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Dynamic Evolution of *Achromobacter xylosoxydans* in a Patient with Leukemia Receiving Antibiotic Treatment

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Sir,

Members of the *Achromobacter* genus are Gram-negative opportunistic pathogens that are ubiquitous in soil and water ecosystems [1]. Initially isolated from patients with chronic otitis media [1], *Achromobacter xylosoxidans* has since been linked to severe

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Conflicts of Interest: The authors declare no conflict of interest.

Access to data

The Whole Genome Shotgun project was deposited in GenBank with accession numbers JAXQNU000000000, JAXQNV000000000, and JAXQNW000000000 for Ax114, Ax115, and Ax130, respectively.

Ethical statement

All procedures performed in this study met the ethical standards of Hospital de Clínicas Jose de San Martín, Buenos Aires, Argentina, and the 1964 Declaration of Helsinki and further amendments. Informed consent was obtained from the patient involved in the study.

acute and chronic infections in high-risk individuals, including those with compromised immunity and chronic debilitating conditions such as cancer, diabetes, renal disease, or lung disease. *A. xylosoxidans* is also now recognized as an emerging pathogen in cystic fibrosis patients that is usually resistant to many antibiotics [1, 2]. *A. xylosoxidans* can infect multiple anatomical sites, such as soft tissues, bones, wounds, skin, eyes, middle ear, urinary tract, gastrointestinal tract, lower respiratory tract, bloodstream, and central nervous system, suggesting that the organism has metabolic adaptability to survive in diverse environments and potentially evade host immune responses [1]. Here, we describe rapid genomic modifications accompanied by the acquisition of antibiotic resistance in *A. xylosoxidans* sequentially isolated from a leukemia patient receiving antibiotic treatment.

A child with a history of chronic myeloid leukemia developed a respiratory infection caused by an *A. xylosoxidans* strain, Ax114, susceptible to meropenem (MIC 1.5 μg/ml), colistin (MIC 2 μg/ml), and ceftazidime-avibactam (MIC 4 μg/ml). Although, at first, the patient responded to the treatment, there were recurrences of the infection that were treated with supplementary antibiotics because the etiologic agent became resistant to meropenem (MIC 24 μg/ml) (strain Ax115) and then to meropenem (MIC 24 μg/ml) and ceftazidime/avibactam (MIC 32 μg/ml) (strain Ax130). The patient could not recover and subsequently died. In brief, the antibiotic treatments attempted were, first a combination meropenem and amikacin, second meropenem, colistin, and vancomycin; and finally, the latter treatment was supplemented with levofloxacin, azithromycin-loaded liposomal amphotericin, ceftazidime/avibactam, and tigecycline. A complete and detailed description of the treatments and outcomes, as well as the resistance profiles of all three isolates (Table S1), are described in the Supplementary material.

The genetic characteristics of all three strains were compared by whole genome sequencing. *In silico* multi-locus sequence typing (MLST) showed that the three genomes were of sequence type 184 (ST184). A core genome phylogeny analysis of 132 *A. xylosoxidans* genomes from GenBank revealed that all three sequenced genomes were closely related (Fig. 1A). The *A. xylosoxidans* 2011Ycf922II and 2015Y70663831 strains originated from a cystic fibrosis sputum sample, were phylogenetically related to this study's isolates.

Eleven mutations were identified in the Ax115 and Ax130 strains compared with the parental strain Ax114 (Table S3, Supplementary material). Five mutations were common to both strains, while one and five were exclusive to strains Ax115 and Ax130, respectively. Six mutations were non-synonymous, and five were intergenic. No mutations in promoter or regulatory sequences were identified. Two of the non-synonymous mutations exclusively found in Ax130 were located within *penA*, a gene encoding peptidoglycan D,D transpeptidase (Table S3). Mutations homologs to this gene have been associated with antimicrobial resistance phenotypes such as reduced susceptibility to cefepime and ceftriaxone [3]. Furthermore, considering that β-lactams inhibit the transpeptidase activity of PBP proteins through a covalent bond, these *penA* mutations most probably are associated to the observed resistance to ceftazidime-avibactam. In addition, non-synonymous mutations were also found in a protein containing the EAL domain that is involved in c-di-GMP degradation, a trait linked to virulence and biofilm formation in the *Burkholderia cepacia* complex, that can be one of the contributors factors to increase antimicrobial resistance seen

in both strains (Ax115 and Ax130) [4](Table S3, Supplementary material). The number of genes that are not present in all three genomes ascended to 209. Strains Ax115 and Ax130 had 36 and 26 unique genes, respectively; 90% coded hypothetical proteins. All three strains shared a class 1 integron containing a *dfrA17* gene cassette. Strains Ax115 and Ax130 possessed a second integron that included *aadB*-$bla_{\text{oxa-2}}$ in the variable region.

Transcriptional analysis carried out by qRT-PCR showed that the mRNA level of $bla_{\text{AXC-1}}$ was 1.5-fold higher in strain Ax130 than in Ax114 (Fig. 1B), while $bla_{\text{OXA-114}}$ mRNA in strains Ax115 and Ax130 was 1.9- and 4-fold higher than in Ax114 (Fig. 1C). No transcripts of genes encoding the efflux pumps *axyEF-OprN*, *axyABM*, *axyXY-OprZ*, and *axyEF-OprN* were detected. Biofilm formation was higher for Ax115 and Ax130 than Ax114 (Fig. 1D). Motility was higher for Ax115 and Ax130 after 24 h incubation at 37°C. However, after 48 h, motility was greater for Ax114 and Ax115 (Fig. 1E).

This study showcases the rapid adaptability of *A. xylosoxidans* to antibiotic exposure during treatment. The similarity between all three isolates observed through whole genome sequence analysis supports the hypothesis that the same strain evolved as antibiotic treatments progressed. This case is particularly interesting because the rapid response to antibiotic exposure included not only the enhancement of resistance to antibiotics used in the subsequent treatments but also quickly modified phenotypic manifestations of potential virulence factors like motility and ability to form a biofilm. This adaptability occurred through genetic modifications that permit bacteria to thrive in the host and resist antibiotics using multiple mechanisms, some direct, like enhancing or acquiring modifying enzymes, and some indirect, like endowing the cells with other phenotypes like modifications in biofilm formation and motility that indirectly may help them to prevent the toxic action of antibiotics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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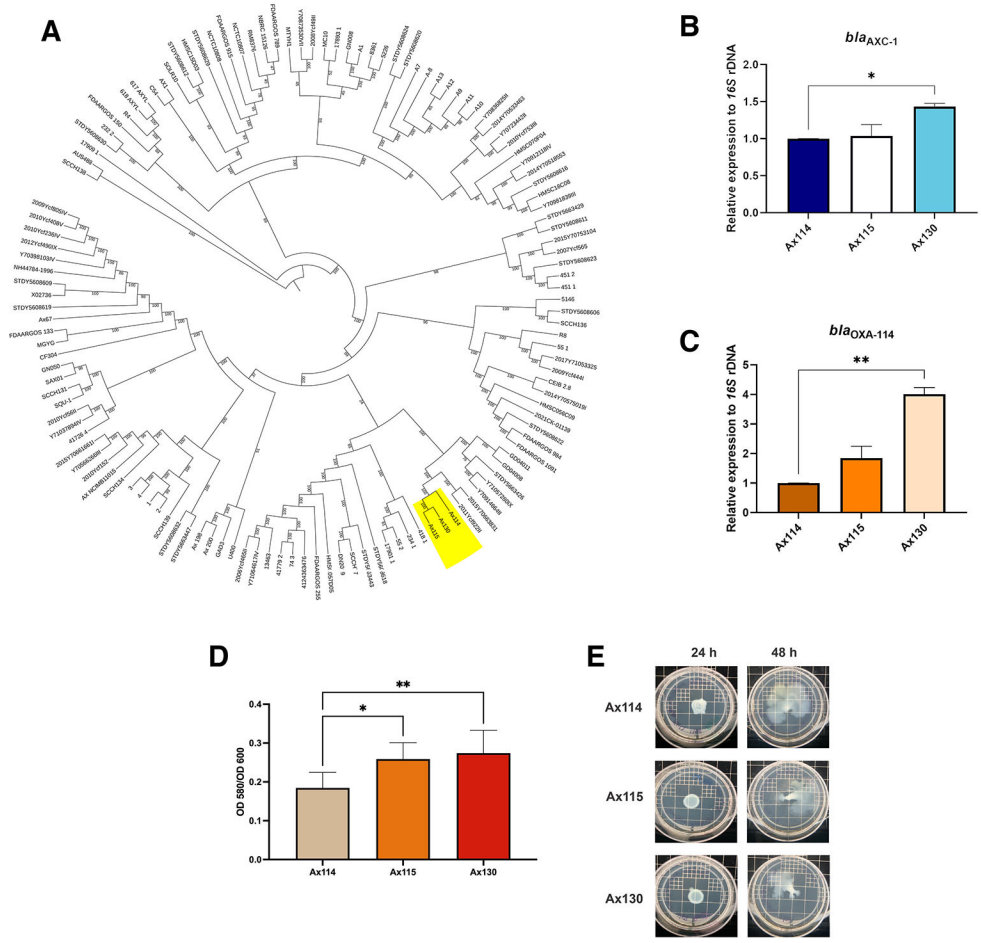

Fig. 1.

A. Core-genome phylogenetic analysis of Ax114, Ax115, Ax130, and 129 genomes of *A. xylosoxidans* available in the NCBI genome database. Table S2 (Supplementary material) shows the list of genomes analyzed. Clean reads generated by whole genome sequencing were *de novo* assembled using SPAdes v3.11.1 [5]. Contigs smaller than 500 bp were discarded to ensure sequencing quality. This procedure resulted in 40, 234, and 216 contigs of the Ax114, Ax115, and Ax130 genomes, respectively. The scaffold N_{50} values were 446.052, 61.480, and 64.505 bp for Ax114, Ax115, and Ax130, respectively. The overall G + C content was 67.49%. The figure displays the maximum likelihood phylogeny of 132 *A. xyloxosidans* sequences. The bootstrap method was used as a supporting method (1000 iterations). The molecular substitution model was GTR. The tree representation was produced with iTOL. Yellow highlights represent genomes sequenced in this study.

B and C. Transcriptional analysis of genes encoding $bla_{\text{AXC-1}}$ (B) and $bla_{\text{OXA-114}}$ (C). RNA was extracted from three independent biological replicates for each strain. cDNA was synthesized and used for qPCR using specific primers for $bla_{\text{OXA-114}}$, $bla_{\text{AXC-1}}$, *axyEF-OprN*, *axyABM*, *axyXY-OprZ*, and *axyEF-OprN* (Table S4, Supplementary material). At least three independent cDNA replicates were tested in triplicate. Data were quantified using the $2^{-\Delta Ct}$ method [20], with 16S RNA polymerase as a normalizer. Differences were determined by ANOVA followed by Tukey's multiple comparison test ($p < 0.05$) using

GraphPad Prism (GraphPad Software v10.0.0, San Diego, CA). **D.** Biofilm formation and motility comparison between Ax114, Ax115, and Ax130. At least three independent replicates were performed from each strain. ANOVA followed by Tukey's multiple comparisons was performed using GraphPad Prism. A P-value < 0.05 was considered significant. Data are presented as mean ± SD. **E.** Cells of Ax114, Ax155 and Ax130 were inoculated on the surface of swimming plates. Plates were inspected and photographed after incubated 24 and 48 hours at 37°C.