



# Genetic determinants of oxytetracycline resistance in *Paenibacillus larvae* from Saskatchewan beekeeping operations

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## INTRODUCTION

**American foulbrood (AFB)** is a fatal infectious disease of honeybee brood caused by spore-forming bacterium *Paenibacillus larvae*<sup>1</sup>. Due to beekeeper reliance on oxytetracycline (OTC) for metaphylactic control of AFB, resistance to OTC has emerged in *P. larvae* isolates<sup>2</sup>.

We have identified 65 OTC – resistant isolates of *P. larvae* from 718 SK honey samples<sup>3</sup> (9.05%), with minimum inhibitory concentration (MIC) ranging from 32 to 256 µg/mL, originating from different geographical regions of the province using broth microdilution technique. Therefore, whole genome sequencing (WGS)-based investigation of OTC-resistance in *P. larvae* has been conducted using subset of isolates, representing different resistance phenotype and geographical regions of SK.

## OBJECTIVES

1. Identify genetic determinants (antimicrobial resistance genes (ARG`s)) in OTC-resistant *P. larvae* isolates from SK beekeeping operations.
2. Evaluate the clonal relationships of OTC-resistant isolates of *P. larvae* across SK.

## MATERIALS AND METHODS

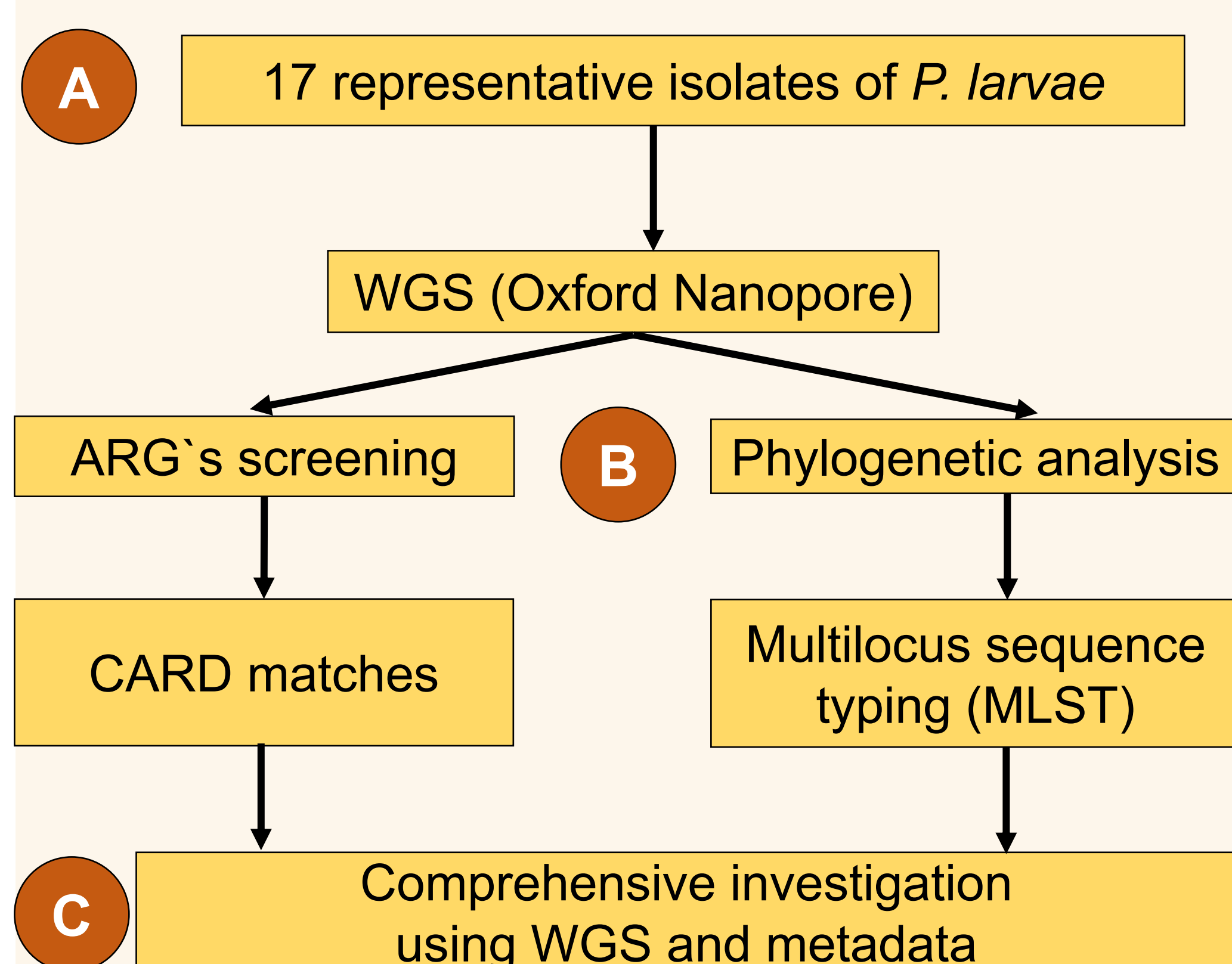


Figure 1. Workflow for WGS analysis of 17 representative *P. larvae* isolates. WGS has been performed using Minion sequencer (A). Subsequently, analysis of data used Comprehensive Antimicrobial Resistance Database (CARD)<sup>6</sup> matches and MLST results to reveal the spread of OTC-resistant *P. larvae* in Saskatchewan (C).

## RESULTS

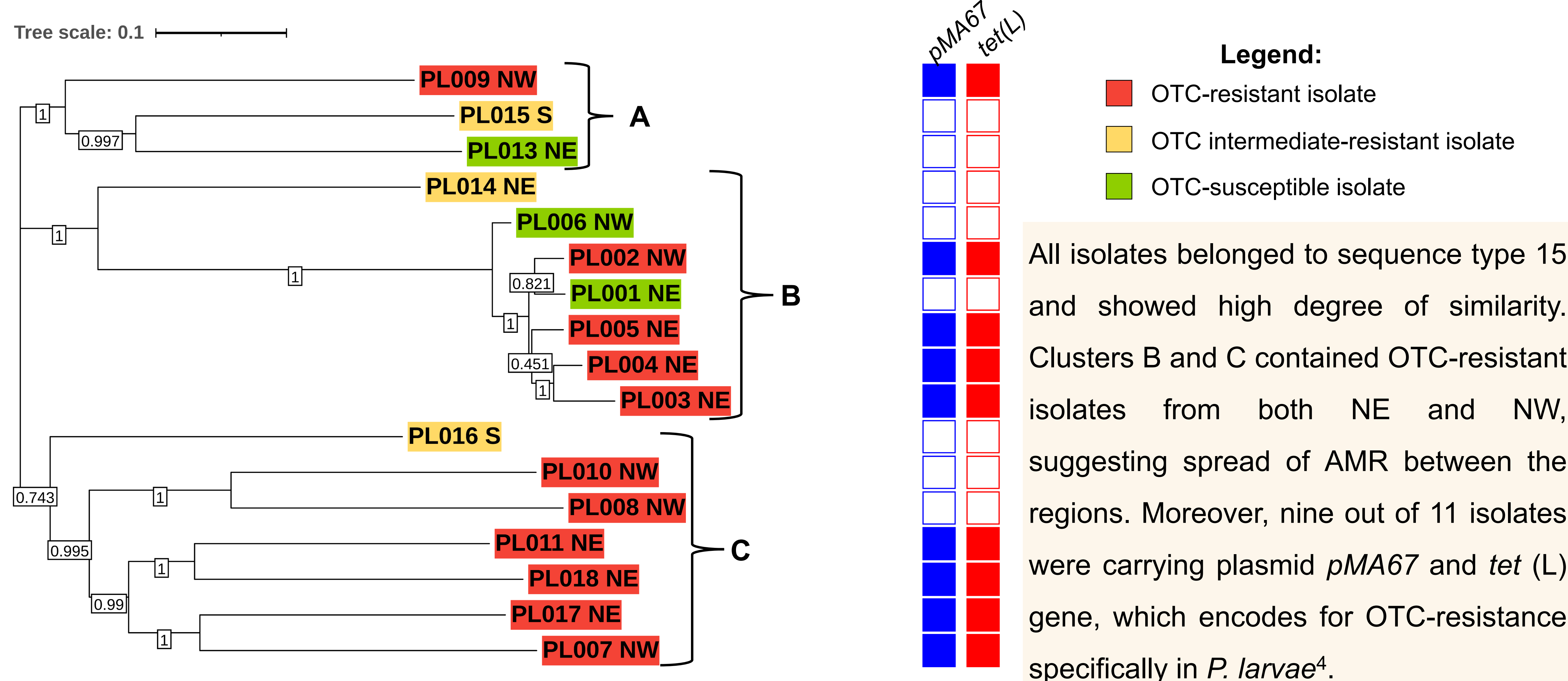


Figure 2. Maximum likelihood phylogenetic tree of 17 *P. larvae* isolates from 11 commercial beekeeping operations in SK generated by iTOL<sup>5</sup> based on binary presence and absence of accessory genes. Geographical region of origin abbreviated after isolates name. Bootstrap values presented in boxes.

Pan-genome of 17 representative isolates of *P. larvae* considered as a closed type pan-genome and consisted of total 14593 genes.

Finally, when combined with the geographical data (**Fig. 3**), we can demonstrate spread of resistance across the different geographical regions of the province.

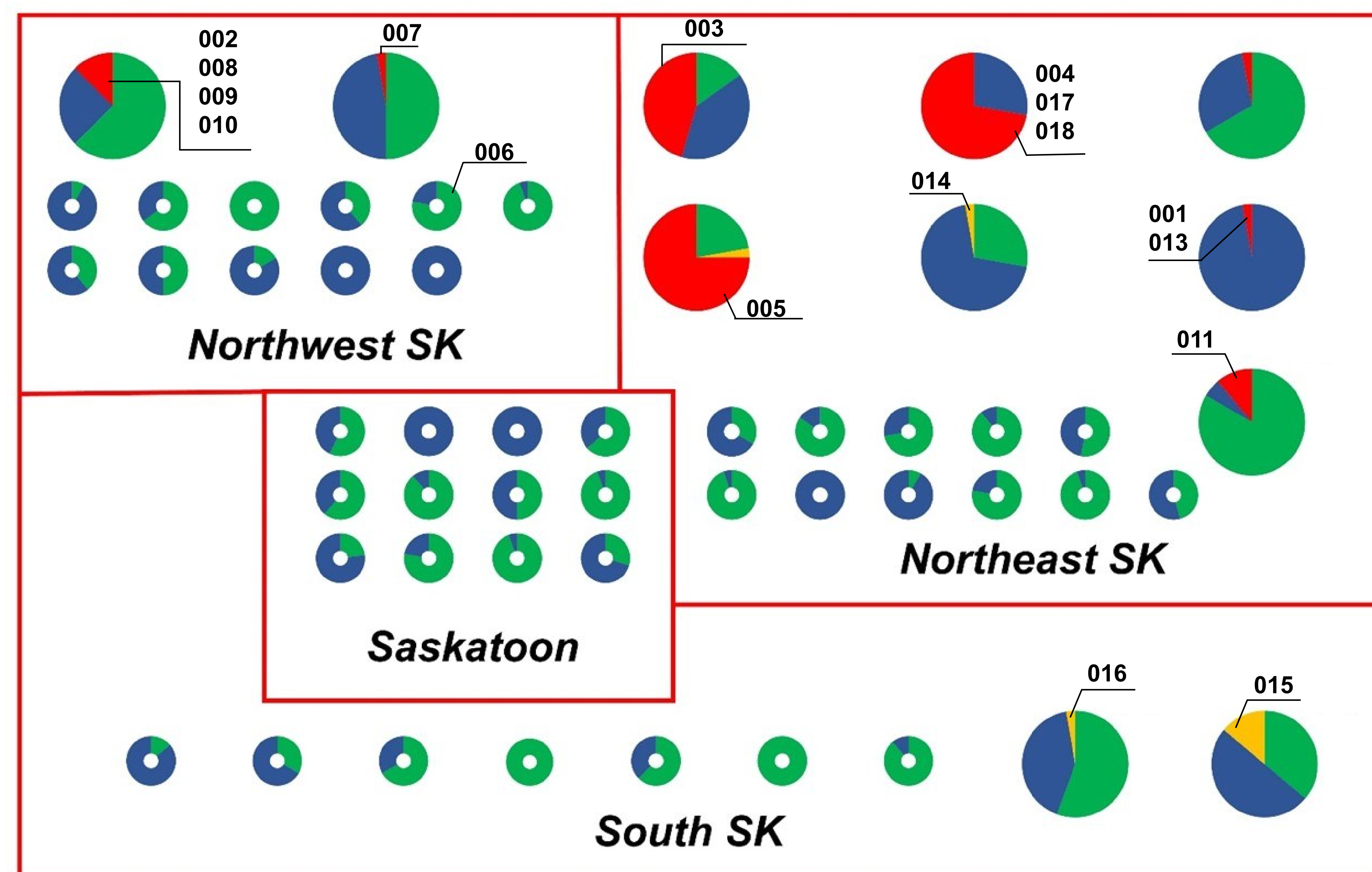


Figure 3. Geographical distribution of OTC-resistance in *P. larvae* isolated from SK beekeeping operations. Operations with OTC-resistance presented in pie charts. Here red, yellow, green and blue colors coding for OTC-resistant, intermediate-resistant, susceptible *P. larvae* isolate or it's absence in the tested honey sample, respectively.

## CONCLUSIONS

Here we demonstrate that previously identified OTC-resistance in *P. larvae* that were isolated from Saskatchewan beekeeping operations is based on ARG (*tet(L)*) and mobile genetic elements (*pMA67*). Similar results have been reported in USA, where the plasmid and the gene have been found in *P. larvae* isolates<sup>4</sup>.

The main limitation of this study small sample size and availability of only essential metadata about isolates.

Future directions will include enhanced sampling and more detailed genetic investigation. Ultimately, by establishing the surveillance we can prevent outbreaks of AFB and reduce unnecessary use of antibiotics in beekeeping industry.

## REFERENCES

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## ACKNOWLEDGEMENTS

