

IN SILICO DETECTION OF CONJUGATIVE PLASMIDS IN *E. COLI* FROM BOVINE

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Abstract

The purpose of this research is to detect the conjugative plasmids in *E. coli* from bovine animals and to study antibiotic resistance genes (ARGs) and virulence genes in plasmids. PlasmidFinder2.1 and VRprofile2 were used to detect plasmids in *E. coli* sequences downloaded from NCBI RefSeq. ARGs and virulence genes were detected by VRprofile2 and OriTfinder. Our results showed that ARGs were mostly found in the conjugative plasmids, while virulence genes were primarily found in non-mobilizable plasmids. Furthermore, the geographical distribution revealed that most *E. coli* downloaded sequences that contained ARGs, and virulence genes came from European countries and Asia.

Introduction

Escherichia coli are bacteria responsible for around 265,000 infections yearly in the United States¹. *E.coli* are found in the microbiome of healthy animals and humans, but they can become virulent in unhealthy individuals. Several strains are pathogenetic and cause symptoms like nausea, vomiting, and abdominal issues. Most of the infections are due to consumption of bovine food products².

The **overuse and misuse of antibiotics accelerate bacteria developing resistance**. Bacteria can acquire resistance by mutations and horizontally gene transfer (HGT)³. HGT can happen through transformation, conjugation, and transduction⁴. Conjugation is a process that bacteria directly transfer their genes from donor to recipient bacteria.⁴

The purpose of this research is to detect conjugative plasmids in *E. coli* from bovine animals and determine their antibiotic resistance and virulence genes, to provide insights for further solutions to the spread of antibiotic resistance and virulence. We hypothesized that the conjugative plasmids may have the most antibiotic resistance and virulence genes.

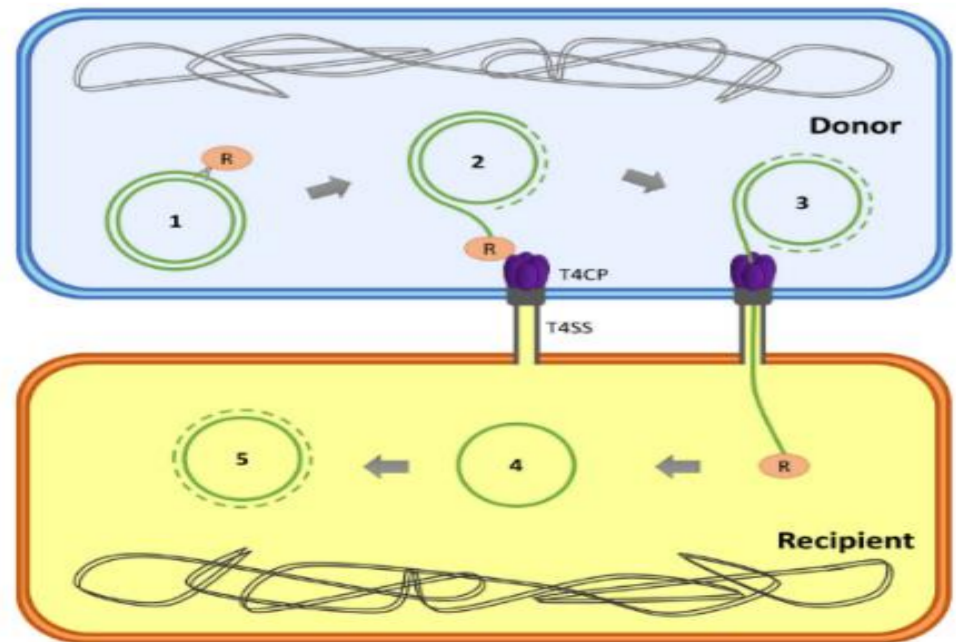
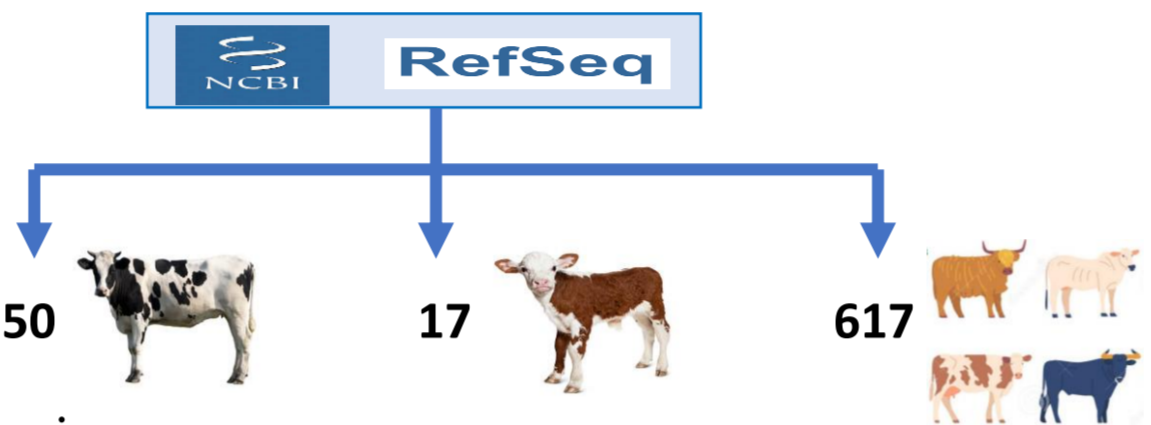


Figure 1. The process of donor bacterium transfers its plasmid to recipient by conjugation.

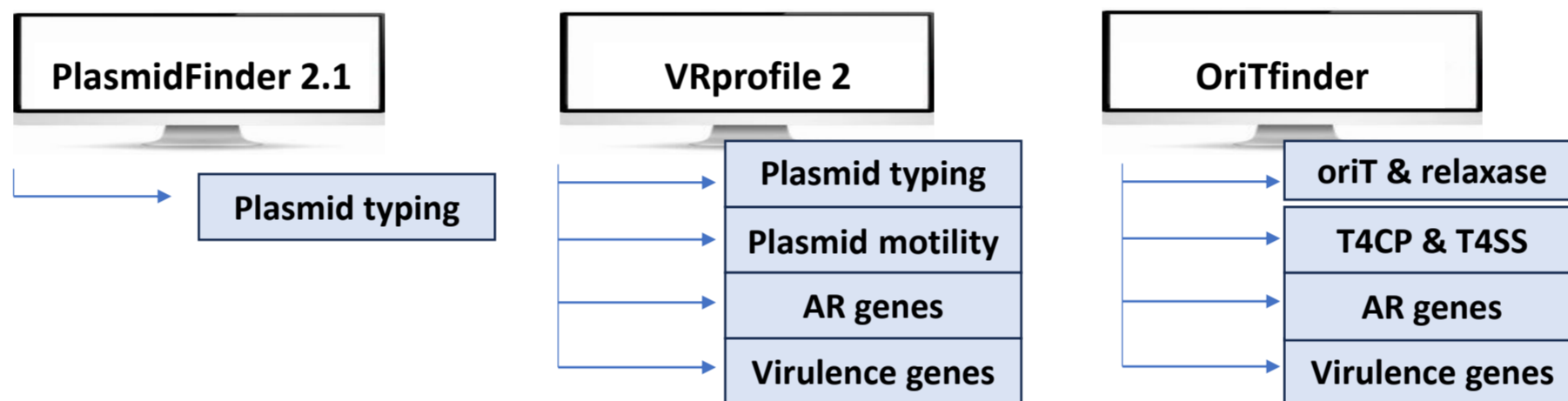
Legend: The relaxase will bind to the origin of transfer in the gene and it will cut the double-strand DNA, making a single-strand DNA. The relaxase will go from one cell to the other by the channel that is formed with T4CP and T4SS (pilus). When the DNA arrives in the other cell, relaxase has a reverse nicking reaction and it will make the DNA circular. Afterwards, the DNA will replicate to be the same as the original⁵.

Methodology

Downloaded 624 possible plasmid sequences of bovine animals



Put the downloaded sequences in:



Results

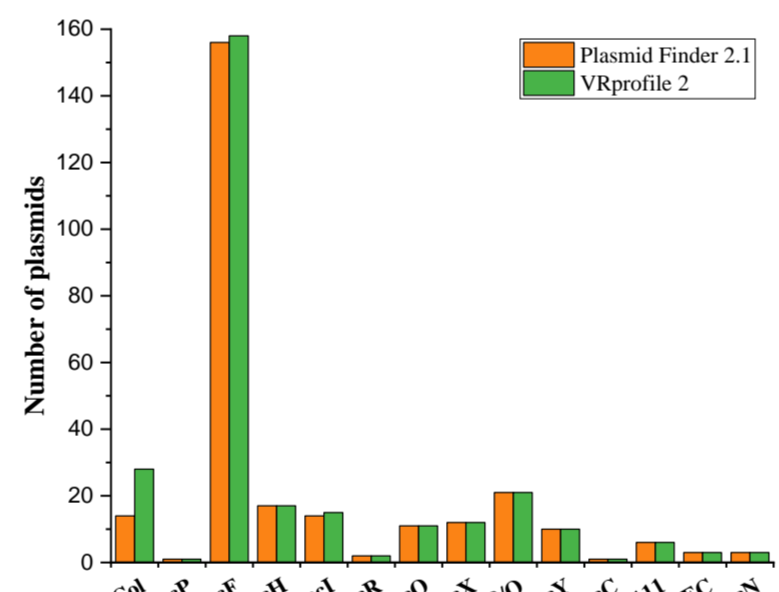


Figure 2. Detection and typing of plasmids

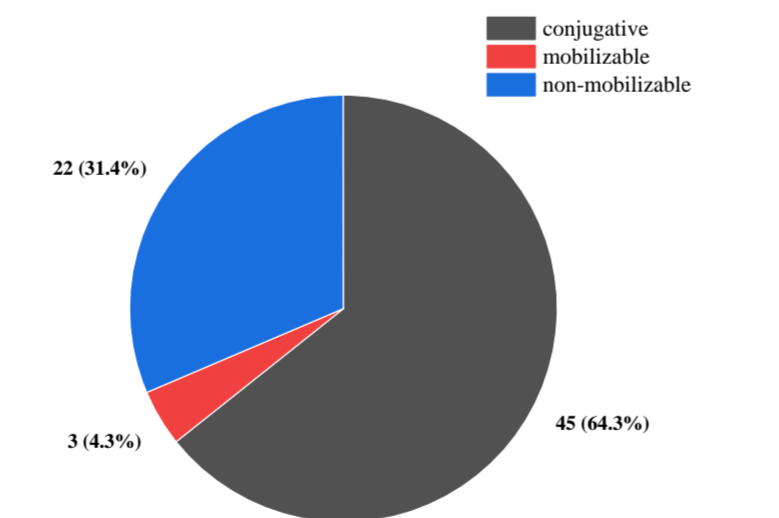


Figure 4. a: ARGs in conjugative, mobilizable, and non-mobilizable plasmids

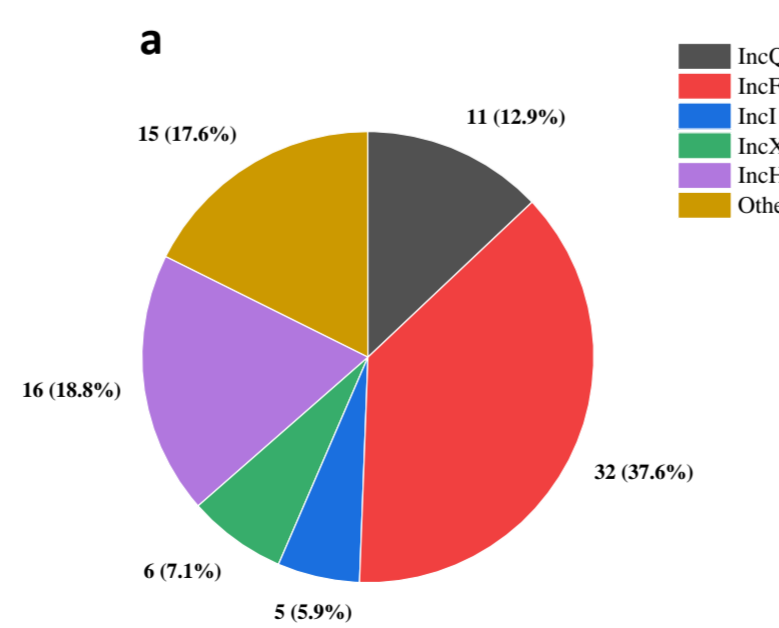


Figure 5. a: ARGs in different plasmid types

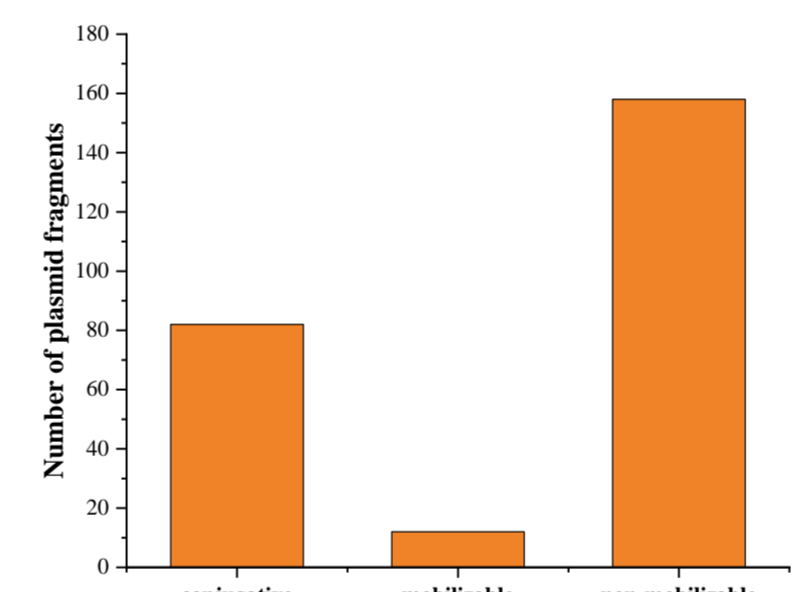


Figure 3. Detection of conjugative plasmids by PlasmidFinder2.1

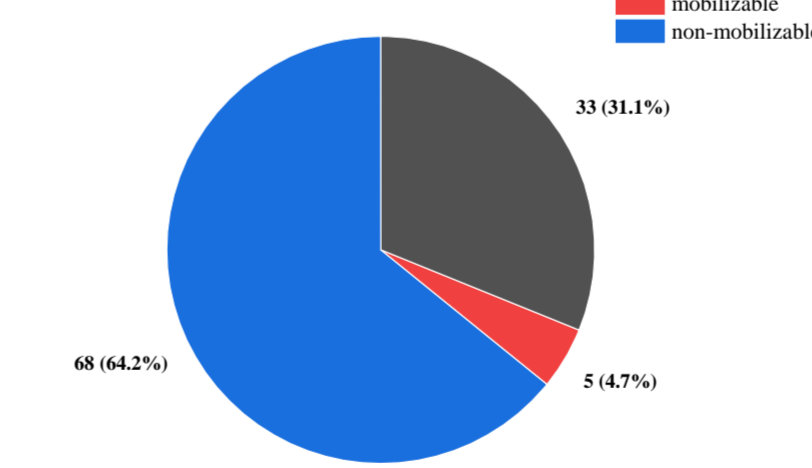


Figure 4. b: Virulence genes in conjugative, mobilizable, and non-mobilizable plasmids

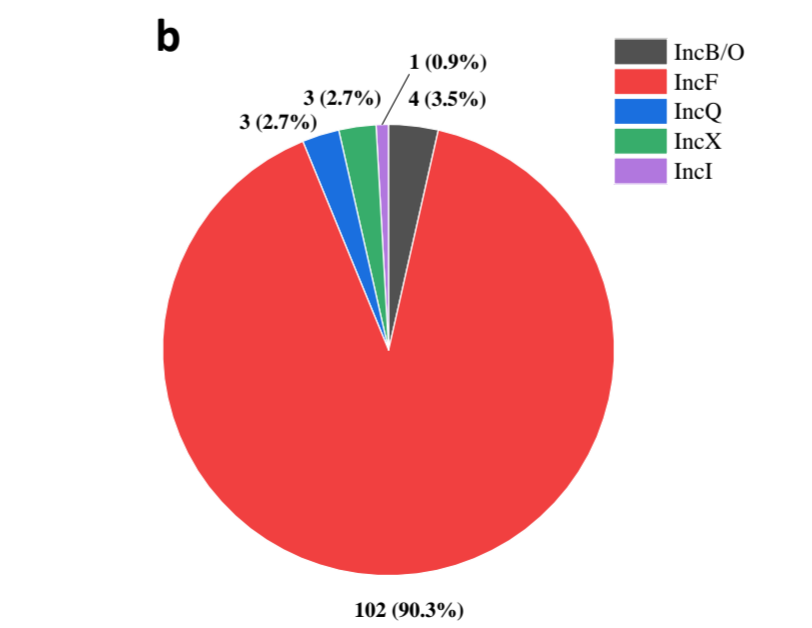


Figure 5. b: Virulence genes in different plasmid types

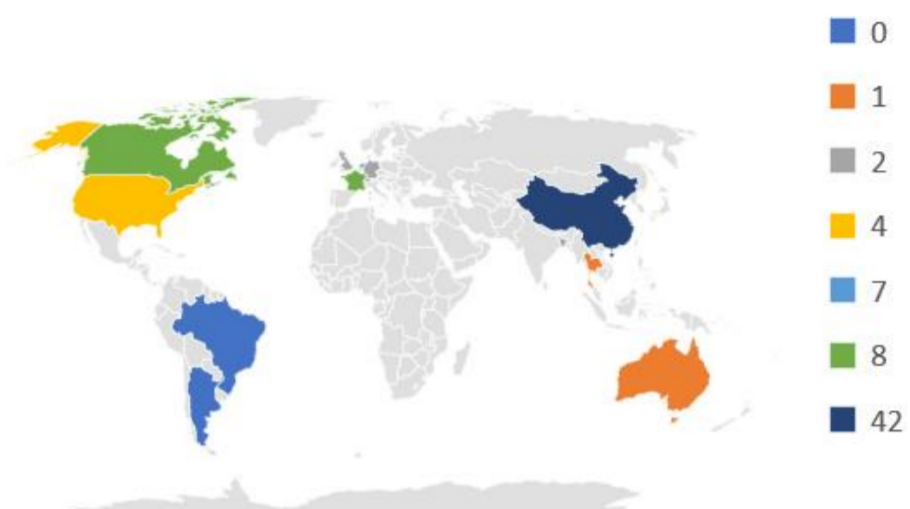


Figure 6. Conjugative plasmids in different countries worldwide

Discussion and Conclusion

The **IncF plasmids** represent one of the most prevalent plasmid groups of the *Enterobacteriaceae*.

- IncF plasmids can have multiple genes that are resistant to major classes of antimicrobials such as beta-lactams, aminoglycosides, tetracyclines, chloramphenicol and quinolone⁶.
- The presence of virulence-associated traits is almost exclusively found in the IncF plasmids⁶.

The **geographical distribution** of all *E. coli* downloaded sequences originated from 15 countries.

- China, France and the Netherlands were the main countries that contained antibiotic resistance genes and virulence genes.
- In a study made in 2013, researchers found that the southern regions of Europe had the highest prevalence of resistance⁷.
- From the 1970s to 2019, antimicrobial resistance doubled in China⁸.

In conclusion, the rising spread of ARGs and virulence genes is mostly attributed to plasmid-conjugation. The workflow of this research can also be used to detect plasmids and their antibiotic resistance and virulence genes in other types of bacteria and animals. Furthermore, an *in vitro* experiment was conducted to understand further the process of conjugation and the spread of antibiotic resistance genes.

References

- Centers for Disease Control and Prevention. (2016). *Escherichia coli (E. coli)*. <https://www.cdc.gov/ecoli/pdfs/CDC-E.-coli-Factsheet.pdf>
- Centers for Disease Control and Prevention. (2019). *E.coli Infection*. <https://www.cdc.gov/healthyets/diseases/ecoli.html#:~:text=day%2Dcare%20facilities,-E.,still%20appear%20healthy%20and%20clean>
- Munita, J. M., & Arias, C. A. (2016). *Mechanisms of Antibiotic Resistance*. Microbiology spectrum, 4(2), 10.1128/microbiolspec.VMBF-0016-2015. <https://doi.org/10.1128/microbiolspec.VMBF-0016-2015>
- Burmeister A. R. (2015). *Horizontal Gene Transfer: Evolution, medicine, and public health*, 2015(1), 193–194. <https://doi.org/10.1093/emph/eov018>
- Getino M, de la Cruz F.(2018). *Natural and Artificial Strategies To Control the Conjugative Transmission of Plasmids*. Microbiol Spectr. doi: 10.1128/microbiolspec.MTBP-0015-2016. PMID: 29327679.
- Yang, Q. E., Sun, J., Li, L., Deng, H., Liu, B. T., Fang, L. X., Liao, X. P., & Liu, Y. H. (2015). *IncF plasmid diversity in multi-drug resistant Escherichia coli strains from animals in China*. Frontiers in microbiology, 6, 964. <https://doi.org/10.3389/fmicb.2015.00964>
- Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). *Escherichia coli in Europe: an overview*. International journal of environmental research and public health, 10(12), 6235–6254. <https://doi.org/10.3390/ijerph10126235>
- Yang, L., Shen, Y., Jiang, J. et al. (2022). *Distinct increase in antimicrobial resistance genes among Escherichia coli during 50 years of antimicrobial use in livestock production in China*. Nat Food 3, 197–205 . <https://doi.org/10.1038/s43016-022-00470-6>