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Genome Note

Genomic characterization of *Achromobacter* genogroup 20 and identification of a potential species-specific marker

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ABSTRACT

Objective: To describe the genome of *Achromobacter* genogroup 20 and explore the presence of resistance determinants.
Methods: Isolate 413638 was identified through *nrdA* and MLST analysis. Antimicrobial susceptibility testing was performed according CLSI 2024. NGS was conducted using Illumina MiSeq, on the NextSeq500 platform with 150 bp paired-end reads, and de novo assembly was assessed using Unicycler (Galaxy). Coding sequences were predicted and confirmed with RAST and BLAST, and resistance determinants were evaluated using Resfinder and manual curation. All *Achromobacter* spp. genomes were obtained from NCBI, and the presence of bla_{OXA} was investigated. Average nucleotide identity (ANI) and tetranucleotide analysis (TETRA) were calculated. Phylogenetic analysis of the new OXA variant was conducted against other species-specific markers in *Achromobacter*.
Results: Isolate 413638 was identified as *Achromobacter* genogroup 20 ST365, showing resistance to third- and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones. The genome included coding sequences for three putative β-lactamases (one new OXA type-class D β -lactamase and two new class C), 32 efflux pump proteins, two aminoglycoside-modifying enzymes and a dihydropteroate synthase; also, substitutions in *par*C and *par*E were detected. The OXA enzyme, designated OXA-1238 (PP446293), differs from OXA-114a by 85 amino acids, with 69% identity. In silico analysis found OXA-1238 variants in three additional *Achromobacter* spp. genomes, with 97% identity. Based on ANI and tetra analysis, the three genomes corresponded to *Achromobacter* genogroup 20.
Conclusion: Several resistance markers were found, probably contributing to the resistance profile observed in *Achromobacter* genogroup 20 ST365. The new OXA variant identified, OXA-1238, could constitute a useful molecular marker for species identification.

Achromobacter spp. are aerobic, non-fermenting, Gram-negative bacteria widely distributed in nature. *Achromobacter* genus currently includes 21 officially designated species and various genogroups [1], being *A. xylosoxidans* the most frequently recovered species. Moreover, clinical isolates are mostly referred to as *A. xylosoxidans* given that phenotypic-based identification through biochemical tests often yields inconclusive results. Accurate species identification relies on genotypic methods, although these are not commonly used in routine identification.

These bacteria are commonly associated with aquatic environments and frequently contaminate aqueous solutions in hospitals, posing a risk of healthcare-associated infections. Indeed, they are increasingly recognized as nosocomial pathogens, particularly affecting immunocompromised individuals and patients with cystic fibrosis [2,3].

Treatment of *Achromobacter* spp. infections is challenging since these bacteria often display multidrug resistance. Additionally,

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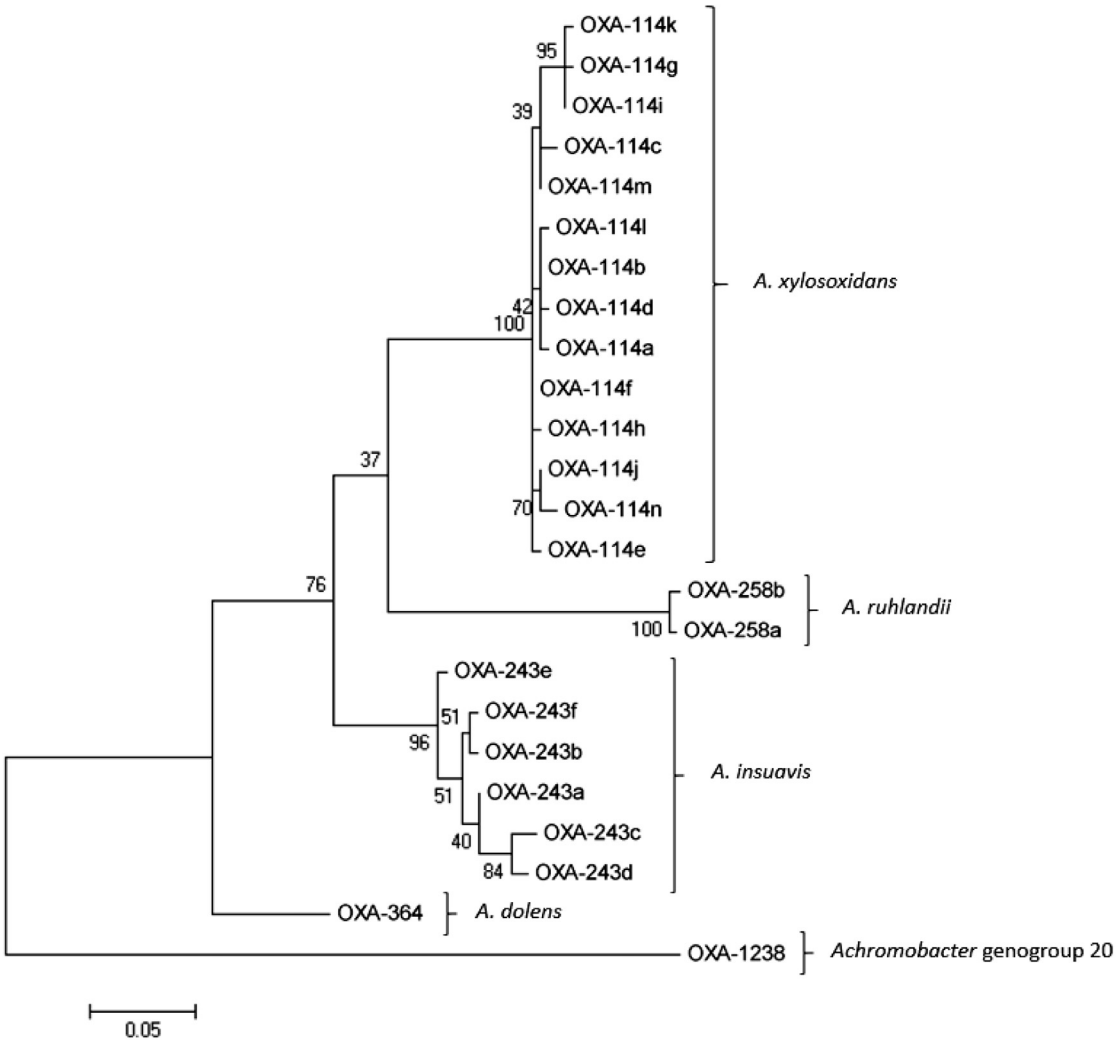

Fig. 1. Phylogenetic tree of OXA variants described in *Achromobacter* spp. using the Maximum Likelihood method.

standard antiseptics and disinfectants may not be effective in eradicating them from hospital environments. Outbreaks and pseudo-outbreaks linked to contaminated fluids, such as chlorhexidine, have been reported [2,3].

Isolate 413638 was recovered from a chlorhexidine solution and was responsible for a nosocomial outbreak in Buenos Aires, Argentina [3]. It was identified as *Achromobacter* genogroup 20 by *nrd*A and belonged to ST365 according to MLST scheme. Antimicrobial susceptibility was determined following CLSI guidelines (https://clsi.org/). The isolate exhibited resistance to third- and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones (table S1 -supplementary material).

The aim of this study was to describe *Achromobacter* genogroup 20 genome and to explore the presence of different resistance determinants.

WGS was performed using Illumina MiSeq NextSeq500 with 150 bp paired-end reads. De novo assembly was conducted with Unicycler (Galaxy). RAST server (https://rast.nmpdr.org/) and BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to predict and confirm all open reading frames (ORFs). The presence of resistance determinants was assessed using Resfinder v.4.6.0 with a threshold for identification of 85% (http://genepi.food.dtu.dk/resfinder) and manual curation. Default parameters were used for all software.

De novo assembly of the Illumina reads generated 224 contigs (>200 bp). The N_{50} and L_{50} values were 60 524 and 35, respectively, and the longest contig was 242 112 bp in length. The genome size was 6 795 788 bp and presented an average GC content of 67.4%. A total of 6562 coding sequences (CDS) were predicted.

Different putative antibiotic resistance genes were identified, including three β-lactamases coding genes, 32 efflux pump genes, two genes coding for aminoglycoside-modifying enzymes, nucleotide substitutions in the quinolone resistance-determining regions (QRDRs) of *par*C and *par*E, and the presence of a dihydropteroate synthase coding gene (table S2 -supplementary material).

β-lactamase ORFs corresponded to a new OXA type-class D β-lactamase and two new putative class C β-lactamases. The novel class D β-lactamase was designated as OXA-1238 and differed from OXA-114a by 85 amino acids, exhibiting 69% identity. In 2008, Doi et al. recommended the detection of $bla_{\text{OXA-114}}$ as a species-specific marker for the accurate identification of *A. xylosoxidans* [4]. Additionally, Papalia et al. and Traglia et al. proposed the amplification and sequencing of other OXA-type genes, specific to *A. ruhlandii* (OXA-258), *A. insuavis* (OXA-364), and *A. dolens* (OXA-243) [5]. A phylogenetic analysis was performed, including the novel OXA-1238 and other OXA variants previously described as

specific markers for other species of *Achromobacter*. The alignment was conducted using ClustalX v.2.1 (http://www.clustal.org/clustal2/), and a dendrogram based on maximum-likelihood estimation was constructed using MEGA v.5.05 software (https://www.megasoftware.net/) (Fig. 1).

The $bla_{\text{OXA-1238}}$ gene was investigated in all *Achromobacter* spp. genomes deposited in the NCBI database (n: 453) (accessed on April 2024). Coding genes for OXA-1238 variants, with a 97% amino acid sequence identity, were identified only in three genomes deposited as *Achromobacter* spp namely strain AONIH1 (ASM290290v1), strain Marseille-Q0513 (ASM1819569v1) and strain Gw_UH_bin_173 (ASM1799053v1).

The average nucleotide identity (ANI) and tetranucleotide analysis (TETRA) were calculated for these genomes using the online tool JSpeciesWS (https://jspecies.ribohost.com/jspeciesws/) (Ribocon GmbH - Version: 4.2.1). Strains of the same species must exhibit a ANI higher than 95–96% using one or both comparison methods, MUMmer or BLAST (ANIm or ANIb, respectively), while TETRA analysis must show a value higher than 99%. Based on ANI and TETRA analysis, all three genomes corresponded to *Achromobacter* genogroup 20 (Tables S3, S4 and S5—supplementary material), despite one genome displayed low quality due to its metagenomic origin. A Genome Blast Distance Phylogeny (GBDP) analysis was conducted among the *Achromobacter* genogroup 20 genomes (the one recovered in this study and those identified from NCBI) and selected reference *Achromobacter* genomes (https://tygs.dsmz.de) (Fig. 1—supplementary material).

Regarding efflux pumps, complete sequences of AxyABM, AxyXY-OprZ and AxyEF-OprN were identified. Previous reports on *A. xylosoxidans* have determined that AxyABM can extrude aztreonam and cephalosporins, while AxyXY-OprZ has a broader spectrum, including aminoglycosides, cefepime, carbapenems, fluoroquinolones, tetracyclines, and erythromycin. AxyXY-OprZ serves as the primary determinant for high-level intrinsic aminoglycoside resistance in *Achromobacter* spp. Additionally, the RND efflux system AxyEF-OprN appears to be involved in fluoroquinolone resistance.

Mutations Q80V in ParC, and T395E and K525S in ParE were identified. Although these mutations are located in the QRDR region, they do not correspond to those previously observed by Furlan et al. in environmental fluoroquinolone-resistant *Achromobacter* spp. isolates [6].

This study provides the first description of *Achromobacter* genogroup 20 genome and an analysis of its resistome. Antibiotic resistance seems to be multifaceted, involving various efflux pumps, enzymes, and their regulatory mechanisms, although further studies are necessary to assess their impact on the resistance profile. Additionally, we identified a novel OXA variant in *Achromobacter* genogroup 20, which could potentially serve as a species-specific marker.

The genome of isolate 413638 was submitted to the GenBank database under the accession number JBICCO0000000000 (Genome assembly ASM4311940v1).

Declaration of competing interests

The authors declare there are no conflicts of interests.

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Ethical approval

The ethics committee of FFyB-UBA approved this study (RESCD-2021–216-E-UBA-DCT). The isolate was delivered anonymized from the hospital to IBaViM-FFyB-UBA, in order to preserve patientś identity.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.11.012.

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