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Letter to the Editor

Emergence of OXA-204 carbapenemase in *Enterobacter cloacae*

Sir,

Carbapenemase-producing Enterobacteriaceae have been reported worldwide in human clinical settings, with OXA-48 being the most widely distributed carbapenemase in Mediterranean countries. Since its first report in a *Klebsiella pneumoniae* isolate in 2001 in Turkey, the bla_{OXA-48} gene proved to have largely disseminated in South Europe, the Middle East and North Africa, thanks both to its location on an epidemic IncL plasmid and the spread of successful OXA-48-producing *K. pneumoniae* lineages. Of note, the bla_{OXA-48}-carrying IncL plasmid did not remain restricted to *K. pneumoniae* but has also been regularly found in *Escherichia coli* and *Enterobacter cloacae*. In parallel, 14 $bla_{OXA-48-like}$ variants have been described so far, such as $bla_{OXA-181}$ and $bla_{OXA-204}$, that are mostly located on other plasmid scaffolds.

In Tunisia, OXA-48-producing *K. pneumoniae* have now become endemically distributed. Tunisia is the first country where the $bla_{OXA-204}$ variant has been identified in Enterobacterales , mostly carried on an IncA/C plasmid [1]. Recent studies in France have demonstrated that $bla_{OXA-204}$ has also successfully spread to both *K. pneumoniae and E. coli*. Here we report the first description of the $bla_{OXA-204}$ gene in *E. cloacae* worldwide.

Between 2014 and 2016, in the 629-bed Sahloul University Hospital (Sousse, Tunisia), 14 *E. cloacae* isolates originating from different wards have been identified as carbapenemase-producers (Table 1). This has been regarded with particular attention since carbapenems are an important option in the treatment of infections caused by *Enterobacter* spp. because of their intrinsic resistance to aminopenicillins and first- and second-generation cephalosporins owing to constitutive production of the chromosomal AmpC β-lactamase. PCR analysis and sequencing revealed the presence of OXA-48 ($n=4$) and OXA-204 ($n=10$) in these 14 isolates. All but one isolate co-produced another β-lactamase enzyme (e.g. CTX-M-15, DHA-1, CMY-4), either alone or in combination (Table 1). Moreover, one isolate (#19990) also produced the VIM-1 enzyme, thus showing the capacity of these *E. cloacae* isolates to cumulate partially overlapping resistance mechanisms.

Minimum inhibitory concentrations (MICs) to carbapenems (Table 1) showed that 11/14 (78.6%) of the isolates presented a non-susceptible phenotype to ertapenem, whilst all remained susceptible to meropenem and imipenem according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. However, all isolates presented meropenem MICs above the EUCAST screening cut-off concentration of 0.125 mg/L, whilst imipenem MICs were below the EUCAST screening cut-off concentration of 1 mg/L for 10/14 isolates. Such clinically susceptible phenotypes have already been described for OXA-48-like-producing Enterobacterales [2] as well as for VIM-1-producing *E. cloacae* [3]. Antimicrobial resistance to non-β-lactam antibiotics as determined by disk diffusion showed that all isolates were resistant to kanamycin and tobramycin, 13/14 to gentamicin and 12/14 to fluoroquinolones. In contrast, all isolates remained susceptible to amikacin and apramycin. MICs showed that all isolates also remained susceptible to colistin as determined by broth microdilution. One isolate (strain 5131) presented reduced susceptibility to tigecycline with an MIC of 1.5 mg/L (Table 1), whilst non-susceptible phenotypes to fosfomycin were observed, most probably due to the presence of the chromosomal *fosA* gene.

S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) followed by Southern blotting using digoxigenin (DIG)-labelled probes proved that the bla_{OXA-48} gene was systematically carried by an IncL plasmid whose size was approximately 60 kb. Multilocus sequence typing (MLST) showed that OXA-48 enzymes were produced by four different sequence types (STs) of *E. cloacae*, including by the high-risk ST114 clone. This suggests multiple transfer of the OXA-48/IncL plasmid, which most probably directly originated from *K. pneumoniae*. In contrast, the prototypic IncA/C plasmid bearing the $bla_{OXA-204}$ gene, along with the bla_{CMY-4} gene, was found in one ST418 isolate only. In all other isolates, the $bla_{OXA-204}$ gene was preceded by an IS*Ecp1* insertion sequence and carried by a 48-kb plasmid that remained untypeable according to the PCR-based replicon typing scheme commercialised by Diatheva (Fano, Italy). Interestingly, seven isolates belonged to the ST182 clone, which first appeared in 2014 and sporadically emerged until 2016. This infrequent clone has already been reported as an NDM-1 carrier in Mexico and China, suggesting that it may be particularly prone to carry carbapenem resistance determinants [4,5]. Whether this clone will successfully disseminate will have to be monitored in the future.

This study is the first description of the $bla_{OXA-204}$ gene in *E. cloacae*. This is a further step in the dissemination of carbapenemase resistance genes in Tunisia following the dissemination of OXA-48- and NDM-1-producing *K. pneumoniae* and the additional emergence of colistin resistance [6–8]. This step has also occurred in a country where OXA-204-producing *K. pneumoniae* have been reported at first and have now become prevalent. These data strongly underline that urgent attention should be paid to the prudent use of antibiotics in clinical settings in Tunisia in order to avoid the emergence of totally drug-resistant isolates.

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Table 1
Characteristics of the 14 OXA-like-producing *Enterobacter cloacae* isolates.

Strain	Isolation date	Ward	Sequence type (ST)	bla_{OXA} gene variant	Additional β-lactam resistance gene(s)	MIC (mg/L)[a]						Plasmid carrying bla_{OXA}-like gene	
						ETP	MEM	IPM	TGC	COL	FOS	Inc type	Size (kb)
5131	16.02.2015	ICU	ST78	48	bla_{DHA-1}	2	0.75	2	1.5	<0.25	48	IncL	60
19990	06.07.2015	Orthopaedics	ST136	48	bla_{VIM-1}	0.5	0.25	0.5	0.38	<0.25	24	IncL	60
27386	15.09.2015	Urology	ST171	48	$bla_{CTX-M-15}$	2	0.75	1	0.5	<0.25	48	IncL	60
36354	13.12.2016	Orthopaedics	ST114	48	$bla_{CTX-M-15} + bla_{DHA-1}$	0.5	0.25	1	0.38	<0.25	4	IncL	60
24882	18.08.2014	Urology	ST182	204	$bla_{CTX-M-15}$	1	0.5	0.75	0.75	<0.25	16	NT	48
4144	06.02.2015	ICU	ST66	204	$bla_{CTX-M-15}$	0.38	0.19	0.75	0.38	<0.25	4	NT	48
11698	18.04.2016	Cardiology	ST418	204	$bla_{CMY-4} + bla_{DHA-1}$	2	0.75	1	1	<0.25	4	IncA/C	200
14711	16.05.2016	Urology	ST182	204	$bla_{CTX-M-15}$	1	0.38	0.75	0.5	<0.25	32	NT	48
17794	16.06.2016	Nephrology	ST182	204	$bla_{CTX-M-15}$	2	0.5	0.75	0.5	<0.25	16	NT	48
18357	21.06.2016	Nephrology	ST182	204	$bla_{CTX-M-15}$	1	0.38	0.5	0.5	<0.25	12	NT	48
19423	01.07.2016	Nephrology	ST182	204	$bla_{CTX-M-15}$	1	0.38	0.5	0.38	<0.25	12	NT	48
22248	30.07.2016	Nephrology	ST182	204	$bla_{CTX-M-15}$	1	0.5	0.5	0.5	<0.25	32	NT	48
22495	01.08.2016	Urology	ST121	204	None	3	0.38	0.5	0.5	<0.25	16	NT	48
30205	18.10.2016	Nephrology	ST182	204	$bla_{CTX-M-15}$	1.5	0.38	0.5	0.38	<0.25	24	NT	48

MLST, multilocus sequence typing; MIC, minimum inhibitory concentration; ETP, ertapenem; MEM, meropenem; IPM, imipenem; TGC, tigecycline; COL, colistin; FOS, fosfomycin; ICU, intensive care unit; NT, not typeable.
[a] MIC breakpoints for susceptible/resistant were as follows: ETP, ≤0.5/>1; MEM, ≤2/>8; IPM, ≤2/>8; TGC, ≤1/>2; COL, ≤2/>2; and FOS, ≤32/>32.

Competing interests

None declared.

Ethical approval

Not required.

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