Whole-Genome Sequence of a *bla*_{OXA-48}-Harboring *Raoultella ornithinolytica* Clinical Isolate from Lebanon

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We analyzed the whole-genome sequence of a *bla*_{OXA-48}-harboring *Raoultella ornithinolytica* clinical isolate from a patient in Lebanon. The size of the *Raoultella ornithinolytica* CMUL058 genome was 5,622,862 bp, with a G+C content of 55.7%. We deciphered all the molecular mechanisms of antibiotic resistance, and we compared our genome to other available *R. ornithinolytica* genomes in GenBank. The resistome consisted of 9 antibiotic resistance genes, including a plasmidic *bla*_{OXA-48} gene whose genetic organization is also described.

The genus *Raoultella* is composed of Gram-negative, oxidase-negative, catalase-positive, nonmotile, capsulated, facultatively anaerobic bacilli belonging to the family *Enterobacteriaceae* (1). It was initially classified in the genus *Klebsiella*, but the current taxonomy was established based on the analysis of the 16S rRNA and *rpoB* gene sequences, thus creating the new genus *Raoultella* (2). Members of the genus *Raoultella* include *Raoultella electrica*, *R. terrigena*, *R. planticola*, and *R. ornithinolytica*. *R. ornithinolytica* can be found in aquatic and hospital environments (3). Reports of *R. ornithinolytica* causing human infections are rare, but human bloodstream infections by this bacterium have been reported (4–6).

Raoultella ornithinolytica CMUL058 was isolated from a stool sample from a 65-year-old male patient with Hodgkin lymphoma, during a study that was aimed at detecting carbapenemase-producing bacteria in patients diagnosed with cancer in Lebanon. It was identified as *R. ornithinolytica* by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique and by comparing its 16S rRNA gene sequence to the nucleotide database in NCBI using BLASTn, and the BLAST result of 99% showed the highest identity to *R. ornithinolytica* strain B6 (accession no. CP004142). The objective of the present study was to utilize whole-genome sequencing (WGS) to analyze the bacterial resistome, the genetic localization of the *bla*_{OXA-48} gene, and the specific features of this multidrug-resistant *R. ornithinolytica* clinical isolate.

Screening for carbapenemase production was carried out with the CarbaNP test (7), and molecular detection was carried out by PCR amplification for most of the carbapenemase genes using primers and PCR conditions previously described (8–10). *R. ornithinolytica* CMUL058 was found to be CarbaNP test positive, and PCR followed by sequencing showed that this strain harbors the *bla*_{OXA-48} gene.

Genomic DNA of *R. ornithinolytica* CMUL058 was sequenced using Miseq Technology (Illumina Inc., San Diego, CA, USA) using the mate pair strategy. The assembly of the mate pair reads was done using A5-miseq and SPAdes (coverage 153 and 98, respectively). Mauve (11) was used to order the scaffolds and compare the genome with the closest related complete reference genome, that of *R. ornithinolytica* strain B6 (accession no. CP004142).

FIG 1 Pangenome of the five *Raoultella ornithinolytica* strains. The flower plot shows the numbers of strain-specific genes found in each genome of each strain (petals) and the number of *R. ornithinolytica* core orthologous genes (center). Accession numbers for *R. ornithinolytica* S12 and B6 are NZ_CP010557 and NC_021066, respectively.

Functional annotation of the assembled genome sequence was done using the RAST (Rapid Annotation using Subsystems Technology) server (12); tRNA sequences were predicted using the findtRNA tool and rRNA predicted by RNAmmer 1.2 (13). Pro-

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TABLE 1 Resistome of *Raoultella ornithinolytica* CMUL058

Antibiotic class	Gene	Size (aa) ^a	Function	% aa identity	E value
Beta-lactams	<i>ampC</i>	437	Class C beta-lactamase (transpeptidase superfamily)	99.77	0
	<i>ampH</i>	385	Beta-lactam-binding protein	100	0
	<i>bla</i> _{TEM-166}	291	Beta-lactamase TEM	99	0
	<i>bla</i> _{OXA-48}	265	Class D carbapenemase-hydrolyzing beta-lactamase OXA-48	100	0
Macrolides	<i>macA</i>	371	Macrolide-specific efflux protein MacA/membrane fusion protein	99.7	0
	<i>macB</i>	648	Macrolide-specific efflux protein MacB/ABC transporter permease	99.53	0
Chloramphenicol	<i>cmr</i>	402	Major facilitator superfamily multidrug/chloramphenicol efflux transporter	99	0
	<i>cat</i>	212	Chloramphenicol acetyltransferase	100	1e−155
Fluoroquinolones	<i>gyrA</i> mutated (S83T)	879	DNA gyrase, A subunit/type IIA topoisomerase	100	0

^a aa, amino acids.

phage sequences were checked for using the PAST tool (14), and large genes were also checked for. All antimicrobial resistance genes, including mutated genes involved in antibiotic resistance, were retrieved by carrying out a BLAST search with the sequences obtained from the genome (chromosomal and plasmidic sequences) against the ARG-ANNOT database using tblastx on Bioedit (15) and BLASTn in GenBank. Antibiotic susceptibility testing was done for all the antibiotics whose resistance genes were retrieved. Last, the genetic environment of the *bla*_{OXA-48} gene in *R. ornithinolytica* CMUL058 was compared with other OXA-48 platforms in NCBI. In addition, the genome of *R. ornithinolytica* CMUL058 strain was also compared with other available genomes (4 in GenBank) to obtain the pangenome and the specific features of our strain.

The genome of *R. ornithinolytica* CMUL058 was assembled into 38 scaffolds, corresponding to a genome size of 5,622,862 bp with GC content of 55.7%. Three of the 38 scaffolds formed a plasmid of ~62 kb with a GC content of 51.2% that contained the *bla*_{OXA-48} gene. The total number of predicted coding sequences

(CDSs) in strain CMUL058 genome was 5,371, with 75 predicted tRNAs and 11 predicted rRNAs. A total of 5,177 genes (96.4%) were assigned to putative functions (by clusters of orthologous groups [COG]), and 194 genes (3.6%) were identified as having unknown functions. The *R. ornithinolytica* CMUL058 genome contained a total of three prophage regions, which represents 2.14% of the total bacterial genome content.

The *R. ornithinolytica* CMUL058 genome was then compared to the other available genomes of *R. ornithinolytica* present in GenBank by CMG-biotools (16) and was found to be most closely related to that of *R. ornithinolytica* strain TNT (BioProject NA242950), followed by *R. ornithinolytica* strain B6 (BioProject NA187030), with 77.9% and 77.0% similarity, respectively.

The pangenome of *R. ornithinolytica* consisted of 7,256 gene families with 3,607 core genes (Fig. 1). Compared to the four genomes present in GenBank, *R. ornithinolytica* CMUL058 genome had 507 specific genes, including the *bla*_{OXA-48} gene located on the plasmid, a nicotinamidase-encoding gene, and other genes belonging to the phages found in this strain. In addition, this

FIG 2 Graphical circular map of the plasmid and organization of the genetic environment of the *bla*_{OXA-48} gene. (A) Circular map of the plasmid carrying *bla*_{OXA-48}. From outside to center are plasmid genes, plasmid scaffolds, GC spot, and GC skew. (B) Organization of the genetic environment of *bla*_{OXA-48}.

strain had some large genes, including an 8.5-kb hemagglutinin gene and an ~5-kb α -2-macroglobulin gene.

R. ornithinolytica CMUL058 was resistant to ertapenem and meropenem and intermediately resistant to imipenem, with a MIC of 3 mg/liter, and showed high-level resistance to fluoroquinolones (ciprofloxacin and ofloxacin MICs = 12 mg/liter). Phenotypic antimicrobial susceptibility showed that the strain was susceptible to aztreonam, aminoglycosides (amikacin, gentamicin, and tobramycin), and colistin.

Eight antibiotic resistance genes were detected in the genome; mutations known to confer resistance to quinolones were detected in the quinolone resistance-determining region of the DNA gyrase gene (*gyrA*) in the genome. The resistance genes covered a total of four different antibiotic families (Table 1).

*bla*_{OXA-48} was found to be located on a plasmid, as reported previously in several studies (16, 17). The plasmid of *R. ornithinolytica* CMUL058 (accession no. [CVRH01000036](#) to [CVRH01000038](#)) displayed the closest resemblance (99.98% identity with 100% coverage) to a *bla*_{OXA-48}-bearing plasmid from *Klebsiella pneumoniae* (accession no. [KC335143](#)); the *bla*_{OXA-48} gene and associated genes displayed organization similar to that of the plasmid E71T from *K. pneumoniae*. The *bla*_{OXA-48} gene was found to be part of the transposon Tn1999.2 (accession no. [KC335143](#)), which is depicted in Fig. 2.

Few reports have described the emergence of carbapenemases in *Raoultella*, including IMP-8, OXA-48, and NDM-1 in *R. planticola* (18–20) and KPC and NDM-1 in *R. ornithinolytica* (21, 22). Here, we report the first detection of a *bla*_{OXA-48} gene in an *R. ornithinolytica* clinical isolate. The presence of the same plasmid as that of *Klebsiella pneumoniae* harboring the *bla*_{OXA-48} gene in *R. ornithinolytica* is noteworthy, since it is the first detection of this gene in this species that reflects the rapid dissemination of this plasmid in bacteria.

Nucleotide sequence accession number. The chromosome and plasmid sequences of *Raoultella ornithinolytica* CMUL058 isolate have been deposited in GenBank under the accession numbers [CVRH01000001](#) to [CVRH01000035](#) and [CVRH01000036](#) to [CVRH01000038](#), respectively.

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We declare that we have no conflicts of interest.

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