Applying a **hybrid sequencing** strategy to a **One-Health** scenario for **colistin resistance** transmission among pigs, calves and the farmer

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INTRODUCTION

Colistin has been used in **Spain** during the last decades in **veterinary medicine** for the **treatment** of **enterobacteria infections**

Nowadays, colistin is considered a **last resort treatment for multi-drug resistant (MDR) gram-negative** bacteria causing infections in human

The aim of this work is to study colistin resistant *E. coli* isolated in a **mixed farm** (4 swine, 13 bovine and the farmer)

MATERIALS & METHODS

DNA extraction
QIAGEN UltraClean Microbial Kit

Sequencing
ONT MinION + SQK-RBK004
Illumina MiSeq 300-cycle kit v2
FAST5→ Albacore+Porechop→ FASTQ/BC

Assembly	Annotat.	Visualisation	Genome charact.
Flye - Nanopore	Abricate	Bandage	Prokka
Minimap2 - Nanopore	ISFinder	BRIG	BUSCO
Racon - Nanopore	Phaster	FigTree	CGE DTU MLST
Medaka x2 - Nanopore	PGAP	SnapGene Viewer	SerotypeFinder
Racon - Illumina	Prokka		VirulenceFinder
	OriTFinder		CSIPhylogeny
			Clermontyping

RESULTS

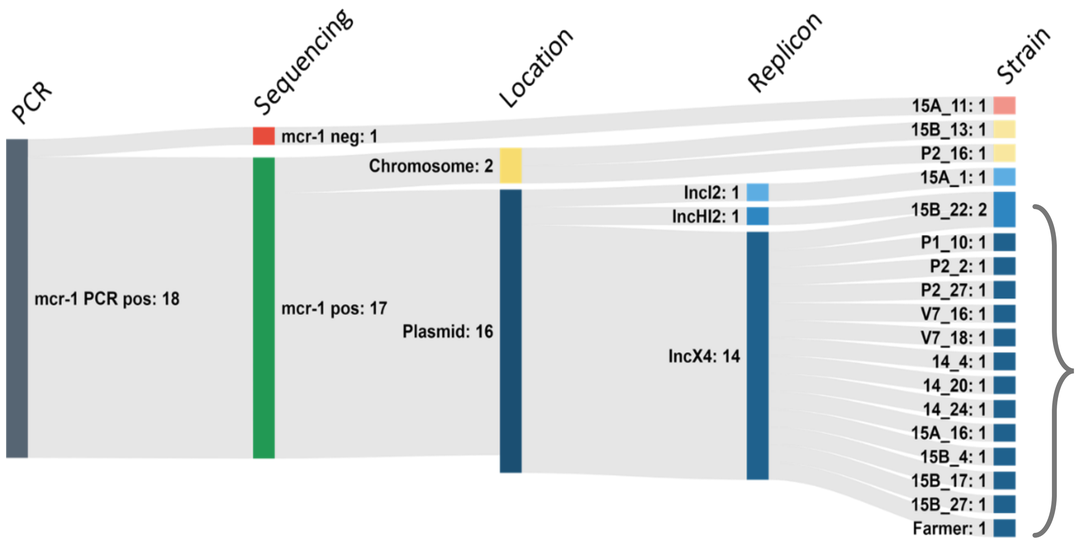


Figure 1. *Sankey diagram from the strains positive for mcr-1 gene build with http://sankeymatic.com/. pos, positive; neg, negative*

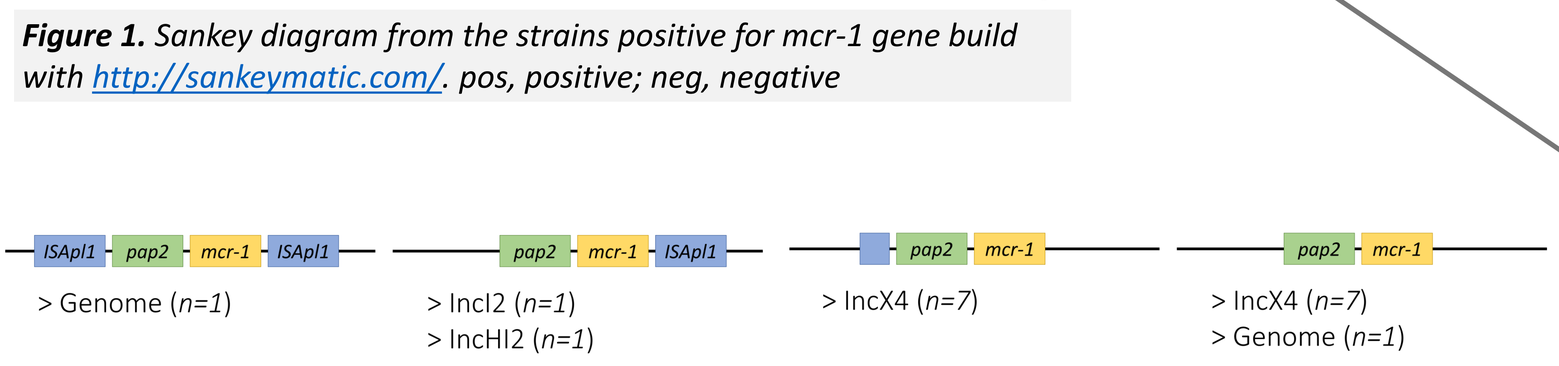


Figure 3*. Genomic structures found nearby mcr-1 gene*

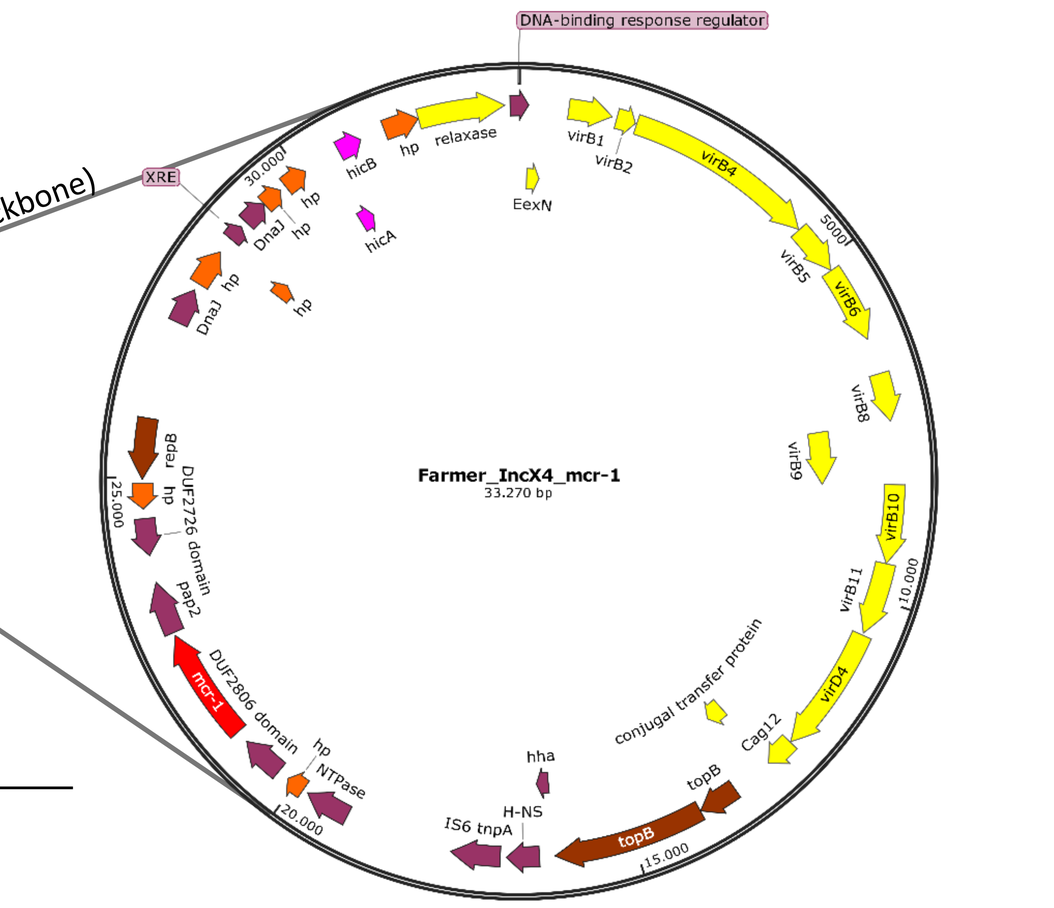
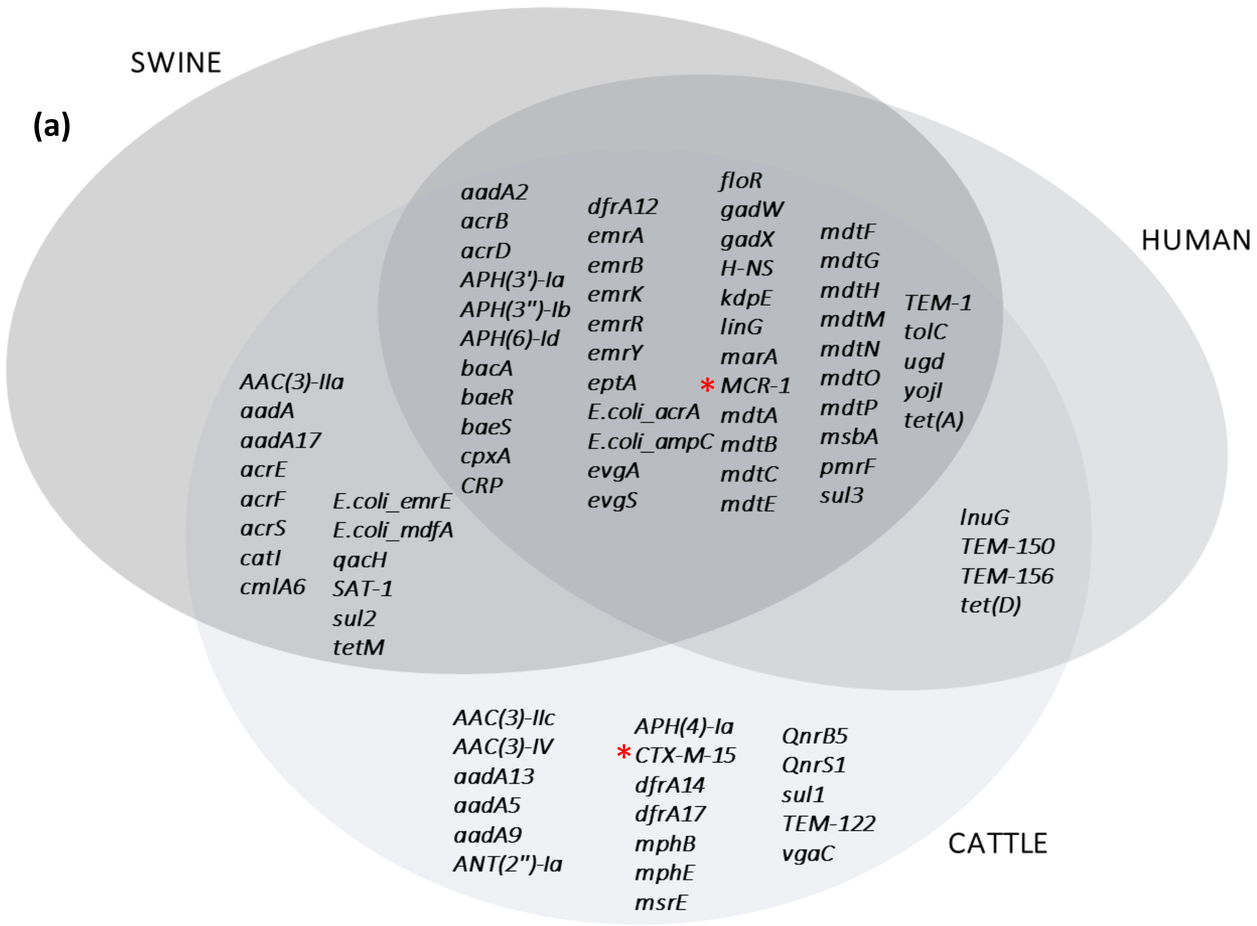


Figure 2. *Prokaryotic Genome Annotation Pipeline (PGAP) annotation of the IncX4 plasmid from the Farmer visualized with SnapGene Viewer*

- Type IV Secretion System (conjugation)
- Toxin-Antitoxin system (plasmid maintenance and stability)
- Plasmid replication elements
- Resistance to colistin



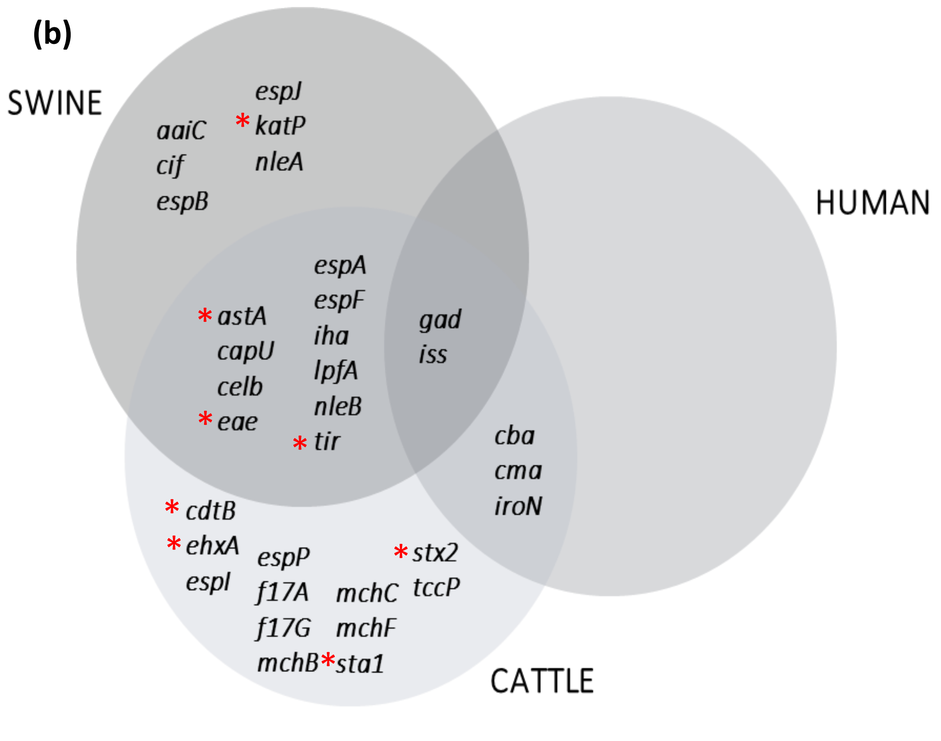


Figure 4. *Venn diagrams of the **(a)** Antibiotic Resistance Genes (ARGs) and **(b)** virulence factors described in this study*
**most concerning ARGs and virulence factors for human health*

CONCLUSIONS

1. This mixed farm resulted in a hotspot for ARGs and virulence factors: all isolates were MDR to virtually all families of ATBs, and some strains coded for life-threatening virulence factors such as shiga toxin or cytolethal distending toxin
2. Cattle showed the highest diversity of ARGs, virulence factors and plasmid-mediating antibiotic resistance
3. A hybrid sequencing approach (Nanopore/Illumina) is a perfect strategy in order to perform a Whole Genome Sequencing from *E. coli*