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Ongoing dissemination of OXA-244 carbapenemase-producing *Escherichia coli* in Switzerland and their detection

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ABSTRACT

OXA-244 is a derivative of OXA-48 showing weaker carbapenemase activity, compromising the detection of corresponding producers in clinical laboratories. Since 2017, the Swiss National Reference Center for Emerging Antibiotic Resistance noticed an increased identification of OXA-244-producing *Escherichia coli* ($n=15$) within the country. Different methods (biochemical and immunoassay tests, screening culture media) were tested for the detection of OXA-244 producers. Whole genome sequencing was used to investigate the genetic relatedness between the isolates and the genetic structures at the origin of the acquisition of the $bla_{OXA-244}$ gene. The mSuperCARBA® medium and the NG-Test CARBA5 assay were found to be suitable tools for detecting all OXA-244-producing isolates. Other selective media did not perform optimally. Among the fifteen strains, five sequence types were identified, with ST38 being predominant. The $bla_{OXA-244}$ gene was located on the chromosome for all isolates. Overall, detection of OXA-244 producers is challenging and specific guidelines must be followed.

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1. Introduction

Resistance or reduced susceptibility to carbapenems in *Enterobacterales* may be driven by different mechanisms, among which the production of carbapenemases represents the main threat. Carbapenem-hydrolyzing class D carbapenemases of the OXA-48 type are predominant in many European countries, including in France, Belgium, The Netherlands, and Switzerland (Zurfluh et al., 2015; Pitout et al., 2020). Although the bla_{OXA-48} encoding gene has been initially identified from *Klebsiella pneumoniae* (Poirel et al., 2004a), it is not only widespread in that species but also in *Enterobacter cloacae* (Peirano et al., 2018) and *Escherichia coli* (Gauthier et al., 2018). As a consequence of location of OXA-48-like gene in *E. coli*, OXA-48 enzymes variants producers are associated with hospital- but also with community-acquired infections (Nordmann et al., 2011), rendering the control of their spread almost impossible.

A series of OXA-48 variants has been reported, the most commonly identified being OXA-181 (Castanheira et al., 2011), OXA-232 (Potron et al., 2013b), OXA-204 (Potron et al., 2013a), OXA-162 (Kasap et al., 2013) and OXA-244 (Oteo et al., 2013; Potron et al., 2016). OXA-244 is a single-point mutant derivative of OXA-48 (Arg214Gly) that possesses a weaker carbapenemase activity as compared to that of OXA-48 (Potron et al., 2016). As a consequence, OXA-244 producers may exhibit low carbapenem MIC values, thus making their detection quite challenging. OXA-244 was initially identified from a *K. pneumoniae* clinical isolate in Spain in 2012 (Oteo et al., 2013). Subsequently, this variant was identified worldwide, including Germany (Valenza et al., 2014; Hans et al., 2019), the Netherlands (Van Hattem et al., 2016), the United Kingdom (Findlay et al., 2017), France (Hoyos-Mallecot et al., 2017), Russia (Fursova et al., 2015) and Colombia (Abril et al., 2019). In 2019, the Robert Koch Institute reported an outbreak of OXA-244-producing *E. coli* in Germany (Hans et al., 2019).

At the Swiss National Reference Center for Emerging Antibiotic Resistance, where all enterobacterial clinical isolates suspected to produce an acquired carbapenemase are sent by all Swiss clinical laboratories (either when a PCR-based detection of carbapenemase genes is performed, when the NG-Carba5 assay leads to a positive result with any type of carbapenemase, or when only a reduced susceptibility to carbapenems was observed), multiple OXA-244-producing *E. coli* isolates were recovered during the 2017-2019 period from different parts of Switzerland. Our aim was to evaluate the reliability of different

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screening media and phenotypical tests for detecting the above OXA-244-producing *E. coli* isolates. The whole genomes of those isolates were also analyzed in order to investigate the genetic relatedness of the strains and their $bla_{OXA-244}$ genetic environment.

2. Materials and methods

2.1. Clinical strains and molecular analysis

A total of fifteen OXA-244 carbapenemase-producing *E. coli* were received during a ca. 2-year period (January 2017-October 2019) (Table 1). Those isolates had been recovered from 15 patients who had been hospitalized in different parts of Switzerland (Aarau, Basel, Bern, Fribourg, Lausanne, Luzern and Sion). They were mostly from urinary tract infections or gut flora (rectal swab). Identification was confirmed using the EnteroPluri-Test® (Liofilchem S.R.L., Roseto degli, Italy). Susceptibility testing was performed by disc diffusion and E-test®, following EUCAST recommendations (EUCAST, 2020). Detection of carbapenemase genes, including the $bla_{OXA-244}$ gene, was first performed by PCR and sequencing of the corresponding amplicons (Microsynth, Balgach, Switzerland), as previously reported (Potron et al., 2016).

2.2. Laboratory detection of OXA-244-producing E. coli

The ability to recover OXA-244-producing *E. coli* strains was investigated using the following screening media: ChromID Carba Smart® plates, ChromID ESBL® plates (bioMérieux, La Balme-les-Grottes, France) and mSuperCARBA® plates (CHROMagar, Paris, France) (Nordmann et al., 2012; Girlich et al., 2013; Garcia-Quintanilla et al., 2018). The ChromID Carba Smart® is a biplate designed to detect all carbapenemase producers, including those producing OXA-48, with an excellent sensitivity (Lee et al., 2019). ChromID Carba Smart plates possess two half parts, one being supplemented with a carbapenem molecule while the other half is supplemented with temocillin. OXA-48-like producers being resistant to temocillin are mostly identified on that half part, while they may not be detected on the side supplemented with a carbapenem in some cases. The mSuperCARBA® medium contains ertapenem and allows efficient detection of all types of carbapenemase producers. The ChromID ESBL® plate contains a cephalosporin and has been developed to select strains resistant to broad-spectrum cephalosporins. We added here this medium for comparison since many OXA-48-like producers coproduce an ESBL. After initial overnight broth culture, different concentrations of each strain were plated on each medium, ranging from 10^8, 10^6, 10^4, 10^2, 10^1 CFU/mL.

Table 1
Clinical characteristics and origins of OXA-244 *E. coli* isolates.

Isolate	Isolation date	Specimen	Origin
N11	2017	Drain	Lausanne
N460	2019	Urine	Lausanne
N511	2019	Rectal swab	Basel
N481	2019	Urine	Luzern
N816	2019	Stool	Lausanne
N861	2019	ENT*	Aarau/Egypt travel
N293	2018	Urine	Bern
N658	2019	Urine	Luzern
N832	2019	Urine	Luzern
N871	2019	Urine	Zürich
N846	2019	Rectal swab	Sion
N717	2019	Rectal swab	Lausanne
N790	2019	Urine	Fribourg
N824	2019	Cervical smear	Luzern
N867	2019	Urine	Luzern

* ENT = Ear-Nose-Throat.

2.3. Rapid diagnostic tests

2.3.1. Carbapenemase activity

The detection of carbapenemase production may rely on the detection of carbapenem hydrolysis. The performances of the Rapidec Carba NP test (bioMérieux) which principle is based on detection of imipenem hydrolysis (Poirel and Nordmann, 2015) and of the β-Carba test (BioRad, Cressier, Switzerland) which principle is based on the use chromogenic carbapenem hydrolysis (Decousser et al., 2017) were tested.

2.3.2. Lateral flow immunoassay

The NG-Test CARBA5 assay (NG Biotech, Guipry, France) is an immunochromatographic test that can detect the most common carbapenemases (Boutal et al., 2018; Hopkins et al., 2018).

2.3.3. Whole genome sequencing (WGS)

Genome comparison of all OXA-244-producing isolates was evaluated by WGS using an Illumina MiSeq benchtop sequencer. Reads from sequencing were assembled using CLC Genomic Workbench 7 (Qiagen, Courtaboeuf, France). Then, the genetic environment of $bla_{OXA-244}$ was analyzed by NCBI blast and SnapGeneViewer (GSL Biotech LLC, Chicago). Multi Locus Sequencing Type (Wirth et al., 2006) were determined from those genomic data, as well as the *E. coli* phylogroup (Clermont et al., 2000).

2.3.4. Genetic environment of $bla_{OXA-244}$ genes

Mating-out assays were performed using OXA-244-producing *E. coli* as donors and *E. coli* J53 (azide resistant) as recipient to identify transmissible vector of those OXA-244 genes. Selection was made using Luria Bertani agar plates supplemented with ampicillin (100 μg/mL) and sodium azide (100 μg/mL).

3. Results and discussion

3.1. Detection of OXA-244-producing E. coli using selective media and rapid diagnostic tests

The growth of the fifteen OXA-244-producing *E. coli* isolates was evaluated using the several screening media (Table 2). Fourteen out of the 15 isolates grew on the mSuperCarba selective medium. By contrast, only a single out of the 15 isolates grew on the side supplemented with temocillin in the ChromID Carba Smart plate, while no isolate grew on the side supplemented with the carbapenem molecule in the ChromID Carba Smart plate. Different detection thresholds were obtained (Table 2), ranging for instance from 10^2 to 10^8 using the mSuperCarba medium. Those different detection thresholds somehow correlated with the variable MICs values of ertapenem for those isolates (Table 3).

On the other hand, eleven isolates grew on the ChromID ESBL plates, which is not designed for selecting carbapenemase producers but rather ESBL producers. This high recovery rate by using the ChromID ESBL medium is related to the high rate of ESBL producers among this collection of OXA-244 producers.

Of note, all isolates remained susceptible to meropenem and imipenem, highlighting the utility of the SuperCarba medium ertapenem as selective agent. Also noteworthy is that fact that all isolates remained susceptible to the ceftazidime/avibactam combination, as expected (Table 3).

The β-Carba test gave positive results only for ten isolates, being those exhibiting the highest MICs of ß-lactams, and especially to broad-spectrum cephalosporins (Table 2). By contrast, all isolates gave negative results when using the Rapidec Carba NP test, even those exhibiting the highest MICs of carbapenems such as isolate N511 (MIC of imipenem at 2 μg/ml). Use of the NG Carba immunoassay test was found to be suitable for the detection of all OXA-244-producing isolates,

Table 2
Detection threshold of screening media (SuperCarba[a], CarbaSmart[b], ESBL[c]) and results of Rapid test (Biochemical (Rapidec[d] and B-CarbaTest[e]) and Immunoassay (NG-Test[f])) of OXA-244 isolates.

Isolate	Screening media (Detection threshold; CFU/mL)			Rapid test (positive or negative)		
	mSuperCarba[a]	CarbaSmart[b]	ESBL[c]	Rapidec[d]	B-CarbaTest[e]	NG-Test[f]
N11	10^2	/	10^2	-	+	+
N460	10^2	/	10^2	-	+	+
N511	10^4	/	10^2	-	+	+
N481	10^2	/	10^1	-	+	+
N816	10^2	10^8	10^1	-	+	+
N861	10^2	/	10^2	-	+	+
N293	10^6	/	10^2	-	-	+
N658	10^2	/	10^1	-	+	+
N832	10^6	/	10^2	-	+	+
N871	10^2	/	10^2	-	+	+
N846	10^2	/	10^1	-	+	+
N717	10^8	/	/	-	-	+
N790	/	/	/	-	-	+
N824	10^8	/	/	-	-	+
N867	10^6	/	10^2	-	-	+

SuperCarba[a], mSuperCARBA® (CHROMagar, Paris, France); **CarbaSmart**[b], ChromID Carba Smart® (BioMerieux, Geneva).
ESBL[c], ChromID ESBL® plates (bioMérieux, Geneva) ; **Rapidec**[d], Rapidec Carba NP test (bioMérieux, Geneva).
B-CarbaTest[e], β-Carba test (BioRad, Cressier) ; **NG-Test**[f] , NG-Test CARBA5 assay (NG Biotech, Guipry, France).
/ = No growth ; - = Negative ; + = Positive.

the OXA-244 enzyme being well-recognized as an OXA-48-like resistance determinant, as expected.

3.2. Whole genome sequence analysis

WGS was performed either to determine the resistome of those isolates, but also to evaluate their clonal relationship. Among the fifteen isolates, eleven distinct resistomes were observed. Those resistomes encompassed a large series of resistance determinants, including those encoding resistance to ß-lactams (Table 4), tetracylines, aminoglycosides, chloramphenicol, macrolides, trimethoprim and sulfonamides (data not shown). Interestingly, ESBL genes were mostly $bla_{CTX\text{-}M\text{-}14}$ or its derivative $bla_{CTX\text{-}M\text{-}27}$, rather than $bla_{CTX\text{-}M\text{-}15}$ which is the most frequently identified CTX-M encoding gene in clinical strains worldwide.

Genotyping allowed the identification of five different ST (ST38, ST69, ST963, ST58, ST10) with a majority of the isolates belonging to ST38. Most of the isolates (13/15) belonged to phylogroup D, associated to extra-intestinal infections (Table 4).

3.3. Genetic support of the $bla_{OXA\text{-}244}$ gene

Mating-out assays performed with all 15 isolates as donors resulted unsuccessful. This finding suggested a chromosomal location of the $bla_{OXA\text{-}244}$ gene as previously shown (Potron et al., 2016). Analysis of WGS sequences showed that the $bla_{OXA\text{-}244}$ was actually surrounded by insertion sequences (IS*1R* and IS*1999*), forming a transposon being itself inserted in the chromosome, and truncating either the HDH intrinsic endonuclease encoding gene alone (ST38, ST58, ST963 and ST10) (Genbank CP041538.1), and for isolates belonging to ST69 truncating both the HDH endonuclease encoding gene and a membrane protein encoding gene (Genbank KT444705.1). Such insertion into these chromosomal genes had been already reported either for $bla_{OXA\text{-}244}$ (Potron et al., 2016) or for $bla_{OXA\text{-}48}$ (Turton et al., 2016) in *E. coli*. Such common feature suggests that the $bla_{OXA\text{-}244}$ and $bla_{OXA\text{-}48}$ genes are derivatives of each other, originally corresponding to the same structure captured from a common progenitor, known to be *Shewanella* spp., and were not separately mobilized (Poirel et al., 2004b, Tacão et al., 2018).

4. Conclusion

Most of the OXA-244-producing isolates remained susceptible to carbapenems here. Therefore, the detection of those carbapenemase producers based on a the unique criterium of resistance to carbapenems will fail. Among the culture media tested for screening carbapenemase producers was the SuperCarba. Since most of the OXA-244 producers could not grow on the ChromID Carba plate may be explained not only by the low MICs of carbapenems observed, but also by a reduced resistance levels to temocillin compared to other OXA-48-like producers.

We showed here that accurate detection of OXA-244 by using the immunoassay NG-Test CARBA5 assay is valid, as shown by using another immunoassay the OXA-48 K-set (Dortet et al., 2016a). Immunological detection is here of interest for two main features of OXA-244 (i) its weak carbapenemase activity, and (ii) the chromosomal location of its gene very likely as a single copy leading to low level of its production.

The majority of currently-available diagnostic tests are specific and sensitive with regard to OXA-48 and OXA-181, which are the most commonly identified carbapenem-hydrolyzing class D ß-lactamases (CHDL) in Enterobacterales at least in Europe. However they cannot as well be applied to the detection of OXA-244. This might explain a possible under-detection, examplified by the low prevalence of OXA-244-producing isolates (0.7%) observed in France in 2016 (Hoyos-Mallecot et al., 2017). These difficulties have been previously underscored by

Table 3
Plasmid-mediated ESBL and AmpCs and MICs (mg/L) of β-lactams, temocillin and colistin for OXA-244-producing *E. coli* isolates.

Isolate		ESBL	AmpC	MEM[a]	ETP[b]	IPM[c]	CTX[d]	CAZ[e]	CAZ/AVI[f]	TEM[g]	CST[h]
N11	$bla_{OXA\text{-}244}$	+	-	0.25	1.5	0.19	>32	3	0.125	48	<0.12
N460	$bla_{OXA\text{-}244}$	+	-	0.38	1.5	0.19	>32	3	0.125	48	<0.12
N511	$bla_{OXA\text{-}244}$	+	-	1.5	0.38	2	>32	1.5	0.064	48	<0.12
N481	$bla_{OXA\text{-}244}$	+	-	0.75	0.38	1	>23	1.5	0.19	48	<0.12
N816	$bla_{OXA\text{-}244}$	+	-	2	>32	0.75	>32	4	0.75	96	<0.12
N861	$bla_{OXA\text{-}244}$	+	-	0.25	0.75	0.25	>32	2	0.25	96	<0.12
N293	$bla_{OXA\text{-}244}$	+	-	0.094	0.25	0.19	>32	2	0.094	12	<0.2
N658	$bla_{OXA\text{-}244}$	+	-	0.125	0.38	0.25	>32	2	0.094	24	<0.12
N832	$bla_{OXA\text{-}244}$	+	-	0.125	0.38	0.5	>32	2	0.125	32	<0.12
N871	$bla_{OXA\text{-}244}$	+	+	1	>32	0.5	>32	16	0.19	48	<0.12
N846	$bla_{OXA\text{-}244}$	-	+	0.25	0.5	0.25	>32	16	0.125	32	<0.12
N717	$bla_{OXA\text{-}244}$	-	-	0.047	0.19	0.19	0.12	0.19	0.032	16	<0.12
N790	$bla_{OXA\text{-}244}$	-	-	0.047	0.12	0.25	0.094	0.125	0.064	12	<0.12
N824	$bla_{OXA\text{-}244}$	+	-	0.094	0.25	0.38	0.12	0.25	0.064	48	<0.12
N867	$bla_{OXA\text{-}244}$	+	-	0.094	0.19	0.25	16	1.5	0.064	24	<0.12

MEM[a], meropenem; ETP[b], ertapenem; IPM[c], imipenem; CTX[d], cefotaxime ; CAZ[e], ceftazidime; CAZ/AVI[f], ceftazidime/avibactam; TEM[g], temocillin ; CST[h], colistin.
EUCAST breakpoints (2020) : ETP : S ≤ 0.5, R > 0.5 ; IMP : S ≤ 2, R > 4 ; MEM : S ≤ 2, R > 8 ; CAZ : S ≤ 1 , R > 4 ; CAZ/AVI : S ≤ 8, R > 8 ; TEM : S ≤ 8, R > 8 (2019) ; CST : S ≤ 2, R > 2 (mg/L).

Table 4
Genetic characteristics of OXA-244 *Escherichia coli* isolates.

Isolate	*B*-Lac[a]	Phylogroup	ST[b] type
N11	$bla_{OXA-244}$ $bla_{CTX-M-14}$ bla_{TEM-1B}	D	38
N460	$bla_{OXA-244}$ $bla_{CTX-M-14}$ bla_{TEM-1B}	D	38
N511	$bla_{OXA-244}$ $bla_{CTX-M-14}$ bla_{TEM-1B}	D	38
N481	$bla_{OXA-244}$ $bla_{CTX-M-14}$ bla_{TEM-1B}	D	38
N816	$bla_{OXA-244}$ $bla_{CTX-M-14}$ bla_{TEM-1B}	D	38
N861	$bla_{OXA-244}$ $bla_{CTX-M-14}$ bla_{TEM-1B}	D	38
N293	$bla_{OXA-244}$ $bla_{CTX-M-27}$	D	38
N658	$bla_{OXA-244}$ $bla_{CTX-M-27}$	D	38
N832	$bla_{OXA-244}$ $bla_{CTX-M-27}$	D	38
N871	$bla_{OXA-244}$ bla_{CMY-2} bla_{TEM-1B}	D	38
N846	$bla_{OXA-244}$ bla_{CMY-2}	D	963
N717	$bla_{OXA-244}$	D	69
N790	$bla_{OXA-244}$	D	69
N824	$bla_{OXA-244}$ bla_{TEM-33}	B1	58
N867	$bla_{OXA-244}$ $bla_{CTX-M-15}$	A	10

B-Lac[a] , extended-spectrum beta-lactamase; ST[b], sequence type.

Dortet et al. (2016b) showing that molecular tests were among the most efficient methods for detection of $bla_{OXA-244}$-positive isolates.

The bla_{OXA-48} carbapenemase gene is the most widespread CHDL encoding gene in *Enterobacterales,* including in *E. coli* (Giani et al., 2012). It is mainly identified on a single ca. 63-kb IncL/M-type plasmid (recently renamed IncL plasmids) that conjugates at very high frequency, favoring its dissemination within different enterobacterial species (Potron et al., 2014). Nevertheless, the bla_{OXA-48} gene has also been identified in a chromosomal position in *E. coli*, this integration being mediated by IS*1R* insertion sequences (Beyrouthy et al., 2014; Turton et al., 2016). The $bla_{OXA-244}$ gene has so far always been identified as well in a chromosomal position in *E. coli* (Oteo et al., 2013; Hoyos-Mallecot et al., 2017), but was identified on a plasmid in *Klebsiella* spp. (Oteo et al., 2013; Fursova et al., 2015). It is possible that selection of OXA-244 isolates we analyzed belong mostly to the ST38 background as identified in other countries (Findlay et al., 2017; Hoyos-Mallecot et al., 2017; Abril et al., 2019; Hans et al., 2019) suggesting the wide spread of a successful clone a derivative of an OXA-48 clone.

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