

## XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024

Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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## **DISSEMINATION OF ANTIBIOTIC RESISTANCE MODULES BY IS26-MEDIATED TRANSPOSITION IN *Acinetobacter baumannii* PLASMIDS**

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*Acinetobacter baumannii* (Ab), a Gram-negative opportunistic pathogen, is a leading cause of nosocomial infections. The rapid rise in multidrug-resistant (MDR) Ab strains underscores the critical need to understand the pathogen's evolutionary dynamics within clinical environments. The acquisition of antimicrobial resistance genes (ARGs) is frequently associated with mobile genetic elements (MGEs) such as plasmids, transposons, and insertion sequences. Ab242, Ab244, and Ab825 are epidemiologically related MDR Ab strains belonging to the clonal complex CC15 prevalent in our region. They were isolated in a hospital from Rosario and characterized in our laboratory. Ab825 and Ab242 are carbapenem-resistant (carbR), harboring resistance plasmids pAb825\_36 and pAb242\_25, respectively. These plasmids contain a *bla*OXA-58 and *TnaphA6* resistance module (RM) conferring resistance to carbapenems and aminoglycosides, respectively. Additionally, they carry an aminoglycoside resistance gene (*aacC2e*), bracketed by two IS26 elements, which imparts gentamicin resistance. *Ab244*, a *carbS* strain, houses a different plasmid, pAb244\_7, where IS26 forms a composite pseudo-transposon (Tn6925) carrying the ARGs *bla*TEM and *aacC2e*, conferring resistance to  $\beta$ -lactams and gentamicin. This pseudo-transposon was found in plasmids present in other related strains belonging to the CC15 from Latin America. In this work, we combine PCR amplification and sequencing, transformation assays and new MinION long-read data (Oxford Nanopore), to characterize the plasmid content carried by these clinical strains. We focused on the analysis of different transposon-like structures formed by IS26 elements, an insertion sequence belonging to the IS6 family, which is often implicated in the spread of ARGs among Ab and other Gram-negative bacteria. We show experimental evidence indicating that IS26 mediates various intragenomic rearrangements in these strains, involving various non-replicative translocable units (TUs) for the mobilization of ARGs. Thus, leading to multiple plasmid variants, some harboring both ARGs, some with only one, and others with none of them, all coexisting within the same cell population. Moreover, we observed that the resistance genes are not lost after THG mechanisms, even in the absence of antibiotic selective pressure. Overall, our results provide insights into the roles and mechanisms of IS26 in the dissemination of antibiotic resistance genes and further support the idea that MGEs enhance genetic diversity and genome plasticity in bacteria, thereby affecting their adaptability and evolution.

Palabras clave: *Acinetobacter baumannii* - resistance plasmids - IS26 modules - transposition - blaOXA-58