Genomic characterization of *Staphylococcus aureus* with methicillin-resistant phenotypes from chicken and pork in Upper Northern Thailand using long-read whole genome sequencing

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1 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 β-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.

2 Objective

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

3 Methods

Sample Collection
341 meat samples
(chicken: 61, pork: 280)

Isolation and Identification
of *S.aureus*

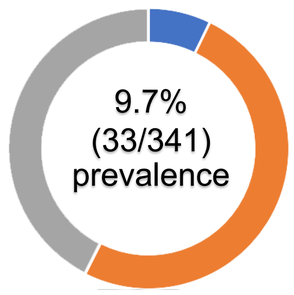
Phenotypic resistance to cefoxitin and oxacillin was determined by broth microdilution **MIC testing**

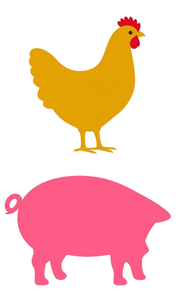
DNA extraction
High-molecular weight DNA extraction

Long-read sequencing
Genome assembly
Genome annotation

4 Results

4.1 Prevalence of *S. aureus*



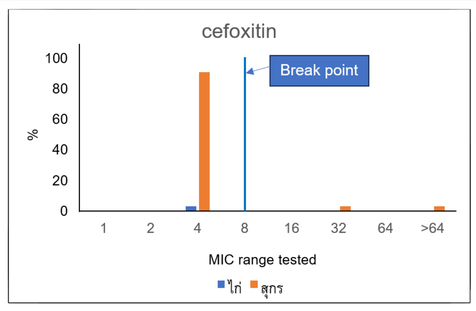


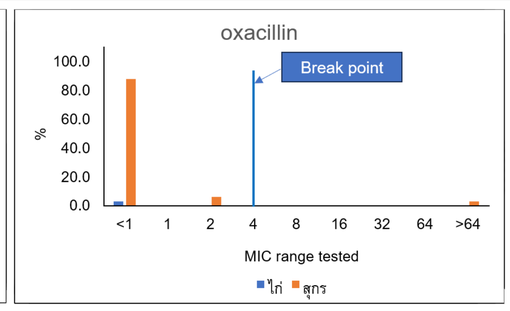
1.6%
(1/61)
chicken

11.4%
(32/280)
pork

S. aureus was detected in 9.7% (33/341) of meat sample, with a higher prevalence in pork than chicken

4.2 Phenotype resistance





Isolated	Antibiotic MIC (µg/mL)		Remark
	cefoxitin	oxacillin	
Barcode 2	>64	>64	resistance to both cefoxitin and oxacillin (MIC >64 µg/mL)
Barcode 3	32	1	resistant to cefoxitin (32 µg/mL) but susceptible to oxacillin (<1 µg/mL)

4.3 Long read WGS and genome assembly

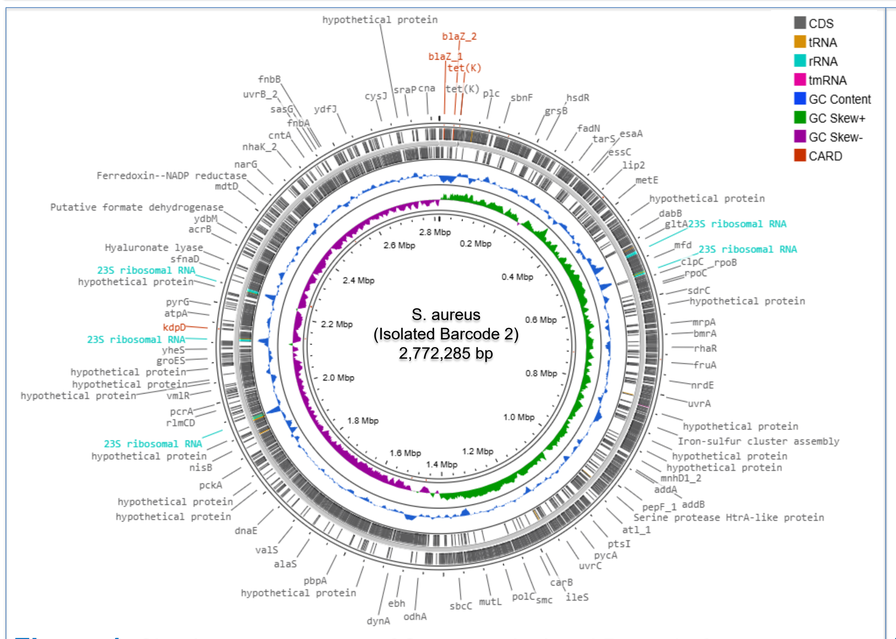


Figure 1. Circular genome map of S. aureus isolated Barcode 2 generated from long-read whole-genome sequencing.

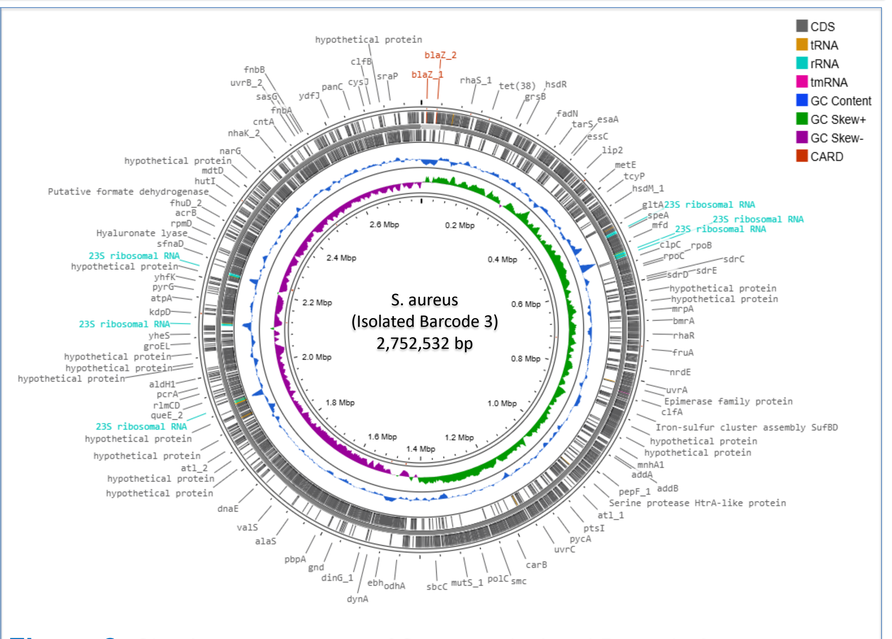


Figure 2. Circular genome map of S. aureus isolated Barcode 3 generated from long-read whole-genome sequencing.

4.4 Genomic characteristics

Feature		Barcode 2	Barcode 3
Species (best hit)		***S. aureus***	***S. aureus***
ANI (best hit : *S. aureus*)		**0.996**	**0.995**
Sequence type (ST)		**ST6**	**ST672**
Genome size (Mb)		**2.77**	**2.75**
Circular contigs (no.)		**3**	**2**
AMR gene	***blaZ* (β-lactamase)**	✓	✓
	***tet* (K) (tetracycline resistance)**	✓	-
	mecA	-	-
	mecC	-	-

4.5 Phenotype-Genotype Discordance

PENOTYPE
- **cefoxitin resistance**
- **oxacillin resistance**

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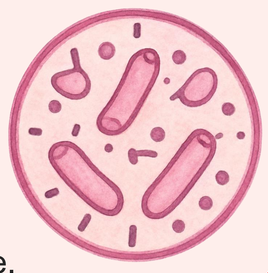
GENOTYPE
- ***mecA* : Not detected**
- ***mecC* : Not detected**

Interpretation

Phenotypic resistance to cefoxitin and/or oxacillin was observed despite the absence of *mecA* and *mecC* genes. The detection of *blaZ* in both isolates suggests a potential contribution of non-mec-mediated β-lactam resistance mechanisms to the observed phenotype.



5 CONCLUSIONS

- ✓ *blaZ* and *tet(K)* were identified among the isolates
- ✓ No *mecA* or *mecC* genes were detected despite phenotypic resistance to cefoxitin and oxacillin.
- ✓ The observed phenotype–genotype discordance suggests possible non-mec -mediated resistance mechanisms

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6 ACKNOWLEDGMENTS

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7 REFERENCES

Clinical and Laboratory Standards Institute. 2023. Performance standards for antimicrobial susceptibility testing, 33rd ed CLSI supplement M100 Clinical and Laboratory Standards Institute, Wayne, PA.

Jain, M., Olsen, H. E., Paten, B. and Akeson, M. 2016. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome biology*, *17*(1): 239.

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., ... & McArthur, A. G. 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic acids research, 51(D1): D690-D699.

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, *30*(14): 2068-2069.