# Genomic Heterogeneity of *orfX-SCCmec* Junction Insertions Driving MRSA Diagnostic Escape from Rapid PCR Assays

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## Background

Rapid molecular MRSA screening targets the *orfX-SCCmec* junction to ensure diagnostic specificity by discriminating against "empty" *SCCmec* cassette remnants and methicillin-resistant coagulase-negative staphylococci.
However, "diagnostic escape" occurs when structural alterations disrupt PCR detection of phenotypically resistant strains. We characterized the genomic architecture of these PCR-negative, phenotypically resistant MRSA isolates to identify vulnerabilities in rapid screening platforms widely utilized for infection prevention and control programs.

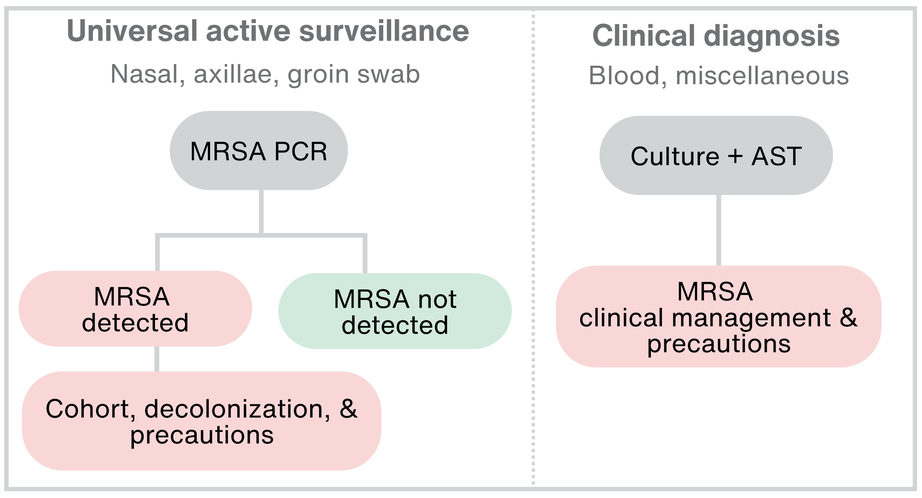


**Figure 1**. MRSA detection pathways and clinical and infection control implications

## Objectives

To characterize the genomic basis of diagnostic escape in PCR-negative, phenotypically resistant MRSA and assess its implications for the rapid screening platforms used in universal active surveillance.

## Methods

Routine MRSA screening samples (n=1368) were analyzed over a 3-month period. Diagnostic escape samples were defined as Cepheid Xpert MRSA NxG PCR negative (*SCCmec* not detected, *mecA/C* detected) but culture positive with confirmed phenotypic resistance. 11 MRSA isolates from 10 unique patients were sequenced using Oxford Nanopore Technologies to resolve the structural architecture of the *orfX-SCCmec* junction.

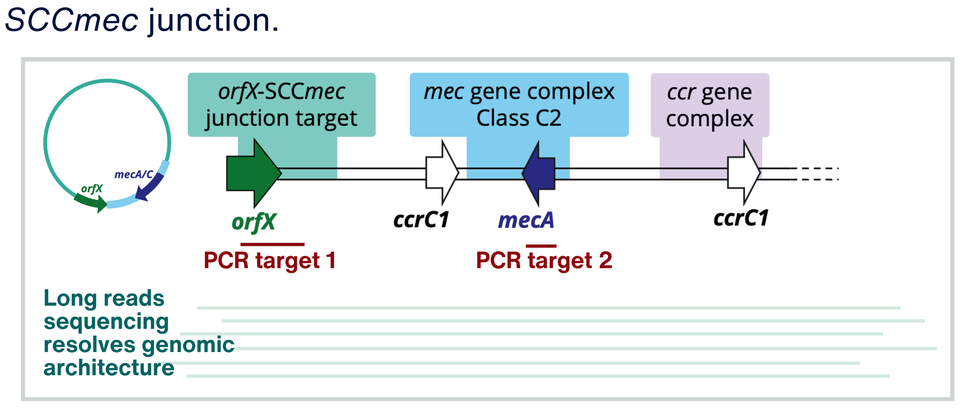


**Figure 2**. Schematic of the *SCCmec cassette* showing the two PCR target regions interrogated by the Cepheid Xpert MRSA NxG assay. Long-read Oxford Nanopore sequencing was used to resolve the genomic architecture of the cassette.

## Results

Majority of the genomes belonged to ST45 lineage. Comparative genomic analysis revealed significant structural heterogeneity at the *orfX-SCCmec* junction, with three distinct architectural variants resulting in diagnostic escape. The majority (n=7) harbored an SCCmec Type XIV (5A) configuration featuring a ~15 kb interspecies J3 region potentially derived from *Staphylococcus haemolyticus*. Two additional Type XIV (5A) variants had expanded cassette sizes (66 kb and 76 kb). Of note, two isolates had a unique ccrB1A1C1 configuration with a ~4.8kb interspecies J3 region, suggestive of a novel SCCmec variant.

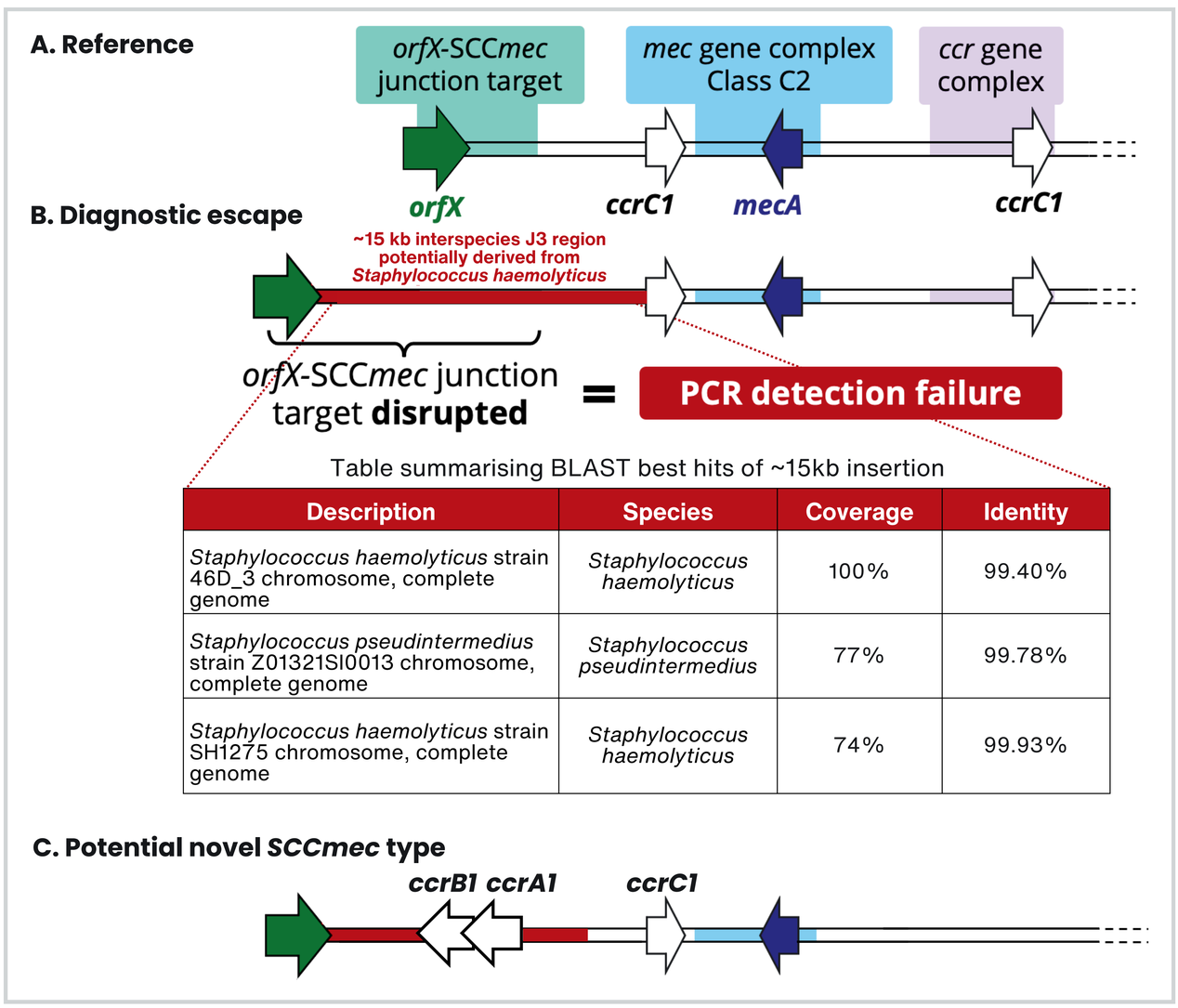


Table summarising BLAST best hits of ~15kb insertion

| Description | Species | Coverage | Identity |
|---|---|---|---|
| *Staphylococcus haemolyticus* strain 46D_3 chromosome, complete genome | *Staphylococcus haemolyticus* | 100% | 99.40% |
| *Staphylococcus pseudintermedius* strain Z01321SI0013 chromosome, complete genome | *Staphylococcus pseudintermedius* | 77% | 99.78% |
| *Staphylococcus haemolyticus* strain SH1275 chromosome, complete genome | *Staphylococcus haemolyticus* | 74% | 99.93% |

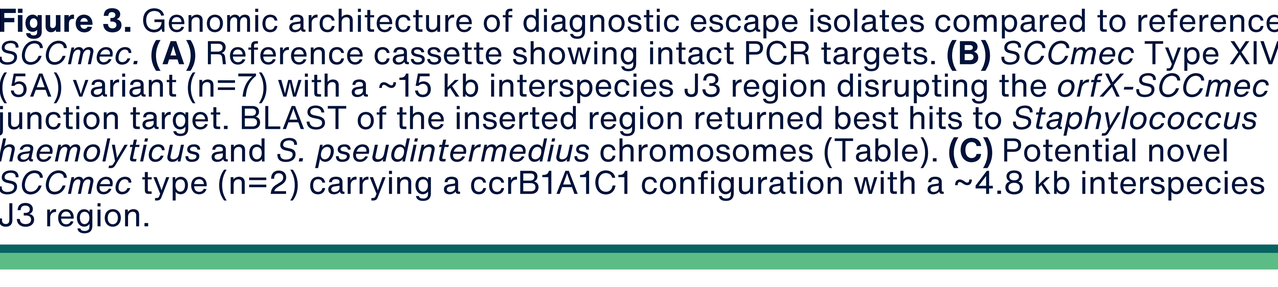


**Figure 3.** Genomic architecture of diagnostic escape isolates compared to reference *SCCmec.* **(A)** Reference cassette showing intact PCR targets. **(B)** *SCCmec* Type XIV (5A) variant (n=7) with a ~15 kb interspecies J3 region disrupting the *orfX-SCCmec* junction target. BLAST of the inserted region returned best hits to *Staphylococcus haemolyticus* and *S. pseudintermedius* chromosomes (Table). **(C)** Potential novel *SCCmec* type (n=2) carrying a ccrB1A1C1 configuration with a ~4.8 kb interspecies J3 region.

## Conclusion & clinical implications

Our findings suggest that the orfX-SCCmec junction possesses greater genomic plasticity than previously recognized, representing a systemic diagnostic vulnerability rather than an isolated clonal event. These structural variations compromise diagnostic sensitivity, resulting in potential risk of silent MRSA transmissions. Continuous genomic surveillance is necessary to ensure diagnostic tools remain relevant against evolving pathogens.

**Acknowledgement**

This work was supported by A*STAR's Epidemic Preparedness Horizontal Technology Coordinating Office (EP HTCO; FY22_CF_HTCO SEED_ID_GIS_22714013). Additional financial support was provided by the SingHealth Duke-NUS Pathology Academic Clinical Programme (ACP), the Nurturing Clinician Researcher Scheme (NCRS), and the ACP Collaborative grant. This research was also supported by the Faculty of Science, Chiang Mai University. We also acknowledge the Amazon Web Services Health Equity Initiative (HEI) Program for providing cloud credit support that facilitated the computational analyses.