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Abstract No.
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Acquired 16S rRNA methyltransferase *armA* maintained for a decade in a veterinary hospital via an IncR plasmid

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Antimicrobial resistance (AMR) is one of the major threats to Public Health that our society is facing nowadays. Aminoglycosides remain one of the most effective antibiotic classes to fight infections caused by gram negative bacteria. However, the emergence of the acquired 16S rRNA methyltransferases jeopardizes the use of such needed antibiotics.

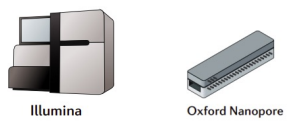
Horizontal gene transfer through plasmids plays a pivotal role in AMR. This phenomenon is especially relevant in hospital environments, since they are a major reservoir for plasmid-mediated antibiotic resistance. IncR plasmids that carry different AMR genes have been reported worldwide within the *Enterobacteriaceae* family. Therefore, the aim of the present study was to perform a comparative analysis of IncR plasmids to understand their epidemiological dynamics.

Methods

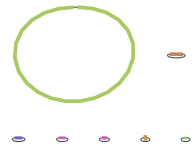
1 Screening of aminoglycoside resistant isolates processed during 2018 and 2019 at Hospital Clínico Veterinario Complutense (HCVC)

2 Antibiotic susceptibility testing via broth microdilution method

3 Whole Genome Sequencing of isolates presenting high level resistance to aminoglycosides using Illumina and Nonopore technologies

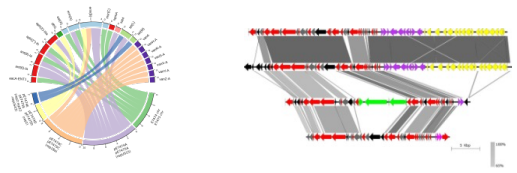


4 Assembly was performed with long reads using Flye and polished with Medaka. Assemblies were corrected with short read data using Pilon



5 Taxonomic clasification of the isolates was carried out with mlst. Assignation to a phylogenomic group was performed using Snippy, gubbins and RAxML

6 Genomic structures were annotated using Prokka and compared with Easyfig and GCviewer



Results

Of the 1451 isolates processed by the microbiology laboratory at HCVC, 30 *Enterobacteriaceae* isolates presented resistance to aminoglycosides. Aminoglycoside susceptibility tests of those 30 isolates were performed and only isolate 3472, that was recovered from the wound of a horse, showed a high level of resistance. Full antibiotic resistance pattern of this isolate was assessed (Table 1). Interestingly, resistance to β-lactams and fluoroquinolones can be observed.

Antimicrobial	AMK	NEO	SMX	TMP	CIP	TET	MERO	AZI	NAL	FOT	CHL	TGC	TAZ	COL	AMP	GEN
MIC (mg/l)	>256 R	16 I	>1024 R	>32 R	>8 R	>64 R	0.12 S	>64 R	>128 R	2 I	64 R	2 S	2 S	<0.1 S	>64 R	>32 R

Table 1. Minimum inhibitory concentration (MIC) of isolate 3472 to the different antimicrobials tested.

Taxonomic characterization of isolate 3472 showed that it belongs to the *Enterobacter cloacae* complex, being specifically an *Enterobacter hormaechei* ST171. Further classification of the isolate shows that it belongs to *Enterobacter hormaechei* subsp. *xiagfangensis* phylogenomic group (Figure 1).

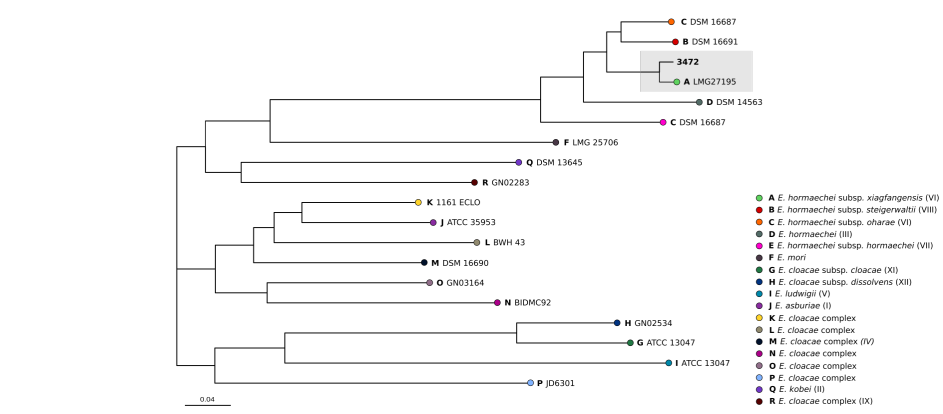
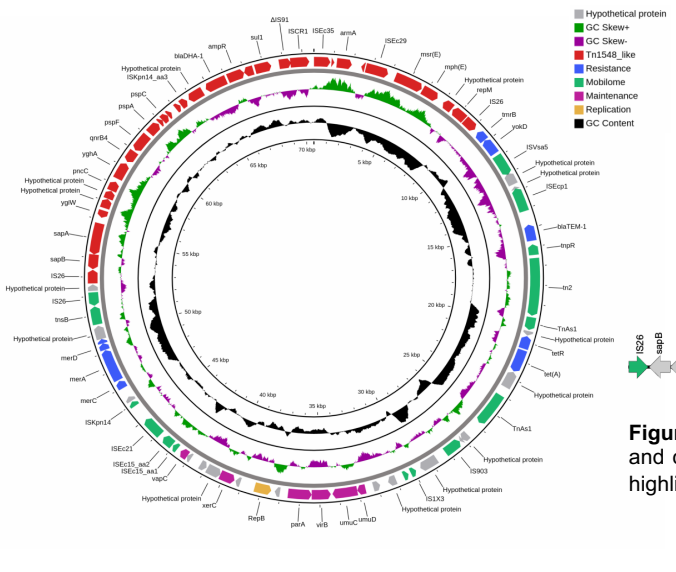


Figure 1. Phylogenetic analysis identifies isolate 3472 as most closely related to *Enterobacter harmaechei* subsp. *xiagfangensis* (Group A) .



The genome of isolate 3472 consists of a chromosome of 4747 kb and five plasmids ranging between 2 and 186 kb, including an IncR plasmid of 70 kb. This IncR plasmid is of great interest because it harbours the resistance genes *armA*, bla_{DHA-1} and *qnrB4*, among others, in a Tn1548-like element (Figure 2).

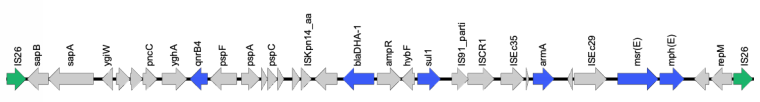


Figure 2. Genomic structure of IncR plasmid belonging to isolate 3472 (left) and close view of Tn1548-like structure with antimicrobial resistance genes highlighted (right).

This Tn1548-like element is identical to the one found by Hidalgo *et al*.[1] in five isolates collected in the same veterinary hospital throughout 2008 to 2010, and was also embedded in an IncR plasmid. WGS of these strains and plasmid comparison of their IncR plasmids with the one from isolate 3472 showed that they are are extremely similar – 100% coverage, 99.9% identity (Figure 3).

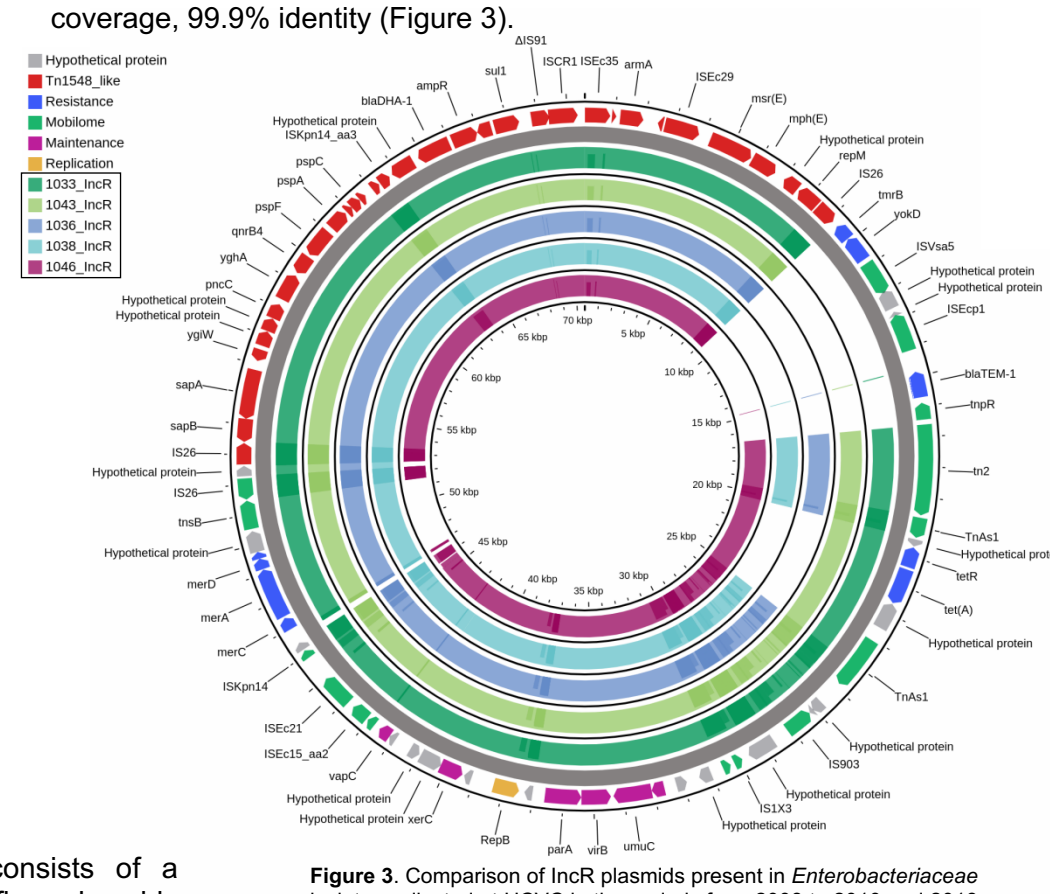


Figure 3. Comparison of IncR plasmids present in *Enterobacteriaceae* isolates collected at HCVC in the periods from 2008 to 2010 and 2018 to 2020.

Conclusions

We report evidence of the horizontal transfer of an IncR plasmid between different bacterial species from the *Enterobacteriaceae* family. This IncR plasmid is responsible for the maintenance and spread of *armA* in a single veterinary hospital for a decade. In addition, clinical treatment of infections caused by enterobacteria harbouring this plasmid may be endangered due to the association of *armA* with resistance determinants to β-lactams and fluoroquinolones in a Tn1548-like element.

[1] Hidalgo, L. et al. *Klebsiella pneumoniae* sequence type 11 from companion animals bearing ArmA methyltransferase, DHA-1 β-lactamase, and QnrB4. *Antimicrob. Agents Chemother.* **57**, 4532–4534 (2013)