

# Genomic Characterization of *Staphylococcus aureus* with Methicillin-resistant Phenotypes from Chicken and Pork in Upper Northern Thailand Using Long-read Whole Genome Sequencing

Kamonrat Krutharote<sup>1,\*</sup>, and Saranya Wutthiosot<sup>1</sup>

## Abstract

**Background:** *Staphylococcus aureus* is a significant member of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*), posing major concerns for public health and food safety. In particular, strains resistant to  $\beta$ -lactam antibiotics, such as methicillin, referred to as methicillin-resistant *Staphylococcus aureus* (MRSA), are of critical importance. Whole-genome analysis of MRSA provides in-depth insights into genetic diversity, transmission dynamics, and antimicrobial resistance with high accuracy.

**Objective:** To comprehensively characterize the genomic features of *Staphylococcus aureus* isolates exhibiting methicillin-resistant phenotypes from chicken and pork in Upper Northern Thailand using long-read whole genome sequencing (WGS).

**Methods:** A total of 341 meat samples were tested for *S. aureus*. All detected *S. aureus* isolates were examined for phenotypic resistance to ceftiofur and oxacillin using the broth microdilution method for minimum inhibitory concentration (MIC) determination. Isolates exhibiting methicillin-resistant phenotypes were analyzed using long-read WGS on the Oxford Nanopore MinION Mk1C platform. The genomes were de novo assembled using Flye and subsequently annotated using Prokka.

**Results:** *S. aureus* was isolated from 9.7% (33/341) of meat samples, with a markedly higher prevalence in pork (11.4%; 32/280) compared to chicken (1.6%; 1/61). Antimicrobial susceptibility testing was performed on all 33 isolates, revealing that 6.1% (2/33) of the isolates exhibited methicillin-resistant phenotypes. One isolate showed high-level resistance to both ceftiofur and oxacillin (MIC >64  $\mu$ g/mL), while the other was resistant to ceftiofur (32  $\mu$ g/mL) but remained susceptible to oxacillin (<1  $\mu$ g/mL). These phenotypically resistant isolates were subsequently selected for whole genome sequencing. Long-read WGS generated high-quality genome assemblies (~2.7–2.9 Mb) comprising two circular contigs. All isolates were confirmed as *S. aureus* based on average nucleotide identity (ANI) values (~0.996), with sequence types ST6 and ST672 identified by multilocus sequence typing (MLST). Genomic analysis detected the  $\beta$ -lactamase gene *blaZ* in all isolates and *tet(K)* in selected isolates. However, neither *mecA* nor *mecC* genes were identified. The observed discordance between phenotypic and genotypic resistance suggests the involvement of non-*mec*-mediated mechanisms.

**Conclusion:** This study demonstrates the genetic diversity and antimicrobial resistance profiles of *S. aureus* isolated from chicken and pork in Northern Thailand. Although multiple resistance genes, particularly *blaZ* and *tet(K)*, were identified, no classical methicillin resistance genes (*mecA* or *mecC*) were detected despite the presence of phenotypic resistance to ceftiofur and oxacillin in some isolates. This discrepancy between phenotypic and genotypic resistance suggests the involvement of non-*mec*-mediated mechanisms. The application of Long-read WGS provides enhanced resolution for genomic characterization and highlights its value in antimicrobial resistance surveillance within a One Health framework.

**Keywords:** *Staphylococcus aureus*, Methicillin-resistant phenotype, Long-read whole genome sequencing, Antimicrobial resistance

<sup>1</sup> Veterinary Research and Development Center (Upper Northern region), Department of Livestock Development, Lampang, Thailand

\* Corresponding author; E-mail: aofst123@gmail.com