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Unravelling 16S rRNA methyltransferase-carrying plasmid dynamics in wastewater treatment plants by Nanopore Whole Genome Sequencing analysis

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Background: Waste water treatment plants (WWTPs) play a critical role in antibiotic resistance dissemination, since they are niches where different bacterial elements from diverse origins converge. 16S rRNA methyltransferases (methylases) confer high-level resistance to all clinically relevant aminoglycosides, including plazomicin. The aim of this work was to dissect the genomic structure of the plasmids harboring this resistance mechanism in *E. coli* from WWTPs by Illumina-Nanopore WGS analysis and assess their association with worldwide disseminated plasmids.

Materials/methods: Twenty-five *E. coli* isolates high-resistant to aminoglycosides from two WWTPs in Barcelona were fully sequenced by Illumina technology (MiSeq), and 6 representative isolates were sequenced by Nanopore platform (MinION). Hybrid assemblies were performed with Unicycler. Prokka was used to annotate the methylase-carrying plasmids, backbone comparison was carried out by BRIG and variable region analysis was performed by EasyFig. SNP-trees were obtained for plasmids of the same replicon type to analyze the global plasmid dissemination using SMALT, RAXML and Microreact.

Results: All *E. coli* isolates harbored the methylase gene *rmtB*, except two that harbored *armA*. *rmtB*-carrying isolates belonged to sequence types (ST) 1196, which has been associated to clinical settings and carbapenemase production, and ST224, a multidrug-resistant sequence type adapted to human and animal niches. Two IncFII plasmid types, with similarities to pHN7A8 and pC15-1a, were the carriers of *rmtB*. Among ST1196 isolates, both plasmid types were present, but *rmtB* was located on pHN7A8; whereas ST224 *E. coli* harbored pC15-1a but not pHN7A8. On the one hand, pHN7A8 originated in Asia and has never been described in Europe. It has a conserved structure, being a key element in the dissemination of *rmtB* in human environments. On the other hand, pC15-1a is disseminated in different sources worldwide and has a dynamic resistance gene content.

Conclusions: Our results show the high prevalence of methylases in WWTPs in Spain and the flux of methylase genes from conserved plasmids and bacteria to new genetic platforms and hosts that are flexible and adaptable. This process favors the emergence, selection and dissemination of successful high-risk associations that can reach eventually new ecological niches.

