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Letter to the Editor

Emergence of OXA-484, an OXA-48–type beta-lactamase, in Switzerland

Editor: Stefania Stefani

Since their first identification in 2004, OXA-48–type carbapenemases have become one of the most common types of carbapenemases and have disseminated worldwide [1]. To date, 32 different OXA-48–type variants have been identified in Enterobacterales (http://bldb.eu/), with the OXA-48, OXA-181, and OXA-232 variants being the most prevalent globally [1]. Variant OXA-484 differs from the OXA-48–type variants OXA-181, -232, and -924 by a single amino acid at position 214, which is located within the β5-β6 loop [2]. The gene encoding OXA-484 was initially identified in an unidentified plasmid in an ST14 *Klebsiella pneumoniae* isolate in the UK in 2015 [3] and, more recently, it was described in ST410 *Escherichia coli* isolates from Germany and Switzerland, encoded on ~51 kb IncX3 plasmids [2–4].

Five Enterobacterales isolates, which were submitted to the Swiss National Reference Center for Emerging Antibiotic Resistance (NARA) between October 2020 and November 2021 for the investigation of carbapenem resistance, were identified as harbouring the $bla_{\text{OXA-484}}$ gene. Briefly, positivity for an OXA-48–like enzyme was firstly assessed using the NG-Test CARBA 5 test (NG Biotech), and subsequent polymerase chain reaction (PCR) analyses and sequencing identified $bla_{\text{OXA-484}}$. These comprised four *E. coli* isolates and one *Klebsiella aerogenes*. The isolates were obtained from screening swabs from four patients in four different cantons of Switzerland, with one patient harbouring both an OXA-484–positive *E. coli* and *K. aerogenes*. Susceptibility testing, performed by broth microdilution, showed that isolates were resistant to ertapenem but remained susceptible to both imipenem and meropenem (Table 1). Isolates were inoculated onto mSuperCARBA™ agar (CHROMagar™) and yielded growth. Illumina whole-genome sequencing (WGS) and analyses were performed on all five isolates, as previously described [5], and identified that the four *E. coli* strains represented three different sequence types (STs), with the ST410 high-risk clone [6] being represented by two isolates; the *K. aerogenes* isolate was ST396. Two *E. coli* isolates additionally harboured a plasmid-mediated $bla_{\text{CMY-42}}$ gene (EC_2 and EC_4) and one possessed the ESBL-encoding gene, $bla_{\text{CTX-M-15}}$ (EC_1). The OXA-48–type variants, OXA-48, OXA-181, OXA-232, and OXA-484, were cloned into the pCR-Blunt II-TOPO vector (Invitrogen) and transformed into *E. coli* Top10. Susceptibility testing of recombinant strains showed that OXA-484 exhibited the same phenotype as OXA-232, with increased minimum inhibitory concentrations (MICs) to piperacillin but reduced MICs to the carbapenems and temocillin (Table 1). This correlates with previously published data that identified the S214G substitution in OXA-232 (changing OXA-232 to OXA-484) as resulting in reduced hydrolysis of imipenem and temocillin, with a reported imipenem kcat/Km of 20 ± 6.9 mM-1.s-1 [7]. Conjugation experiments performed using *E. coli* J53 as a recipient, followed by confirmatory PCR analyses and amplicon sequencing, identified that an IncX3 plasmid was present in the OXA-484–positive transconjugants from four of five strains, with the plasmid in the remaining transconjugant (EC_3) being of unknown replicon type. The *E. coli* transconjugants remained susceptible to carbapenems but exhibited resistance to piperacillin, piperacillin-tazobactam, and temocillin, as is observed with most OXA-48–type enzymes [1,3]. Subsequently, one representative isolate harbouring the IncX3 plasmid (KA_1), and isolate EC_3, with the plasmid of unknown replicon type, were selected for Nanopore sequencing. Hybrid assemblies were performed using Unicycler, as previously described [5], resulting in complete plasmid sequences for both isolates. The plasmids were designated pOXA-484_IncX (KA_1) and pOXA-484_IncF (EC_3), and sequences were deposited in GenBank (Accession no's. OP594534 and OP594535). Mapping of short read data using CLC Genomics Workbench revealed that the four IncX3-type plasmids were highly similar to the 51,480 bp representative plasmid in KA_1, and to a previously published plasmid, pOXA-484_EC-JS316 [2], exhibiting >99% coverage and identity to both. These ~51 kb plasmids are highly similar to the IncX3 plasmids responsible for the dissemination of $bla_{\text{OXA-181}}$ in Enterobacterales, all of which also harbour the *qnrS1* gene and a truncated ColKP3 replicon (likely a remnant from the previous plasmid location, the 7.6 kb OXA-181 plasmid pKP3-A) [8,9]. Plasmid pOXA-484_IncF was 165,017 bp; harboured three replicons, FIA, FII (pRSB107), and FIB (AP001918); and possessed genes encoding two type II toxin-antitoxin systems, *pemIK* and *ccdAB*. It also harboured the resistance genes *ermB, aadA2, mphA, dfrA12*, and *sul1*, in addition to $bla_{\text{OXA-484}}$ and *qnrS1*, that were also identified in pOXA-484_IncX. The ~13 kb region of both plasmids (pOXA-484_IncX and pOXA-484_IncF) containing $bla_{\text{OXA-484}}$ and *qnrS1* were identical to each other as well as the previously described pOXA-484–JS316 [2], and were flanked by IS26 elements, suggesting that this may have been transposed from an IncX3-type plasmid into pOXA-484_IncF. Conjugation frequencies of the OXA-484 plasmids into *E. coli* J53 ranged from 10^{-6}–10^{-7} for the *E. coli* isolates but were significantly lower at 10^{-9} for the *K. aerogenes* isolate. For comparison purposes, conjugation experiments were also performed using a clinical *E. coli* isolate harbouring the highly transmissible and well characterised plasmid, pOXA-48a [10], and conjugation frequencies were found to be 10^{-4}, illustrating that the OXA-484 plasmids in this study exhibited lower transmissibility.

This study describes the emergence of OXA-484 in Switzerland on the highly successful IncX3 plasmids [8] and, additionally, on a novel multi-replicon IncF-type plasmid. Position 214 in OXA-48–type enzymes has been reported as being important for beta-

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Table 1
Genotypic and phenotypic characteristics of the five OXA-484 isolates, their J53 transconjugants, and *Escherichia coli* Top10 harbouring pTOPO plasmids with bla_{OXA-48}, $bla_{OXA-181}$, $bla_{OXA-232}$, and $bla_{OXA-484}$

Isolate	Species	ST	Resistance Genes	Replicons	ETP	IPM	MEM	PIP	PTZ	TEM	FOX	CAZ	ATM
KA_1	*K. aerogenes*	396	$bla_{OXA-484}$, *qnrS1*, *fosA*	X3, ColKP3[a]	1	1	0.125	>256	128	64	256	0.25	≤0.06
EC_1	*E. coli*	10	$bla_{OXA-484}$, $bla_{CTX-M-15}$, *qnrS1*, *tetA*	X3, FII (pCoo), I1, ColKP3[a]	0.5	0.25	≤0.06	>256	128	64	4	16	16
EC_2	*E. coli*	410	$bla_{OXA-484}$, bla_{TEM-1}, bla_{CMY-42}, *qnrS1, sul2, tetB, strA, strB, aac(3)-lld*	X3, Y, ColKP3[a], FIA, FIB(AP001918), FII(pAMA1167-NDM-5), I, Q1	2	0.5	0.125	>256	>256	>256	>256	>256	128
EC_3	*E. coli*	1722	$bla_{OXA-484}$, bla_{TEM-1}, *catA1*, *ermB*, *aadA2, tetB, sul1, dfrA12, qnrS1, mphA*	FIA, FII (pRSB107), FIB (AP001918), ColKP3[a]	1	0.25	≤0.06	>256	128	64	4	0.25	0.125
EC_4	*E. coli*	410	$bla_{OXA-484}$, bla_{CMY-42}, *ermB*, *aadA5*, *tetB, sul2, sul1, dfrA17, qnrS1, ermB, mphA, strA, strB*	X3, Y, ColKP3[a], FIA, FIB (AP001918), FII (pAMA1167-NDM-5), I, Q1	2	1	0.25	>256	>256	256	>256	256	128
J53	*E. coli*	NA	NA	NA	≤0.06	0.125	≤0.06	1	1	4	4	0.125	0.125
KA_1Tc	*E. coli*	NA	$bla_{OXA-484}$, *qnrS1*	X3, ColKP3[a]	0.125	0.25	≤0.06	128	64	64	4	0.25	0.125
EC_1Tc	*E. coli*	NA	$bla_{OXA-484}$, *qnrS1*	X3, ColKP3[a]	0.125	0.25	≤0.06	256	64	64	4	0.25	0.125
EC_2Tc	*E. coli*	NA	$bla_{OXA-484}$, *qnrS1*	X3, ColKP3[a]	0.125	0.25	≤0.06	256	64	64	4	0.25	0.125
EC_3Tc	*E. coli*	NA	$bla_{OXA-484}$, *qnrS1*, *emrB*, *aadA2*, *mphA*, *dfrA12*, *sul1*	FIA, FII (pRSB107), FIB (AP001918), ColKP3[a]	0.25	0.25	≤0.06	256	64	64	4	0.25	0.125
EC_4Tc	*E. coli*	NA	$bla_{OXA-484}$, *qnrS1*	X3, ColKP3[a]	0.125	0.25	≤0.06	256	64	64	4	0.25	0.125
Top10	*E. coli*	NA	NA	NA	≤0.06	0.125	≤0.06	2	2	8	4	0.25	0.125
pTOPO OXA-48	*E. coli*	NA	NA	NA	0.25	0.5	0.125	64	64	256	8	0.5	0.125
pTOPO OXA-181	*E. coli*	NA	NA	NA	0.25	0.5	0.125	64	64	256	8	0.5	0.125
pTOPO OXA-232	*E. coli*	NA	NA	NA	0.125	0.25	≤0.06	128	128	64	8	0.5	0.125
pTOPO OXA-484	*E. coli*	NA	NA	NA	0.125	0.25	≤0.06	128	128	64	8	0.5	0.125

ETP, ertapenem; IPM, imipenem; MEM, meropenem; PIP, piperacillin; PTZ, piperacillin-tazobactam; TEM, temocillin; FOX, cefoxitin; CAZ, ceftazidime; ATM, aztreonam. Tc, transconjugant.
[a] Partial gene.

lactamase activity and the R214G amino acid change in OXA-484 (relative to OXA-48) that hinders the hydrolysis of both carbapenems and temocillin [7]. This is evidenced by the low MICs exhibited by the five OXA- 484–positive isolates and the *E. coli* recombinant strains. This exemplifies the problems faced in the detection of some OXA-48–type variants.

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Ethical approval

Not required

Competing interests

None declared

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