In Silico Analyzing of Conjugative Plasmids in *Salmonella* from Poultry

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Introduction

Salmonella enterica **is a common foodborne pathogen. It can infect humans and animals, mostly transmitted to humans through contaminated poultry products and represents one of the leading causes of bacterial foodborne diseases. According to a CDC report, *Salmonella* causes 1.3 million illnesses, 26,500 hospitalizations, and 420 deaths in the US every year, causes an approximate economic loss of over 14 billion dollars annually for the poultry industry. In particular, frequent detection of antibiotic resistance and virulence factors in *Salmonella* of poultry origins is a major concern for food safety. Plasmid conjugation, one of the means for horizontal gene transfer (HGT), contributes to the spread of antibiotic resistance genes (ARGs) and virulence genes among bacteria (Heuer & Smalla 2012), as it is more efficient to transfer genes between bacteria than transformation and often has a broader host range than bacteriophage transduction (von Wintersdorff et al. 2016). However, little is known about conjugative plasmids in bacteria of poultry origins. Plasmid typing methods are normally based on recognizing the replication region and mob genes encoding for relaxases. For example, Carattoli et al. (2005) developed a PCR method based on replicons of the major plasmid incompatibility groups among *Enterobacteriaceae*. Alvarado et al. (2012) applied a degenerate primer MOB typing (DPMT) method to classify γ-Proteobacterial plasmids in clinical and environmental settings based on relaxase. The new typing methods such as plasmid multi-locus sequence typing (pMLST) and whole genome sequence based methods were also based on the replication region or mob genes (Rozwandowicz et al. 2018). In this research, we were interested in detecting both transmissible and non-transmissible plasmids from Salmonella species from poultry and classifying plasmids based on replicon typing. The conjugative transfer regions of conjugative plasmids typically consist of four modules: an origin of transfer (oriT) region, a relaxase gene, a type IV coupling protein (T4CP) gene and a gene cluster for the bacterial type IV secretion system (T4SS) apparatus (Burrus 2017). Thus, we can confirm the conjugative plasmids by detecting all these four modules by in silico methods. To build up a workflow to comprehensively study plasmids, we applied PlasmiFinder 2.1, VRprofile 2, and oriTfinder to plasmid-based sequencing data of poultry source *Salmonella* from the NCBI Refseq database for detecting and typing plasmids, for detecting conjugative plasmids and for analyzing the ARGs and virulence genes in plasmids.**

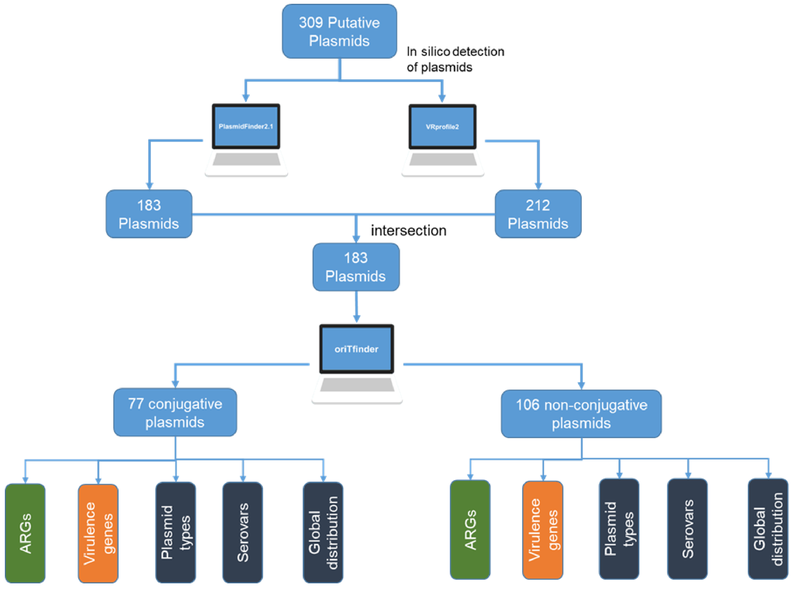

Fig 1. Workflow of detecting and analyzing plasmids

Materials & Methods

Experiment approach

The possible plasmid sequence from *Salmonella* of poultry-origins were downloaded from NCBI Refseq database. Plasmids were detected by both plasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/) and VRprofile 2 (https://tool2-mml.sjtu.edu.cn/VRprofile/) to improve the detection accuracy. The conjugative plasmids were detected by oriTfinder (https://tool-mml.sjtu.edu.cn/oriTfinder/oriTfinder.html) and VRprofile 2 from the detected plasmids. The ARGs and virulence genes were identified by VRprofile 2 and oriTfinder.

Results

One hundred eighty-three plasmid fragments were detected from 309 downloaded sequences (59.22 %) by both plasmidFinder 2.1 and VRprofile 2. Among them, 77 conjugative plasmid fragments were identified (42.08%). These plasmids belonged to 8 typing groups (Col, IncA/C, IncF, IncH, IncI, IncL/M, IncQ, and IncX).

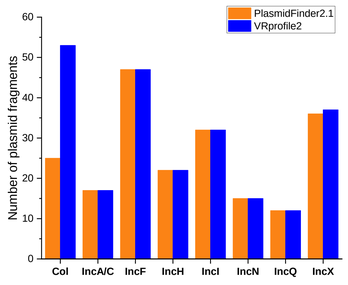

Fig 2. Typing of plasmids

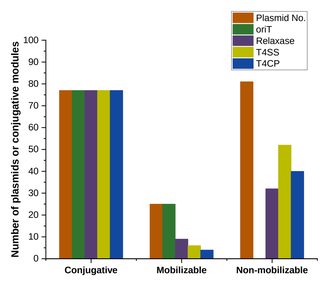

Fig 3. Plasmids and their conjugative modules

Forty-nine plasmid fragments were detected to carry virulence genes, while 105 plasmid fragments were found to carry ARGs, and 28 plasmid fragments were detected both ARGs and virulence genes.

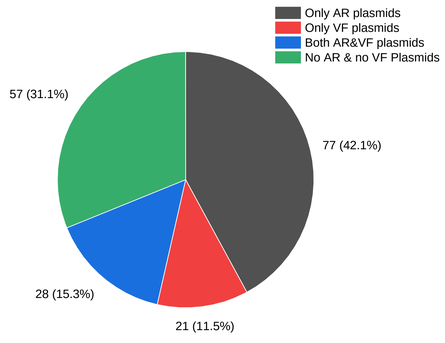

Fig 4. Number of plasmids to carry ARG and virulence genes

The 183 plasmids came from 19 countries or regions (Fig. 5) and they contained at least 27 serovars (Fig. 6).

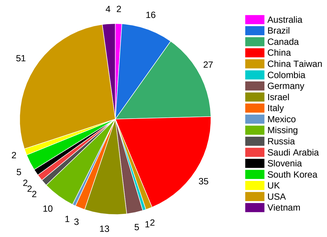

Fig 5. The sources of detected plasmids

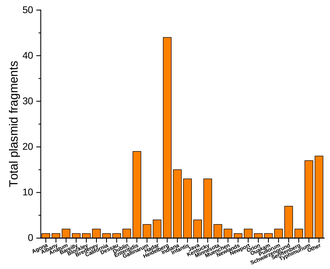

Fig 6. Different serovars for detected plasmids

Discussion

In this study we applied *in silico* tools to characterize plasmids from the NCBI database. We have built a workflow for studying conjugative plasmids easily and efficiently. Conjugative plasmids played an important role in spreading ARGs and virulence genes among bacteria through horizontal genes transfer. Using both VRprofile2 and oriTfinder, we detected 77 conjugative plasmid fragments out of 183 plasmid fragments (42.08%). This number was higher than those from previous studies, which reported that 14% to 23% of the known plasmids were conjugative (Al-Hejin et al. 2019; Coluzzi et al. 2022). The reason from the discrepancy is unknown and this needs further investigation.

One hundred five plasmids (57.4%) were detected with resistance, suggesting the high potential for spread of ARGs in *Salmonella* of poultry. This may explain the frequent detection of ARGs in poultry. Similarly, 26.8% of the detected plasmids contained virulence genes and may also contribute to the frequent detection of virulence *Salmonella* in poultry.

Conclusions

In conclusion, this research has built a workflow to apply *in silico* tools for detecting and typing plasmids, for detecting conjugative plasmids and for analyzing the ARGs and virulence genes in plasmids in *Salmonella* of poultry origins. This research evaluated the plasmids and the spread of plasmid-carrying ARGs and virulence in *Salmonella* from poultry. The workflow can be also used for further detecting plasmids and predicting the spread of ARGs and virulence genes in other bacteria.

References

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