First report of carbapenemase OXA-181-producing Serratia marcescens

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Journal of Global Antimicrobial Resistance 26 (2021) 205–206

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

First report of carbapenemase OXA-181-producing *Serratia marcescens*

Editor: Professor A Tsakris

Sir,

Among the carbapenemases, OXA-48-like enzymes (Ambler class D) have disseminated worldwide or even become endemic in some areas, such as OXA-48 in Tunisia and the whole Mediterranean basin [1]. OXA-181, first described in 2007, has emerged as the second most frequent OXA-48-like enzyme after OXA-48. It is commonly acknowledged that OXA-48-like enzymes originate from *Shewanella* spp., but OXA-48 and OXA-181 followed different evolutionary paths, with $bla_{\text{OXA-48}}$ genes usually associated with Tn*1999* and IncL plasmids, and $bla_{\text{OXA-181}}$ mostly associated with Tn*2013* and IncX3 plasmids [1]. These enzymes/genes were mostly reported in *Klebsiella pneumoniae* and *Escherichia coli*, but also in other Enterobacterales, notably OXA-48, but not OXA-181, in a few *Serratia marcescens* isolates [2]. Long considered a harmless saprophyte, *S. marcescens* is now recognised as an important pathogen presenting intrinsic resistance (narrow-spectrum penicillins, amoxicillin/clavulanic acid, cephalosporins, macrolides, tetracyclines and colistin) and having a propensity to survive in clinical environments [3,4].

Between 2017 and 2019, four temocillin-resistant isolates were identified as *S. marcescens* by VITEK® (bioMérieux, Marcy-l'Étoile, France) at Sahloul Hospital in Sousse, Tunisia. They were mostly isolated from young patients (three of four) from the cardiology and orthopaedic wards (Table 1). Using a specific PCR, the presence of OXA-48-like enzymes was confirmed in the four isolates [5], which presented reduced susceptibility to imipenem, ertapenem and meropenem, with minimum inhibitory concentrations (MICs) of 0.5, 0.25 and 0.125 mg/L, respectively. All four isolates were submitted to whole-genome sequencing (WGS) using a NovaSeq instrument (Illumina Inc., San Diego, CA, USA) (BioProject **PRJNA702027**). Resistance genes and replicon content were inferred using ABRicate v.1.0.1 (https://github.com/tseemann/abricate) on reads assembled using Shovill v.1.0.4. WGS analysis revealed that isolates 53643 and 53646 were OXA-48-producers, while isolates 53644 and 53645 were OXA-181-producers (Table 1). Isolates 53644 and 53646 were further sequenced using MinION technology (Oxford Nanopore Technologies, Oxford, UK), and assembly both of Illumina short reads and Nanopore long reads was performed using Unicycler [6]. Hybrid analysis of isolate 53644 showed that $bla_{\text{OXA-181}}$ was located together with the *qnrS1* gene on an IncX3/ColKP3 plasmid of 51 475 bp. This plasmid showed 100% homology over 30 361 bp with *E. coli* plasmid pABC381-OXA-181 (GenBank **MK412919**) described in the United Arab Emirates [7]. In agreement with the absence of identification of any known replicon (especially IncL) in the Illumina data, hybrid analysis of isolate 53646 revealed the presence of a unique contig of 5 191 506 bp, thus demonstrating the atypical chromosomal location of the $bla_{\text{OXA-48}}$ gene.

To elucidate the phylogenomic distribution of these four isolates in the global OXA-48-like-producing *S. marcescens* epidemiology, all publicly available genome assemblies of OXA-48-producing *S. marcescens* were retrieved from the NCBI database and analysed (Supplementary Table S1). A total of 15 isolates were found, which all carried $bla_{\text{OXA-48}}$ and presented an IncL replicon. Two isolates originated from patients in Madrid, Spain [2], one from a human sample in Lebanon, and twelve from environmental swabs collected in a hospital in Pakistan. Assemblies were de novo annotated with Prokka v.1.14.6 and pangenome analysis was performed with the Roary pipeline v.3.13.0 using a Protein BLAST identity of 80% and a core definition of 90%. A maximum likelihood tree was constructed from the core gene alignment produced by Roary using RAxML v.8.2.12 with default parameters. The corresponding phylogenetic tree based on the matrix presence/absence of a gene was visualised using iTOL v.5.5.1 (http://itol.embl.de/itol.cgi) (Supplementary Fig. S1). Pairwise single nucleotide polymorphism (SNP) distances were calculated from core genome alignments generated using snp-dists (https://github.com/tseemann/snp-dists). In all, isolates strongly clustered by country, however the paucity of WGS data of carbapenemase-producing *S. marcescens* isolates prevents any further interpretation from this phylogenetic tree. Among the Tunisian isolates, the two OXA-48-positive isolates were identical (no SNP difference) and the two OXA-181-positive isolates differed by only 1 SNP, while there was around 95 SNPs difference between the two groups.

Up to now, OXA-48-like enzymes in clinical settings have been principally detected in epidemic lineages of *E. coli* and *K. pneumoniae*, and more rarely in other Enterobacterales. However, a significant circulation of carbapenemase-producing *S. marcescens* clones was recently reported in a Spanish healthcare institution, similarly to our report from Tunisia. The two OXA-48-producing isolates were collected 2 years apart and their close genetic relatedness suggests long-term persistence of the same clone within the hospital. Of note, *S. marcescens* is capable of colonising, even silently and over long periods of time, different surfaces (such as sink traps) or liquids (such as chlorhexidine) [3,4]. In contrast, the two OXA-181-producing isolates were collected within a week, most likely indicating nosocomial transmission between two different wards. These two situations strongly reinforce the need for stringent monitoring to avoid larger outbreaks. Interestingly, three of the four patients were children, in line with the description of several *S. marcescens* outbreaks in paediatric hospitals. Finally, this is the first description of an OXA-181 enzyme produced by *S. marcescens*, an association that has most probably been favoured by the efficient spread of $bla_{\text{OXA-181}}$-carrying IncX3 plasmids. Preventing the emergence and transmission of carbapenemase-producing *S. marcescens* will be a challenge in the future because of the persistent charac-

https://doi.org/10.1016/j.jgar.2021.06.004

Table 1
Characteristics of the OXA-48- and OXA-181-producing *Serratia marcescens* isolates

Isolate	Sampling date	Sex	Age (years)	Pathology	Ward	Treatment	Carbapenemase gene	Carbapenemase-carrying plasmid
53643	12/10/2017	M	0.33	Cyanogenic heart disease	Cardiology	Imipenem, amikacin	bla_{OXA-48}	–
53644	02/12/2018	M	57	Ischaemia	Cardiology	Imipenem, amoxicillin/clavulanic acid, gentamicin, ciprofloxacin	$bla_{OXA-181}$	IncX3
53645	09/12/2018	M	11	Osteomyelitis	Orthopaedic	Cefotaxime, cefixime, sulfonamide/trimethoprim	$bla_{OXA-181}$	IncX3
53646	29/04/2019	M	9	Osteomyelitis	Orthopaedic	Cefotaxime, gentamicin, ciprofloxacin, fosfomycin	bla_{OXA-48}	–

M, male.

ter of this bacterial species and the selective pressure due to carbapenem use in hospitals.

Funding

This work was supported by internal funding of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES).

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.06.004.

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Revised 4 May 2021