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Whole-Genome Sequence of *Chryseobacterium oranimense*, a Colistin-Resistant Bacterium Isolated from a Cystic Fibrosis Patient in France

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For the first time, we report the whole-genome sequence analysis of *Chryseobacterium oranimense* G311, a multidrug-resistant bacterium, from a cystic fibrosis patient in France, including resistance to colistin. Whole-genome sequencing of *C. oranimense* G311 was performed using Ion Torrent PGM, and RAST, the EMBL-EBI server, and the Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) database were used for annotation of all genes, including antibiotic resistance (AR) genes. General features of the *C. oranimense* G311 draft genome were compared to the other available genomes of *Chryseobacterium gleum* and *Chryseobacterium* sp. strain CF314. *C. oranimense* G311 was found to be resistant to all β-lactams, including imipenem, and to colistin. The genome size of *C. oranimense* G311 is 4,457,049 bp in length, with 37.70% GC content. We found 27 AR genes in the genome, including β-lactamase genes which showed little similarity to the known β-lactamase genes and could likely be novel. We found the type I polyketide synthase operon followed by a zeaxanthin glycosyltransferase gene in the genome, which could impart the yellow pigmentation of the isolate. We located the O-antigen biosynthesis cluster, and we also discovered a novel capsular polysaccharide biosynthesis cluster. We also found known mutations in the orthologs of the *pmrA* (E8D), *pmrB* (L208F and P360Q), and *lpxA* (G68D) genes. We speculate that the presence of the capsular cluster and mutations in these genes could explain the resistance of this bacterium to colistin. We demonstrate that whole-genome sequencing was successfully applied to decipher the resistome of a multidrug resistance bacterium associated with cystic fibrosis patients.

Cystic fibrosis (CF) is the most common autosomal recessive genetic disease and results from mutations within the gene coding for the cystic fibrosis transmembrane regulator (CFTR) protein. This life-threatening disease affects all racial and ethnic groups, though it is more common among Caucasians (1, 2). CF is characterized by hyperproduction of viscous mucus by the affected glands, resulting mainly in impaired respiratory and pancreatic functions. The most common complication of CF involves the chronic respiratory infections caused by bacterial pathogens (3), which are the main reason for the high morbidity and mortality of the disease (4). Traditionally, only a few bacteria were involved in CF lung infections, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. However, many new or emerging opportunistic bacteria have been described in CF patients over the past decade, for instance, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Pandoraea* spp., *Ralstonia* spp., *Inquilinus limosus*, and nontuberculosis mycobacteria, as well as fungi (5). Chronic microbial infection, along with *P. aeruginosa* infections, leads to excessive airway inflammation and the eventual loss of pulmonary function. Colistin is an extremely important antibiotic used in patients with CF upon the first acquisition and for maintenance of chronic *Pseudomonas* infections. Consequently, polymyxin-resistant *P. aeruginosa* clinical isolates are increasingly being reported in CF patients (6, 7). However, although aggressive antimicrobial therapy has often helped to eradicate or minimize the deterioration of lung infections, it has eventually led to the emergence of new and/or atypical multidrug resistance bacteria, including colistin-resistant bacteria in CF. Several colistin resistance bacteria have been reported recently in CF patients, such as *I. limosus* (8), *Brevundimonas diminuta* (9), *Ochrobactrum anthropi* (9), *S. maltophilia*, and *A. xylosoxidans* (8–12).

Members of the genus *Chryseobacterium*, mainly *Chryseobacterium indologenes*, have been documented as opportunistic pathogens known to be associated with nosocomial infections in infants and immunocompromised patients of all age groups and are resistant to colistin (13, 14). There are about 283 reported cases of infections associated with *C. indologenes* (15, 16). In a report by Chen et al., 215 clinical isolates of multidrug-resistant *C. indologenes* were identified after the increasing clinical use of colistin and tigecycline (16), a risk for patients who have undergone extensive administration of antibiotics for a long period (17). Although the source of infection of this microbe is not clear, it has been reported to be acquired nosocomially via medical devices and contaminated water supplies in hospitals (18). *C. indologenes* was also reported from a cohort of CF patients in Italy (19). Thirty-five clinical isolates of *Chryseobacterium* spp. (*C. indologenes*, *Elizabethkingia meningoseptica* [formerly *Chryseobacterium meningosepticum*], and *Chryseobacterium gleum*) were reported from CF patients who were also coinfected by one of the dominating pathogens of CF (*P. aeruginosa* or *Burkholderia cepacia* complex)

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(20). Furthermore, *Chryseobacterium* spp. only susceptible to cotrimoxazole and quinolones were reported in Italian CF patients who had received colistin therapy because of coinfection with *P. aeruginosa* or *B. cepacia* (21). The genetic basis of these multidrug-resistant bacteria remains unknown. Nonetheless, bacterial whole-genome sequencing is an economically feasible tool for deciphering the resistome (22) and has provided unprecedented insight into the evolution of antibiotic resistance (AR) (23).

Here, we report the whole-genome sequence used to decipher the resistome and genomic properties of *Chryseobacterium oranimense* G311, a colistin-resistant Gram-negative bacterium isolated for the first time from the sputum of a 26-month-old child with CF. It should be noted that the patient was coinfected with *S. maltophilia* and *P. aeruginosa* and had received colistin treatment prior to the isolation of this colistin-resistant bacterium. We speculate that colistin therapy led to the selection of this colistin-resistant bacterium; however, we could not isolate any other strain to perform the comparison. The true significance of isolating *C. oranimense* G311 in terms of clinical evolution is difficult to establish; however, it could be clinically significant, especially in immunocompromised patients. We also performed a comparison of the *C. oranimense* G311 genome with the genomes of closely related *C. gleum* ATCC 35910 and *Chryseobacterium* sp. strain CF314.

MATERIALS AND METHODS

Growth conditions and identification. *C. oranimense* was isolated on Columbia agar with 5% sheep blood COS (bioMérieux) medium and was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Microflex; Bruker Daltonic, Bremen, Germany) by using the flex control software (Bruker Daltonics), as previously described (24), and 16S rRNA gene amplification and sequencing as well. Growth was also assessed under other conditions and by using brain heart infusion medium with different salt concentrations, ranging from 0 to 10% NaCl, Trypticase soy agar (TSA) (bioMérieux), extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* medium (ChromID ESBL) (bioMérieux), and *Burkholderia cepacia*-specific CEPACIA medium (AES Laboratory, Combourg, France). Gram staining and electron microscopy were performed.

Antibiotic susceptibility test. Antibiotic susceptibility testing was performed on Mueller-Hinton agar medium (MH) (bioMérieux) according to the Committee for Antimicrobial Testing of the French Society for Microbiology using a Vitek2 auto system (bioMérieux, Marcy l'Etoile, France), and MICs were determined by the Etest method (bioMérieux).

Screening for metallo-β-lactamase activity was performed using the modified imipenem-EDTA (IMI-EDTA) double-disc synergy test and modified Hodge test as described previously (25, 26). Carbapenemase activity was assessed by MALDI-TOF assay. The colistin and imipenem susceptibilities were determined using the Etest strip (bioMérieux) and a 0.5 McFarland inoculum grown on TSA, as previously described (27). The antibiotics used for this study were amoxicillin, ticarcillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, cefoxitin, cefotaxime, ceftriaxone, ceftazidime, aztreonam, gentamicin, tobramycin, amikacin, ciprofloxacin, ofloxacin, and trimethoprim-sulfamethoxazole.

DNA isolation and genome sequencing. *C. oranimense* G311 was grown in Columbia agar with 5% sheep blood (bioMérieux) medium at 37°C for 24 h. The overnight bacterial culture was treated with 500 μl of TE buffer (25 mM Tris-HCl [pH 8.0], 10 mM EDTA [pH 8.0], and 10 mM NaCl) and 1 mg/ml of proteinase K at 37°C, and the genomic DNA was extracted using phenol-chloroform and alcohol precipitation. DNA was then visualized on an ethidium bromide-stained 0.7% agarose gel. The DNA concentration was quantified using the Quant-iT PicoGreen kit (Invitrogen). Bacterial genome sequencing was performed using the Ion Torrent PGM (Life Technologies, Saint Aubin, France) on 1 μg of DNA. A DNA library was constructed using enzymatic fragmentation and adaptor ligation with the Ion Xpress Plus fragment library kit (Life Technologies). Fragment size selection was performed using agarose gel electrophoresis. The distribution of DNA fragment sizes was analyzed with a Bioanalyzer using the High Sensitivity kit (Agilent, Santa Clara, CA). After dilution of the library at 11.62 pM, template preparation, emulsion PCR, and ion sphere particle (ISP) enrichment were performed using the Ion One Touch 200 template kit v.2. The quality of the resulting ISPs was assessed using the Qubit 2.0 fluorometer (Life Technologies), and the ISPs were loaded and sequenced on a 316 chip (Life Technologies). No prior quality filtering was used for the *de novo* assembly, which was performed using Newbler version 2.3 software (Roche) with 90% identity and 50% coverage as overlap.

Genome annotation. For *C. oranimense* G311 genome annotation, contigs were submitted to the Rapid Annotation using Subsystems Technology (RAST) online bioserver (http://rast.nmpdr.org/) (28), and more of the genome was annotated using the EMBL-EBI (The European Bioinformatics Institute) server using default parameters and the standard procedure. ORFans were confirmed by BLASTP (E value 10^{E-3}; identity of ≥30%; coverage of ≥50%) against the nonredundant protein (nr) database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The tRNA and rRNA genes were also verified on the tRNAscan-SE search server (http://lowelab.ucsc.edu/tRNAscan-SE) and RNammer (http://www.cbs.dtu.dk/services/RNAmmer/). All the antimicrobial resistance genes in *C. oranimense* G311 were predicted using our local database Antibiotic Resistance Gene-ANNOTation (29).

Nucleotide sequence accession numbers. The genome sequence was deposited in EMBL under accession numbers CDHM01000001 to CDHM01000015 (EBI accession numbers CEJ67725 to CEJ72111).

RESULTS

Phenotypic properties. *C. oranimense* G311 (Collection de Souches de l'Unité des Rickettsies [CSUR] reference no. P277) (10^4 CFU/ml), along with *P. aeruginosa* (10^4 CFU/ml) and *S. maltophilia* (10^5 CFU/ml), was isolated from the sputum sample of a 26-month-old girl in August 2012. The isolate was pigmented yellow when isolated on Columbia agar with 5% sheep blood COS (bioMérieux) medium at 37°C after 24 h of incubation and correctly identified by MALDI-TOF as *C. oranimense* with a good score (>2.0). The colony size of the CF isolate varied from 0.5 to 1 mm in diameter and was capsulated (Fig. 1). This aerobic, Gram-negative, nonmotile bacillus grew well at 29°C, and growth was also observed at 18°C, 10°C, and 4°C after 2 days, 4 days, and 8 days of incubation, respectively. The isolate was able to grow microaerophilically and in the presence of 5% CO_2 but not under an anaerobic condition. Growth was also observed on Trypticase soy agar, extended spectrum-β-lactamase-producing *Enterobacteriaceae*, and cepacia media at 37°C and 29°C. The isolate also grew at a salt concentration of up to 2% after 24 h of incubation. Although *Chryseobacterium* species have been described to be present in CF patients, *C. oranimense* has not been reported so far, and it was not isolated from any other CF clinical sample in our lab as well. 16S rRNA PCR amplification and sequencing confirmed that the sequence was 99.7% similar to that of *C. oranimense* $H8^T$ (30). The genome sequence confirmed the identification. The phylogenetic tree based on the 16S rRNA sequence was constructed to show the phylogenetic position of CF *C. oranimense* G311 (Fig. 2).

General features of the *C. oranimense* G311 genome. A total of 2,764,904 reads were obtained, leading to 511,490,430 bp of sequence data. The size of the *C. oranimense* G311 genome is 4,457,049 bp, comprising one circular chromosome, with a 37.7% GC content, assembled into 15 contigs. No plasmid was detected.

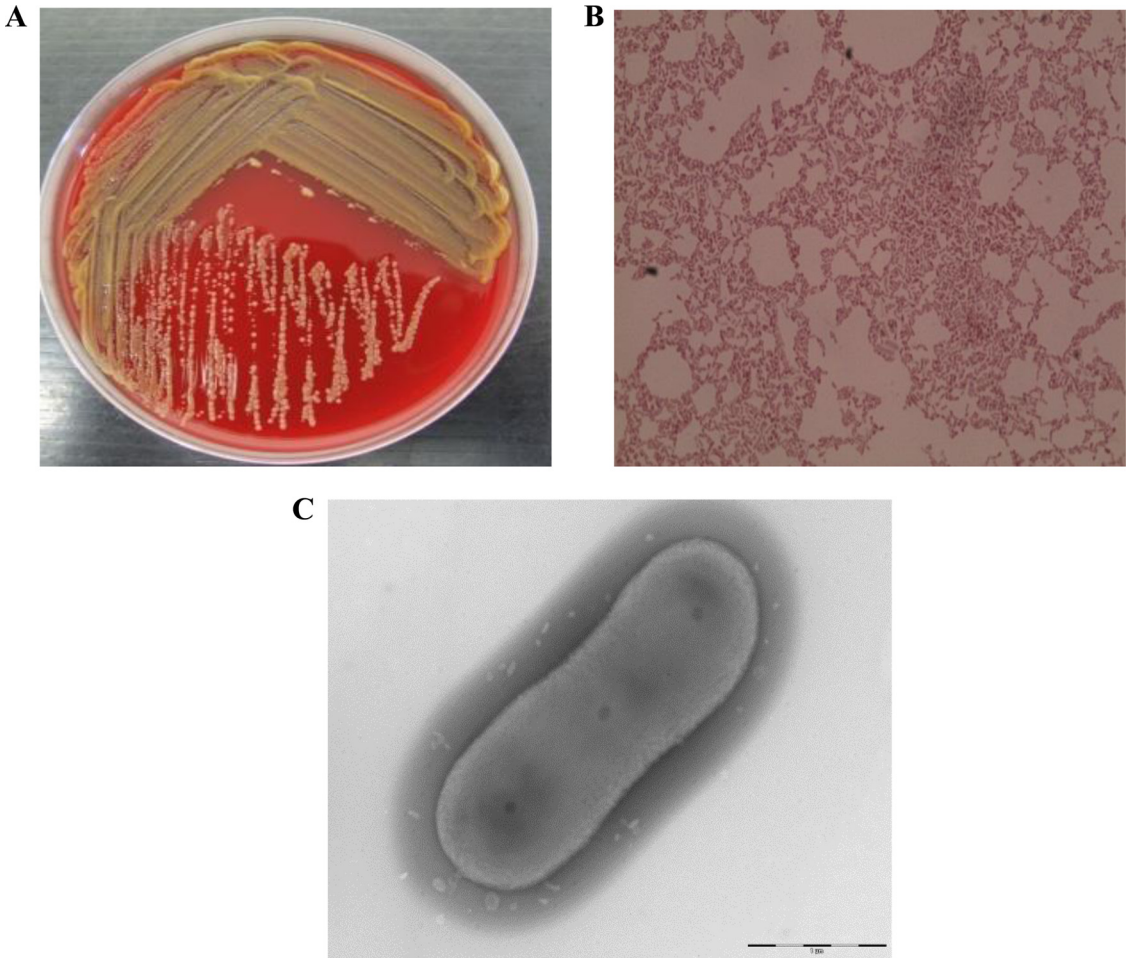

FIG 1 (A) *Chryseobacterium oranimense* G311 yellow isolate on Columbia agar with 5% sheep blood (bioMérieux) at 37°C; (B) Gram staining image of *Chryseobacterium oranimense* G311 viewed at ×100 magnification; (C) transmission electron microscopic image of *Chryseobacterium oranimense* G311 using a Morgani 268D TEM (Philips) at an operating voltage of 60 kV.

A total of 4,475 genes were predicted, including 4,387 protein-coding genes (EBI accession number CEJ67725 to CEJ72111) and 88 RNAs (3 rRNA operons and 85 tRNAs). Of the 4,387 protein-coding genes, 3,004 (68.47%) were assigned a putative function, whereas 216 (4.92%) genes were identified as ORFans and 1,100 were annotated as hypothetical proteins (26.60%). As many as 541 genes had a signal peptide, and 896 transmembrane proteins were detected. A comparison among the general features of the genomes of three *Chryseobacterium* spp. is shown in Table 1. The average nucleotide identities of the *C. oranimense* G311 genome with *C. gleum* ATCC 35910 and *Chryseobacterium* sp. CF314 genomes were 80.68% and 79.72%, respectively. The distribution of genes into COG functional categories and the comparison of *C. oranimense* with *C. gleum* ATCC 35910 and *Chryseobacterium* sp. strain CF using the BLAST Ring Image Generator (31) (BRIG) are presented in Fig. 3, and the distribution of COG categories is presented in Table S1 in the supplemental material.

Resistome of *C. oranimense* G311. The *C. oranimense* G311 isolate was found to be highly multidrug resistant. The isolate was resistant to colistin (MIC of 24 μg/ml) and imipenem (MIC of 12 μg/ml) and also to amoxicillin, ticarcillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, second-generation cephalosporin (cefoxitin), and third-generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime, and aztreonam). *C. oranimense* G311 was also found to be resistant to the aminoglycoside tobramycin but was susceptible to gentamicin and amikacin and was also susceptible to fluoroquinolones ciprofloxacin, ofloxacin, and trimethoprim-sulfamethoxazole (Table 2). The resistome of this multidrug-resistant *C. oranimense* G311 revealed the presence of 27 antibacterial-resistant genes using the Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) database (29). This isolate possesses three different types of β-lactamases, i.e., Amber class A ESBL genes (bla_{CME}-like), Amber class B metallo-β-lactamase (MBL) genes (bla_{GOB}- and bla_{IND}-like), and Amber class C ESBL genes (bla_{ACC}-, bla_{ampH}-, and bla_{CMY}-like). There was little homology between bla_{ACC}-like genes and other beta-lactamases genes (Table 3); thus, there is a possibility that these genes could be novel. They are likely to contribute to the resistance of this bacterium to beta-lactam compounds. We also performed the analysis of the 10-kb sequence upstream and downstream of each AR gene. We found only one putative, 138-amino-acid (aa)-long Holiday junction resolvase (39.1%), located 740 bp upstream of the bla_{ACC} gene, and a 304-aa-long integrase (38.9%), located 2.839 kb downstream of the bla_{ampH}-like gene. Apart from these, there were

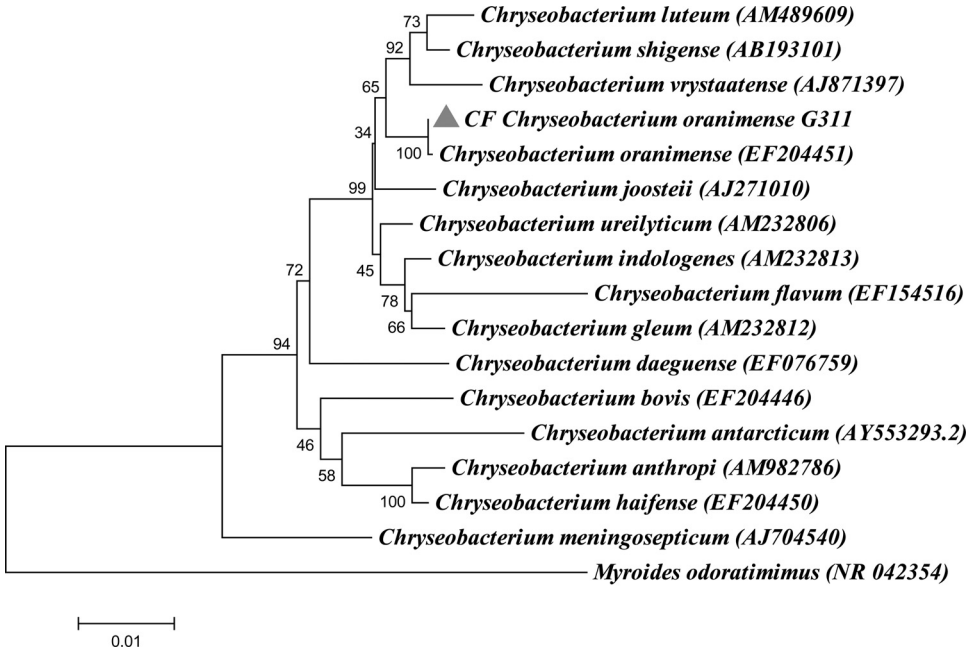

FIG 2 Phylogenetic tree based on 16S rRNA sequence highlighting the phylogenetic position of CF *Chryseobacterium oranimense* G311. *Myroides odoratimimus* was used as an outgroup. Sequences were aligned using ClustalX, and phylogenetic inferences were obtained using the neighbor-joining method within Mega 5 software. Bootstrap values are expressed by percentage of 1,000 replicates with a Kimura 2 parameter test and shown at the branching points. The branches of the tree are indicated by the genus and species name of the type strains followed by the NCBI gene accession numbers.

many hypothetical proteins flanking the AR genes with no BLAST hit; hence, we believe that there is a possibility of those sequences to carry unknown insertion elements or transposases by which the genes were acquired. Tetracycline resistance genes, such as the *otr*-like, *tetC*, and *tetX* genes, were also found, as well as aminoglycoside adenyltransferase genes (*aac6* and *aadK*). Other AR genes included genes conferring resistance to macrolide-lincosamide-streptogramin B (MLS-like), phenicols (*cfr*- and *cmlv*-like), sulfonamide (*sulIII*-like), and rifampin (*arr5*-like) (Table 3). Conversely, *C. oranimense* was found to be susceptible to rifampin (MIC of 0.38 μg/ml) and to fluoroquinolones. We did not find any mutations in the known genes (*gyrA*, *rpoB*, *parC*), which play a role in imparting resistance to these antibiotics. Lastly, a genetic analysis of putative candidate target genes associated with polymyxin resistance (*pmrA*, *pmrB*, *phoP*, and *phoQ* genes) revealed that the *pmrA* gene harbors a single substitution at position eight (E8D),that the *pmrB* gene harbors two substitutions (L208F and P360Q), and that *lpxA* harbors a single substitution (G68D), as shown in Table 4. We speculate that these mutations could likely play a role in colistin resistance exhibited by *C. oranimense* G311.

Specific features of the *C. oranimense* G311 genome. We found an operon (20,162 bp) comprised of modular polyketide synthase genes and a zeaxanthin glycosyltransferase gene. We noted a similar arrangement in the size of 18,518 bp in *C. gleum* ATCC 35910 (Fig. 4), though we did not find this arrangement in the *Chryseobacterium* sp. strain CF314 genome. The presence of zeaxanthin, a carotenoid pigment, in the genome likely explains the yellowish color of our CF isolate. We located the O-antigen biosynthesis cluster in the genome of the *C. oranimense* isolate, as shown in Fig. S1 in the supplemental material (open reading frame [ORF] 569 [CEJ68283] to ORF 601 [CEJ68319]). Similar clusters are present in the genomes of *C. gleum* ATCC 35910 and *Chryseobacterium* sp. strain CF314 (see Fig. S1 in the supplemental material). In addition, a new capsular polysaccharide (CPS) bio-

TABLE 1 General features of the *Chryseobacterium oranimense* G311 genome in comparison to the *Chryseobacterium gleum* ATCC 35910 and *Chryseobacterium* sp. strain CF314 genomes

Species	Database accession no.[a]	Genome size (bp)	% GC content	No. of CDS[b]	No. of RNA	Avg nt identity	No. of *C. oranimense* G311 proteins with: Any similarity	No. of *C. oranimense* G311 proteins with: Up to 80% similarity
Chryseobacterium oranimense G311	CDHM01000001–CDHM01000015	4,457,049	37.70	4,387	88			
Chryseobacterium gleum ATCC 35910	ACKQ02000001–ACKQ02000007	5,569,640	36.80	5,304	79	80.68	1,355	2,435
Chryseobacterium sp. strain CF314	AKJY01000001–AKJY01000119	4,484,672	36.62	4,182	54	79.72	1,449	1,952

[a] The genome sequences were deposited in the Whole Genome Sequence (WGS) database: 15 contigs for *Chryseobacterium oranimense* G311, 7 contigs for *Chryseobacterium gleum* ATCC 35910, and 119 contigs for *Chryseobacterium* sp. strain CF314.
[b] CDS, coding sequences.

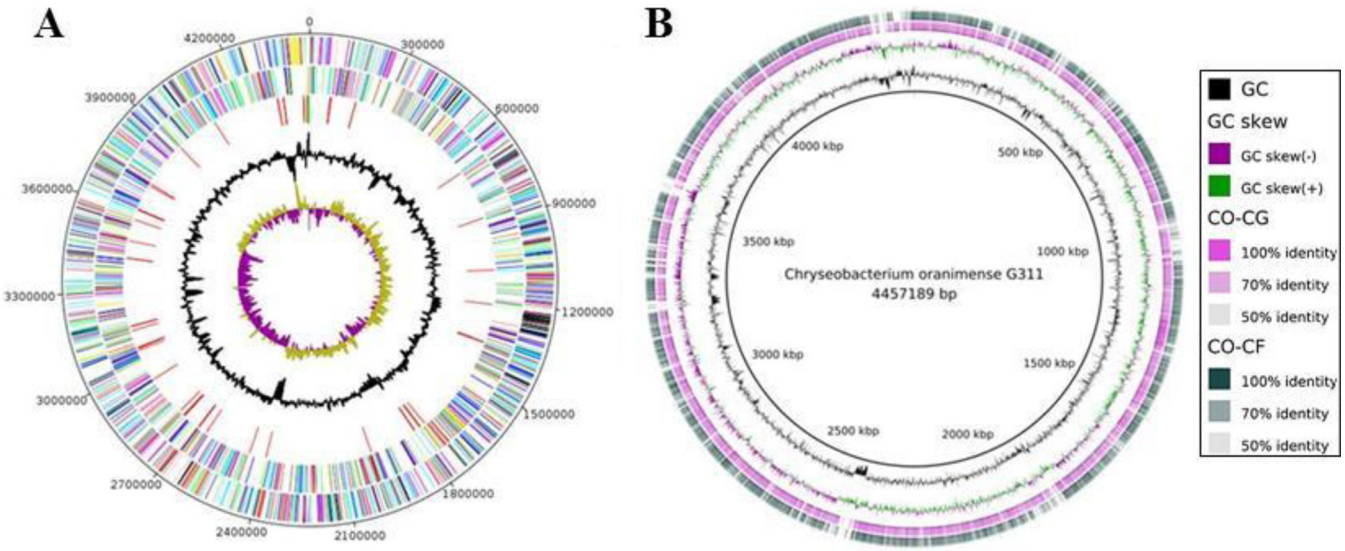

FIG 3 (A) Graphical circular map of the *Chryseobacterium oranimense* G311 genome. Circle range is from 1 (outer) to 5 (inner). Circle 1, positive strand; circle 2, negative strand; circle 3, tRNA (red) and rRNA (green); circle 4, GC; circle 5, GC skew. All genes are color coded according to Cluster of Orthologous Group (COG) functions, shown in the table with number of genes for each COG using BRIG software. (B) Comparison of the *Chryseobacterium oranimense* G311 (CO) genome with *Chryseobacterium gleum* ATCC 35910 (CG) and *Chryseobacterium* sp. strain CF314 (CF) genomes using RAST.

synthesis was identified in the genome of *C. oranimense* (see Fig. S2 in the supplemental material) (ORF 1284 [CEJ68992] to ORF 1317 [CEJ69025]). We could not find a similar cluster in the other genomes analyzed. The list of all the genes of O-antigen-like and K-antigen-like clusters is shown in Table S2 in the supplemental material.

DISCUSSION

Chryseobacterium species are found in a variety of habitats and essentially ubiquitous, though some are opportunist pathogens (32). *C. oranimense* has been reported to be isolated from raw milk (30), yet this is the first report of *C. oranimense* in humans, i.e., from a CF patient. *Chryseobacterium* species are multidrug resistant, with most intrinsically resistant to penicillin, first- and second-generation cephalosporin, aztreonam (14, 33), and colistin (16), and have been reported to be acquired nosocomially via medical devices and contaminated water supplies in hospitals (18). Using a polyphasic approach, some studies have reported the presence of unusual bacteria, such as *Acinetobacter* spp., *Bordetella* spp., *Comamonas* spp., *Rhizobium* spp., *Herbaspirillum* spp., *Moraxella* spp., *I. limosus*, and *Chryseobacterium* spp., in the sputum samples from CF patients (34). Although the emergence of new multidrug-resistant, Gram-negative bacteria in CF lung infections has been relatively low, the incidence is increasing considerably, presenting a serious challenge for the development of effective and appropriate antibiotic therapies when they are misidentified. It is known that *Chryseobacterium* spp. cause infections in immunocompromised patients (13, 14), and their existence in CF airways has been reported over the last 10 years (34). One study reports *E. meningoseptica* and *C. indologenes* as the most frequently isolated species, followed by *C. gleum* and coinfections with at least one Gram-negative bacterium, such as *P. aeruginosa*, *A. xylosoxidans*, *S. maltophilia*, or *B. cepacia* complex in CF patients (34). Many of the isolates in the above-named study were found to be resistant to imipenem but were not checked for resistance to colistin, whereas our multidrug isolate was found to be resistant to both imipenem and colistin. As the life expectancy of CF patients has increased, antimicrobial pressure has also experienced an increase, and, consequently, more multidrug-resistant microorganisms are being isolated from the CF lung microbiota. Importantly, as these bacteria have developed multiple mechanisms of antibiotic resistance, they must be identified correctly for designing therapeutic treatments.

The genomic comparison of *C. oranimense* G311 with the available genomes of *Chryseobacterium gleum* ATCC 35910 and *Chryseobacterium* sp. strain CF314 (35) revealed similar genome

TABLE 2 Antibiotic susceptibility pattern in the *Chryseobacterium oranimense* G311 genome

Antibiotic[a]	Pattern
AMX	9/R
TIC	10/R
AMC	10/R
TCC	15/R
FOX	11/R
CTX	9/R
CRO	14/R
CAZ	25/S
ATM	7/R
IMP	15/R (12 μg/ml)
CN	40/S
TOB	9/R
AK	30/S
CIP	38/S
OFX	30/S
SXT	37/S
CT	10/R (24μg/ml)

[a] AMX, amoxicillin; TIC, ticarcillin; AMC, amoxicillin-clavulanic acid; TCC, ticarcillin-clavulanic acid; FOX, cefoxitin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; ATM, aztreonam; IMP, imipenem; CN, gentamicin; TOB, tobramycin; AK, amikacin; CIP, ciprofloxacin; OFX, ofloxacin; SXT, sulfamethoxazole-trimethoprim; CT, colistin.

TABLE 3 Antibiotic resistance genes in the *Chryseobacterium oranimense* G311 genome

Antibiotic class	ORF/EBI gene identifier	Putative gene	GC (%)	Size (aa)	Function	Best BLAST hit organism in GenBank	% aa identity	E value
Beta-lactams	55/CEJ67779	*penA*-like	41.5	663	Penicillin-binding protein	*Chryseobacterium gleum* ATCC 35910	95.8	0.0
	1366/CEJ69070	bla_{IND}-like	40.2	241	Metallo-beta-lactamase IND-4	*Chryseobacterium indologenes*	93.7	3.00E–165
	2824/CEJ70497	bla_{ACC}-like	40.9	514	Beta-lactamase	*Chryseobacterium luteum*	95.2	0.0
	4186/CEJ71833	bla_{ampH}-like	38.6	420	Beta-lactamase	*Chryseobacterium vrystaatense*	94.7	0.0
	390/CEJ68108	bla_{mecA}-like	41.3	668	Penicillin-binding protein 2a (PBP-2a)	*Chryseobacterium indologenes* NBRC 14944	86.0	0.0
	1132/CEJ68841	bla_{CME}-like	37.5	292	Beta-lactamase	*Chryseobacterium vrystaatense*	87.0	0.0
	1161/CEJ68870	bla_{GOB}-like	39.3	330	Beta-lactamase	*Chryseobacterium luteum*	88.1	0.0
	2092/CEJ69789	bla_{ACC}-like	36.5	462	Beta-lactamase	*Chitinophaga pinensis*	41.6	2.00E–106
	3736/CEJ71390	bla_{CMY}-like	34.5	439	Penicillin-binding protein beta-lactamase class C	*Chryseobacterium hispalense*	91.5	0.0
Aminoglycoside	1104/CEJ68813	*aac6*	39.5	91	Aminoglycoside N6 acetyltransferase	*Chryseobacterium luteum*	83.5	5.00E–50
	2703/CEJ70393	*aadK*	41.7	287	Aminoglycoside 6-adenylyltransferase	*Chryseobacterium luteum*	79.4	3.00E–168
Tetracycline	3882/CEJ71533	*otr*-like	43.6	524	*emrB-qacA* family drug resistance transporter	*Chryseobacterium gleum*	97.6	0.0
	1319/CEJ69027	*otr*-like	39.2	601	GTP-binding protein of *typA-bypA*	*Chryseobacterium daeguense*	97.6	0.0
	2344/CEJ70041	*tetC*	42.1	412	Tetracycline efflux protein	*Chryseobacterium luteum*	93.9	0
	2428/CEJ70124	*tetX*-like	42.6	383	FAD-binding monooxygenase/tetracycline resistance protein	*Pedobacter heparinus* DSM 2366	70.3	0.0
MLS	752/CEJ68465	MLS-like	38.7	540	ABC transporter ATP-binding protein	*Chryseobacterium indologenes* NBRC14944	98.7	0.0
	956/CEJ68667	MLS-like	40.7	233	ABC transporter-related protein	*Chryseobacterium luteum*	98.2	5.00E–162
	3478/CEJ71135	MLS-like	39.7	642	ABC superfamily ATP-binding cassette transporter	*Chryseobacterium vrystaatense*	94.8	0.0
	4328/CEJ71973	*ole*-like	42.3	239	ABC-type multidrug transport system	*Chryseobacterium luteum*	96.2	2.00E–165
	2084/CEJ69781	*ole*-like	41.6	303	ATP-binding cassette transporter	*Chryseobacterium luteum*	94.0	0.0
	3848/CEJ71499	*vga*-like	39.4	295	ABC superfamily ATP-binding cassette transporter	*Chryseobacterium indologenes* NBRC 14944	78.3	2.00E–164
Phenicols	925/CEJ68636	*cfr*-like	42.9	344	Cfr family radical SAM enzyme	*Chryseobacterium gleum*	95.9	0.0
	1679/69381	*cmlv*-like	44.0	404	Major facilitator family protein/chloramphenicol resistance protein	*Chryseobacterium vrystaatense*	93.9	0.0
Glycopeptide	2837/CEJ70510	*vanL*-like	37.9	330	D-alanine–D-alanine ligase	*Chryseobacterium luteum*	97.5	0.0
Fluoroquinolones	908/CEJ68619	*qepA*-like	43.1	462	Drug resistance transporter	*Chryseobacterium vrystaatense*	91.1	0.0
Sulfonamide	2063/CEJ69760	*sulIII*-like	37.9	293	Dihydropteroate synthase	*Chryseobacterium vrystaatense*	89.1	3.00E–167
Rifampin	3000/CEJ70672	*arr5*-like	42.0	141	Rifampin ADP-ribosyl transferase	*Chryseobacterium daeguense*	89.3	5.00E–81

(Continued on following page)

TABLE 3 (Continued)

Antibiotic class	ORF/EBI gene identifier	Putative gene	GC (%)	Size (aa)	Function	Best BLAST hit organism in GenBank	% aa identity	E value
Multidrug efflux pumps	248/CEJ67966		41.5	396	MFS superfamily, putative drug resistance transporter	*Chryseobacterium indologenes* NBRC 14944	81.4	0.0
	1382/CEJ69086	*acrB*	42.9	1061	Acriflavin resistance protein	*Chryseobacterium daeguense*	96.9	0.0
	1653/CEJ69355	*norM*	44.4	467	MatE efflux family protein	*Chryseobacterium vrystaatense*	94.0	0.0
	1766/CEJ69467	*acrB*	42.0	790	Multidrug transporter AcrB	*Chryseobacterium gleum*	96.9	0.0
	1767/CEJ69468		44.4	234	Putative efflux system protein	*Chryseobacterium gleum*	97.2	1.00E–145
	1829/CEJ69527	*acrB*	44.0	1052	Acriflavin resistance protein	*Chryseobacterium vrystaatense*	99.9	0.0
	1830/CEJ69528		42.7	368	RND transporter	*Epilithonimonas lactis*	100	0.0
	1911/CEJ69609	*acrB*	38.4	1454	Acriflavin resistance protein B	*Epilithonimonas lactis*	99.9	0.0
	2652/CEJ70346		41.4	483	RND transporter	*Chryseobacterium luteum*	94.1	0.0
	2653/CEJ70347	*acrB*	42.3	1064	Multidrug transporter	*Chryseobacterium hispalense*	98.8	0.0
	3124/CEJ70786	*matE*	41.6	464	Multidrug transporter MatE	*Chryseobacterium luteum*	96.9	0.0
	3569/CEJ71225		43.3	386	RND transporter	*Chryseobacterium luteum*	93.5	0.0
	3570/CEJ71226	*acrB*	43.2	1059	Multidrug transporter	*Chryseobacterium luteum*	97.4	0.0
	3571/CEJ71227		41.9	470	RND transporter	*Chryseobacterium vrystaatense*	95.7	0.0
	4072/CEJ71722	*matE*	39.7	451	Multidrug transporter MatE	*Chryseobacterium luteum*	96.8	0.0
	1440/CEJ69143	*emrB*	40.8	520	ABC superfamily ATP-binding cassette transporter	*Chryseobacterium gleum*	90.5	0.0
	1945/CEJ69643		43.0	349	Putative major facilitator superfamily transporter	*Chryseobacterium indologenes* NBRC 14944	84.5	0.0
	2257/CEJ69954		43.2	352	MFS transporter	*Chryseobacterium vrystaatense*	95.4	0.0
	2307/CEJ70004		38.6	387	MFS transporter	"*Candidatus* Solibacter usitatus"	26.3	4.00E–26
	3981/CEJ71631	*araJ*	42.3	310	Putative major facilitator superfamily transporter	*Chryseobacterium vrystaatense*	83.2	2.00E–176
	3138/CEJ70800	*mdlB*	38.4	553	Putative ABC transporter ATP-binding/permease protein	*Chryseobacterium luteum*	96.9	0.0
	882/CEJ68593		39.7	730	Putative ABC transporter ATP-binding protein	*Chryseobacterium luteum*	97.2	0.0
	1090/CEJ68799		40.6	396	ABC transporter ATP permease protein	*Chryseobacterium vrystaatense*	93.6	0.0

sizes and GC contents, and none of them harbored any plasmid (Table 1). Apart from deciphering the resistome of this atypical bacterium, which will be discussed in details below, we identified three specific features in the *C. oranimense* G311 genome. First, the presence of PKS might play a role in the synthesis of zeaxanthin, a secondary metabolite imparting the yellowish pigmentation of the isolate. *Flavobacterium multivorum* has been widely studied for the production of the xanthophyll carotenoid zeaxanthin, as this species could be used as a commercial source of zeaxanthin (36). High intake of foods providing zeaxanthin is related with lower incidence of age-related macular degeneration (ARMD), mostly for ocular and retinal health. They are used as supplemental antioxidants in treating ARMD (37). The presence of this bacterium, which produces beta-carotene, in a clinical isolate in the context of CF was unexpected, and the role this bacterium may play in the lung microbiota remains to be studied in the future. Second, the lipopolysaccharide (LPS) cluster in the genome of this bacterium could be acquired laterally and to the best of our knowledge was unknown in this genus. This cluster consisted of glycosyltransferases (see Table S2 in the supplemental material) that likely contribute to modification of LPS (38), which is a well-known phenomenon associated with resistance to polymyxins (38–41). Third, *C. oranimense* G311 also harbors a new capsular polysaccharide biosynthesis (K-antigen) gene cluster that was unique to this genome and also acquired laterally. Within this cluster, the *wza* and *wzc* genes have been described as outer membrane lipoprotein and integral inner membrane protein/protein tyrosine kinase, respectively, in some human pathogens, such as *K. pneumoniae* K2 and *Escherichia coli* K-12 (42–45). Another gene product, *ugd*, was identified, which is involved in the produc-

TABLE 4 Known mutations in genes conferring resistance to colistin and corresponding variants in *Chryseobacterium oranimense* G311, *C. gleum* ATCC 35910, and *Chryseobacterium* sp. strain CF314 variants

Gene	Known mutation (reference)	Organism	Mutation		
			C. oranimense G311 (EBI gene identifier no.)	*C. gleum* ATCC 35910	*Chryseobacterium* sp. strain CF314
pmrA	E8D (65)	*Acinetobacter baumannii*	E8D (CEJ70734)	E8D	E8D
	G53S (62)	*Enterobacter aerogenes*	No mutation (CEJ70734)	No mutation	No mutation
pmrB	L208F (64)	*Acinetobacter baumannii*	L208F (CEJ70733)	No mutation	No mutation
	P360Q (64)	*Acinetobacter baumannii*	P360Q (CEJ70733)	No mutation	No mutation
lpxA	G68D (65)	*Acinetobacter baumannii*	G68D (CEJ70978)	G68D	G68D
	Q72K (65)	*Acinetobacter baumannii*	No mutation (CEJ70978)	No mutation	No mutation
	H159D (65)	*Acinetobacter baumannii*	No mutation (CEJ70978)	No mutation	No mutation
	Insertional inactivation (66)	*Acinetobacter baumannii*	No mutation (CEJ70978)	No mutation	No mutation
lpxC	I38T (67)	*E .coli*	No mutation (CEJ70979)	No mutation	No mutation
	P30L (66)	*Acinetobacter baumannii*	No mutation (CEJ70979)	No mutation	No mutation
	Insertional inactivation (66)	*Acinetobacter baumannii*	No mutation (CEJ70979)	No mutation	No mutation
lpxD	M290A (68)	*E. coli*	No mutation (CEJ70980)	No mutation	No mutation
phoP	ND[a]				
phoQ	D179(L/A) (4)	*E. coli*	No mutation (CEJ67802)	No mutation	No mutation

[a] ND, not detected.

tion of UDP-4-amino-4-deoxy-L-arabinose, a compound that renders *E. coli* resistant to cationic antimicrobial peptides (46). The *ugd* produces UDP-glucuronic acid (UDPGA), which plays a role in the production of a sugar derivative, UDP-4-amino-4-deoxy-L-arabinose (L-Ara4N), which is vital for bacterial resistance to polymyxin (38, 47). Capsular clusters in the genus *Flavobacterium* have been reported in *Flavobacterium columnare* ATCC 43622 (48), *Flavobacterium psychrophilum* strain 259-93 (49), and *Zunongwangia profunda* SM-A87 (50).

The resistome of *C. oranimense* G311 comprises a reservoir of diverse β-lactamases, including a class A β-lactamase gene, bla_{CME}, and the *cme-1* gene has been reported to be structurally divergent from other class A enzymes (51) in *E. meningoseptica*. The *cme-1* gene encodes a clavulanic acid-susceptible extended-spectrum β-lactamase that hydrolyzes most of the cephalosporins, such as cefotaxime and ceftazidime, and monobactams, such as aztreonam, though it does not hydrolyze cephamycins and carbapenems. The *C. oranimense* G311 *cme*-like gene clustered with

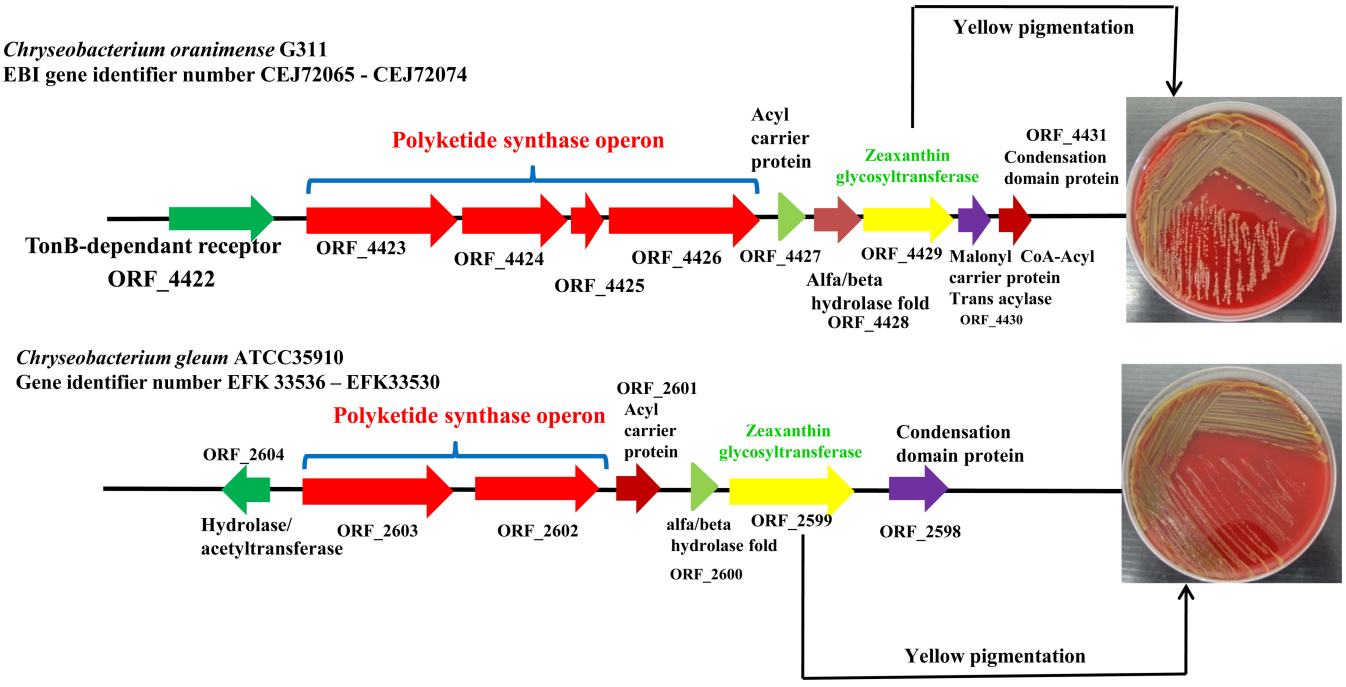

FIG 4 Polyketide synthase operon arrangement in *Chryseobacterium oranimense* G311 and *Chryseobacterium gleum* ATCC 35910.

the *cme-1* gene reported from *E. meningoseptica* (data not shown). Another class A β-lactamase gene, *penA*, encodes penicillin-binding protein PBP-2a, which is a *mecA* gene product that can result in ceftazidime and amoxicillin-clavulanic acid resistance if it is overproduced or mutated (52, 53). We discovered two genes for class B metallo-β-lactamases: a bla_{GOB}-like gene and a putative metallo-β-lactamase bla_{IND} gene. Class B lactamases (generically termed metallo-β-lactamases) employ one or two Zn(II) ions for cleaving the β-lactam ring. The Gob-18 is fully active against a broad range of β-lactam substrates and has been reported from *E. meningoseptica* (54); many more variants of *gob* genes have recently been reported from this species, which is known to be intrinsically resistant to most β-lactams, including carbapenems (55). The bla_{IND-4} gene found in the *C. oranimense* G311 genome is 93.7% similar to bla_{IND-4} from *C. indologenes* 009 (56), an enzyme that is able to hydrolyze carbapenems. We also discovered many class C extended-spectrum β-lactamases (ESBLs), such as bla_{ACC}-, bla_{ampH}-, bla_{ACC-4}-, and bla_{CMY}-like; however, certain genes, such as bla_{ACC}-like, showed similarities with the reported genes from plant sources (57).

The most common mechanism of resistance to colistin is modification of the LPS structure (58). Intrinsic resistance to polymyxins in *Burkholderia cenocepacia* and *Proteus mirabilis* has been linked to alterations in their lipid A structure with the addition at the 4′-phosphate moiety of the LPS of 4-amino-L-arabinopyranose and 4-amino-L-arabinose (L-Ara4N), respectively (59, 60). Such modifications have also been reported for *K. pneumoniae* and *E. coli* (58). In *K. pneumoniae*, the resistance to polymyxin is due to increased production of capsular polysaccharides (61). Recently, it has been demonstrated that in *Acinetobacter baumannii* or in *Enterobacter aerogenes*, acquired resistance to colistin may also be due to mutations in the *pmrA-pmrB* two-component systems (62, 63). Finally loss of LPS production by mutations in the three genes *lpxA*, *lpxC*, and *lpxD* has been associated with the resistance to *Acinetobacter baumannii* (64). Here, we found similar variants of *pmrA* (E8D) (63), *pmrB* (L208F, P360Q) (64), and *lpxA* (G68D) (Table 4) (65) that confer resistance to colistin in *Acinetobacter baumannii* (63–65). Thus, we believe the intrinsic resistance of *C. oranimense* G311 to colistin is due to both alterations in LPS and production of capsular polysaccharides.

Conclusion. In conclusion, we believe that the increased clinical use of nebulized colistin in patients with CF might have led to the selection of this specific colistin-resistant bacterium. Our findings provide insight into the mechanism of colistin resistance in the genus *Chryseobacterium*, as it is well known that many clinically significant species from this genus are intrinsically resistant to many antimicrobial agents. This bacterium could be considered an opportunistic human pathogen in immunocompromised patients. We demonstrate that whole-genome sequencing was successfully applied to completely decipher the resistome of this multidrug-resistant bacterium associated with CF patients.

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