Journal of Global Antimicrobial Resistance 12 (2018) 113–114

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Letter to the Editor

First report of an *Escherichia coli* from Lebanon carrying an OXA-181 carbapenemase resistance determinant

Sir,

The class D carbapenemase OXA-48 was first detected in 2001 from a carbapenem-resistant *Klebsiella pneumoniae* isolate in Istanbul, Turkey [1]. The variant OXA-181, which differs from OXA-48 by four amino acids, was isolated later in India in 2007 from *Enterobacter cloacae* and *K. pneumoniae*. Since then, OXA-181 has been reported in Bangladesh, India, South Africa, Canada, France and New Zealand [2,3]. Here we report the first case of OXA-181-producing *Escherichia coli* identified in Lebanon.

On 26 June 2016, *E. coli* strain EC_AUH_19 was recovered from an 80-year-old male patient admitted to the intensive care unit of the American University Hospital in Beirut (Lebanon). The antimicrobial susceptibility profile showed resistance to cefotaxime and ceftazidime and intermediate resistance to ertapenem. On the other hand, the isolate remained susceptible to amikacin, gentamicin, colistin, tigecycline, meropenem and imipenem (Table 1). Carbapenemase production was confirmed by Carba NP Kit (bioMérieux, La Balme-les-Grottes, France) performed according to the manufacturer's instructions. PCR screening for the bla_{KPC}, bla_{VIM}, bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes was positive for $bla_{OXA\text{-}48\text{-}like}$. The PCR product was sequenced, confirming the result to be $bla_{OXA\text{-}181}$. Multilocus sequence typing (MLST) according to the Pasteur scheme was performed on EC_AUH_19, however it was unsuccessful and the strain was non-typeable. Plasmid typing using a PBRT Kit (Diatheva, Fano, Italy) was performed and the strain was positive for IncFII and IncX3 plasmids. Rifampicin-resistant *E. coli* strain A15 was used as a recipient in a conjugation experiment conducted in mixed broth culture using ampicillin (50 mg/L) and rifampicin (150 mg/L) for selection. Carba NP and PCR confirmed transfer of the OXA-181-encoding plasmid to the recipient. Plasmid analysis was performed by S1 nucleases approach for EC_AUH_19 and its transconjugant, followed by hybridisation with a digoxigenin-labelled $bla_{OXA\text{-}48\text{-}like}$ probe. The analysis confirmed transfer of a plasmid (pSTIB) of ca. 50 kb that hybridised strongly with the $bla_{OXA\text{-}48\text{-}like}$ probe (data not shown). Moreover, the plasmid was typed using the PBRT Kit (Diatheva) and was positive for IncX3, confirming the presence of $bla_{OXA\text{-}181}$ on an IncX3 plasmid. Genomic DNA of EC_AUH_19 and plasmid extraction of the transconjugant was carried out, and sequencing was performed using an Illumina MiSeq platform (Illumina Inc., San Diego, CA). Reads assembly, sequence gap filling and plasmid sequence analysis were performed as described previously [4].

Whole-genome sequencing for EC_AUH_19 was performed to further investigate the potential pathogenicity of the strain. The Pasteur scheme for in silico MLST analysis failed to identify the sequence type owing to missing alleles (three alleles), however the Achtman scheme was successful and resulted in ST940. Moreover, the strain belonged to phylogenetic group D that, along with group B2, was found to be more virulent than those strains belonging to groups A and B1. PlasmidFinder identified five Inc group plasmids including IncFII, IncI1, IncX3, IncFIB and ColKP3. Furthermore, the strain harboured β-lactamase genes including $bla_{OXA\text{-}1}$, $bla_{CMY\text{-}42}$, $bla_{OXA\text{-}181}$ and $bla_{TEM\text{-}1B}$ as well as resistance genes against other antibiotic classes such as *qnrS1*, *mph*(A), *tet*(B), *aadA1* and *dfrA1*.

Plasmid sequence analysis from transconjugant plasmid sequencing confirmed a plasmid designated by pSTIB, which was 51 479 bp in size, highly resembling a previously described plasmid pOXA181 (**KP400525.1**) with 100% coverage and 99% sequence similarity. pSTIB is an IncX3 plasmid that harbours, in addition to $bla_{OXA\text{-}181}$, the *qnrS1* gene that confers low-level resistance to fluoroquinolones (Supplementary Fig. S1). The plasmid backbone is conserved similar to other plasmids belonging to the same family. It consists of the replication initiation site (IncX3-*repB*), the conjugal transfer system (*virB1–virB11*) and the plasmid maintenance system (TaxA, B and C). Moreover, the genetic environment surrounding the $bla_{OXA\text{-}181}$ gene is exactly the same as pOXA181 as described by Skalova et al. [5]. The plasmid sequence was annotated and deposited in GenBank under accession no. **MG570092**.

To our knowledge, this is the first report of an *E. coli* producing OXA-181 in Lebanon. Although dissemination of $bla_{OXA\text{-}48\text{-}like}$ genes is not rare in the Middle East region, the geopolitical

Table 1
Antimicrobial susceptibility profile of *Escherichia coli* strain EC_AUH_19 and the *E. coli* A15_pSTIB transconjugant harbouring the plasmid encoding OXA-181.

E. coli isolate	MIC (mg/L)											
	CTX	CAZ	FEP	IPM	MEM	ETP	GEN	AMK	SXT	CIP	COL	TGC
EC_AUH_19	4	32	0.03	1	0.12	1	0.125	0.03	0.5	0.5	2	0.12
A15_pSTIB	≤0.063	≤0.12	0.03	≤0.12	0.12	0.125	≤0.25	≤0.5	0.5	0.5	≤0.25	0.25
A15	≤0.063	≤0.12	0.03	≤0.12	0.12	≤0.03	≤0.25	≤0.5	≤0.03	≤0.06	≤0.25	0.12

MIC, minimum inhibitory concentration; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; ETP, ertapenem; GEN, gentamicin; AMK, amikacin; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; COL, colistin; TGC, tigecycline.

https://doi.org/10.1016/j.jgar.2018.01.002

circumstances established in the last few years have facilitated the spread of carbapenem resistance genes via stable, self-conjugative plasmids such as pSTIB. These results, in accordance with previous reports, confirm the important role of IncX3 plasmids in the spread of OXA-181 carbapenemase.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2018.01.002.

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Received 8 December 2017

Available online 31 January 2018