b}, bla_{CMY-42}	*aph3-1b*, *aph6-1d*, *aadA1*	None	None	None	None	*sul1*, *sul2*	*dfrA1*	*tetB*	R	R	R	S	S	S	S	R	R	R
62D3	$bla_{OXA-244}$, bla_{CMY-2}	None	None	None	None	None	None	None	None	R	R	R	S	S	S	S	S	S	S
69E6	$bla_{OXA-244}$, bla_{TEM-1b}, $bla_{CTX-M-14b}$	*aadA1*	None	None	*catA1*	None	None	*dfrA1*	None	R	R	R	S	S	S	R	S	R	S
78B5	$bla_{OXA-244}$, bla_{TEM-1b}, $bla_{CTX-M-14b}$	*aadA1*	None	None	*catA1*	None	None	*dfrA1*	None	R	R	R	S	S	S	R	S	R	S
VAL (4 isolates)	$bla_{OXA-244}$, bla_{TEM-1b}	*aph3-1b*, *aph6-1d*, *aadA1*	None	None	*catA1*	None	*sul2*	*dfrA1*, *dfrA14*	*tetB*, *tetD*	R	R	S	S	S	S	R	S	R	R
73G4	$bla_{OXA-244}$, bla_{TEM-1b}, $bla_{CTX-M-27}$	*aph3-1b*, *aph6-1d*, *aph3-1a*	None	None	None	None	*sul2*	*dfrA14*	*tetB*	R	R	R	S	S	S	S	S	R	R
85H4	$bla_{OXA-244}$, bla_{TEM-1b}, $bla_{CTX-M-27}$	*aph3-1b*, *aph6-1d*, *aph3-1a*	None	None	None	None	*sul2*	*dfrA14*	*tetB*	R	R	R	S	S	S	S	S	R	R

[a]Overview of resistance genes detected in the isolates by ResFinder (17). β-Lac, β-lactam; AMG, aminoglycoside; CST, colistin; FOS, fosfomycin; C, chloramphenicol; FQ, fluoroquinolone; SUL, sulfonamide; TR, trimethoprim; TET, tetracycline.

[b]Antimicrobial susceptibilities were determined by the disc diffusion technique and interpreted according to the EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints/). S, susceptible; R, resistant; AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; SXT, sulfamethoxazole-trimethoprim.

[c]The aminoglycoside tested were amikacin, gentamicin, tobramycin, and netilmicin.

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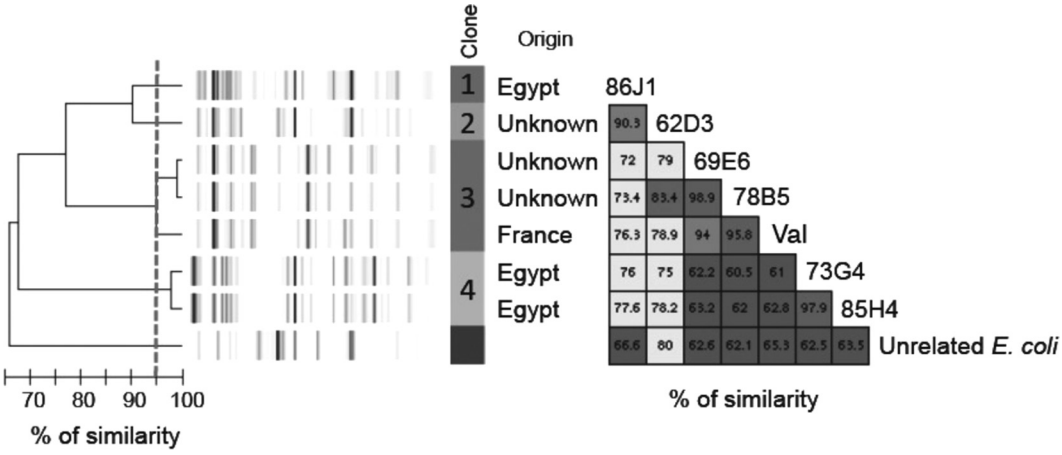

FIG 1 Rep-PCR analysis using the DiversiLab technique, showing a dendrogram and computer-generated image of rep-PCR banding patterns of OXA-244-*Ec* isolates and an *E. coli* isolate of an unrelated strain. As recommended by the manufacturer, a cutoff value of 95% similarity defined a cluster.

oglycosides, only netilmicin, amikacin, tobramycin, and gentamicin were tested. However, different aminoglycoside resistance genes (e.g., *aph3-1a*, *aph3-1b*, *aph6-1d*, and *aadA1*), which confer resistance to other aminoglycosides that were not tested because they were not clinically relevant, were found in some of the isolates. Of note, isolate OXA-244-*Ec* 86J1 was resistant to fluoroquinolones due to substitutions in the quinolone resistance determinant region, as revealed by analysis with the RAST server (rast.nmpdr.org) (18) and comparison with that of *K. pneumoniae* ATCC 13883 (GenBank accession no. DQ673325), i.e., in codons 83 (Ser83-Leu) and 87 (Asp87-Asn) for GyrA and in codons 80 (Ser80-Ile) and 84 (Glu84-Gly) for ParC, which are known to confer fluoroquinolone resistance.

Among the seven isolates, only *E. coli* VAL had no other β-lactam resistance gene besides the $bla_{\text{OXA-244}}$ gene; consequently, that strain was susceptible to cephalosporins (7). For the remaining six isolates, an ESBL gene ($bla_{\text{CTX-M-14}}$ or $bla_{\text{CTX-M-27}}$) or a plasmid-encoded cephalosporinase gene ($bla_{\text{CMY-42}}$ or $bla_{\text{CMY-2}}$) was always associated with the $bla_{\text{OXA-244}}$ gene (Table 2).

Genetic relatedness of OXA-244-*Ec* isolates. The seven OXA-244-*Ec* isolates corresponded to four different clones, as revealed by repetitive element sequence-based PCR (rep-PCR) using the DiversiLab system (bioMérieux), following the manufacturer's recommendations. Two distinct clones were identified among the Egyptian isolates (Fig. 1 and Table 1). Multilocus sequence typing (MLST) results deduced from WGS data using the MLST 1.8 server (https://cge.cbs.dtu.dk/services/MLST) (19) confirmed the rep-PCR results, as each rep-PCR pattern corresponded to a different sequence type (ST), i.e., ST-38, ST-361, ST-1722, or ST-3541 (Table 1).

Genetic environment and support of $bla_{\text{OXA-244}}$ genes. For three strains (78B5, 62D3, and 69E6), no plasmids could be detected after electrophoresis of Kieser-extracted DNA (11) (see Fig. S1 in the supplemental material). Electroporation of the extracted plasmids, as described previously (11), yielded *E. coli* TOP10 transformants for only three strains (86J1, 85H4, and 73G4). However, only ESBL/plasmid-encoded $bla_{\text{AMP-C}}$ genes were found in those transformants. Thus, all of these findings suggest a chromosomal location for the $bla_{\text{OXA-244}}$ gene. PCR mapping of the $bla_{\text{OXA-244}}$ gene flanking sequences showed that all were bracketed by two IS*1R* copies, forming an IS*1R*-made composite transposon named Tn*51098* (Fig. S2A). In all isolates, although they belonged to different rep-PCR patterns or STs, Tn*51098* was inserted into a gene encoding an intrinsic endonuclease from *E. coli*, as described previously (7) (Fig. S2B). Dissemination of *E. coli* isolates harboring a chromosomally located $bla_{\text{OXA-48-like}}$ gene has recently been linked to one ST, namely, ST38 (20, 21). In our study, however, four different STs that have integrated the $bla_{\text{OXA-244}}$ carbapenemase gene into the chro-

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mosome were found, indicating that diffusion could be more related to the mobility of $bla_{OXA-244}$-carrying IS*1R*-made composite transposons (Tn*51098*) than to clonal expansion.

Conclusions. Detection of CPEs remains a challenge for clinical microbiology laboratories (22), especially with OXA-244-*Ec* isolates, since they do not grow on ChromID Carba Smart plates, one of the most used types of medium for the screening of CPEs (7, 23–25). However, as most OXA-244-*Ec* isolates also produced an ESBL, they could grow on ChromID ESBL medium. In the absence of expanded-spectrum hydrolyzing enzymes (such as in *E. coli* VAL), detection of OXA-244-*Ec* strains would rely only on molecular tests directly with rectal swabs (15) and on LFIA tests, such as OXA-48 K-SeT, with cultured bacteria (14). In France, OXA-244-*Ec* strains are still rare, i.e., 0%, 0.3% (two isolates), 0.2% (two isolates), 0.6% (six isolates), and 0.7% (eight isolates) of all OXA-48-like enzymes in 2012, 2013, 2014, 2015, and 2016, respectively, but whether this indicates a real low prevalence or is the result of underdetection is not known.

Accession number(s). The *E. coli* genome sequences of isolates 86J1, 62D3, 69E6, 78B5, 35J9, 73G4, and 85H4, used in this study, were deposited in GenBank under the accession numbers MKGU00000000, MKGY00000000, MKGZ00000000, MKGT00000000, MKGX00000000, MKGV00000000, and MKGW00000000, respectively.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00818-17.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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L.D. holds an international patent for the Carba NP test that was filed on behalf of INSERM Transfert and subsequently licensed to bioMérieux.

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