APPLICATION NOTE

# Empowering health care–associated infection surveillance with bacterial genome sequencing

## Comprehensive identification of pathogenic isolates using Illumina sequencing and the SRST2 BaseSpace™ app

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# Introduction

Health care–associated infections (HAIs) are a major health care concern, especially in critically ill and immunocompromised patients. HAIs not only threaten the patients' health and life but also bring additional economic burden to the patients and the health care system, including direct economic loss and prolonged hospitalization.[1,2] Surveillance of pathogenic bacterial strains in the health care facility environment can help prevent the impact of HAIs. Laboratory methods, such as qPCR and mass spectrometry, provide rapid identification of pathogens, allowing for timely treatment decisions. However, these methods are insufficient for tracing outbreaks or performing transmission investigations.

Next-generation sequencing (NGS) allows for the complete characterization of bacterial genomes, providing information for subtyping, differentiating between isolates, highlighting isolates in a cluster, and antimicrobial resistance (AMR) and virulence markers.[3] Analyzing and interpreting sequencing data quickly allows infection control personnel to respond rapidly to potential outbreaks and trace them back to the source to help prevent further transmission and infection. Following a comprehensive whole-genome sequencing (WGS) workflow that can be completed in two days, the spread of pathogens responsible for HAIs can be efficiently monitored and properly managed.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen with higher morbidity and mortality rates than methicillin-sensitive *S. aureus* strains. Infections caused by hospital-associated methicillin-resistant *S. aureus* (HA-MRSA) strains require longer hospital stays than those caused by hospital-associated methicillin-sensitive *S. aureus* strains and is usually resistant to non-β-lactam as well as β-lactam antibiotics.

To characterize isolates of HA-MRSA or other bacterial species, Illumina offers the SRST2* app as part of a comprehensive WGS solution that includes Illumina library preparation and sequencing (Figure 1). Available in BaseSpace Sequence Hub, the SRST2 app reports the presence of sequence types (STs) from a multi-locus sequence typing (MLST) database[4] and reference genes from a database of sequences for virulence genes, resistance genes, and plasmid replicons with sample-to-sample comparison across > 150 bacterial genera and species. The SRST2 app has been updated to add the Comprehensive Antibiotic Resistance Database (CARD) and ARG-Annot database in addition to ResFinder.

This application note demonstrates the capabilities of the updated SRST2 app to identify and characterize pathogenic isolates, making HAI surveillance accessible to laboratories without prior NGS experience.

* Short-read sequence typing

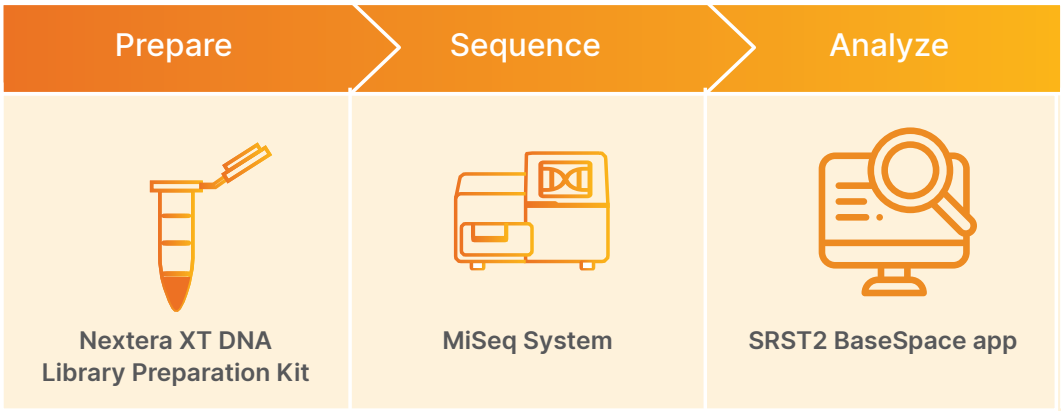



Figure 1: HAI surveillance workflow—The SRST2 BaseSpace app is part of a comprehensive NGS workflow for surveillance of HAIs that includes Illumina library preparation and sequencing.

# Methods

## Samples

For this study *S. aureus* isolates (1 from pleural fluid, 1 from hip fluid, 1 from bone, 1 from unnamed body fluid, and 23 from blood) were collected in 2016 (from multiple clinical sites in Hennepin and Ramsey counties in Minnesota) as part of the normal surveillance by the Minnesota Department of Health (MDH).[5]

## Sample preparation

Isolates were obtained as cryostocks from the MDH, streaked on standard blood agar plates, and incubated at 37°C to obtain single colonies, which were inoculated and grown overnight at 37°C in tryptic soy broth. Genomic DNA was isolated using the QIAamp DNA Mini Kit (QIAGEN, Catalog no. 51304), supplementing the AL buffer with lysostaphin. The DNA concentration and quality were determined using a NanoDrop 2000 spectrophotometer and a Qubit Flex Fluorometer (Thermo Fisher Scientific, Catalog no. Q33327).

## Library preparation and sequencing

Sequencing libraries were prepared using the Nextera™ XT DNA library preparation kit, 24 samples (Illumina, Catalog no. FC-131-1024) and multiplexed using the Nextera XT Index Kit v2 Set A (Illumina, Catalog no. FC-131-2001). WGS was performed on an Illumina MiSeq™ System using a 2 × 300 bp paired-end run.

## Data analysis

Sequencing files were analyzed with the SRST2 app in BaseSpace Sequence Hub.

# Results

Based on multiple databases, the SRST2 app revealed that the isolates can be grouped into two distinct clusters (Figure 2). Several MLST alleles differentiate these two major clusters and subdivide them into smaller clusters of closely related isolates (Figure 3). Using CARD, the SRST2 app detects several resistance genes such as *ErmA*, which is associated with resistance to macrolide antibiotics, one of the most prescribed oral antibiotic classes in the US (Figure 4).[6]

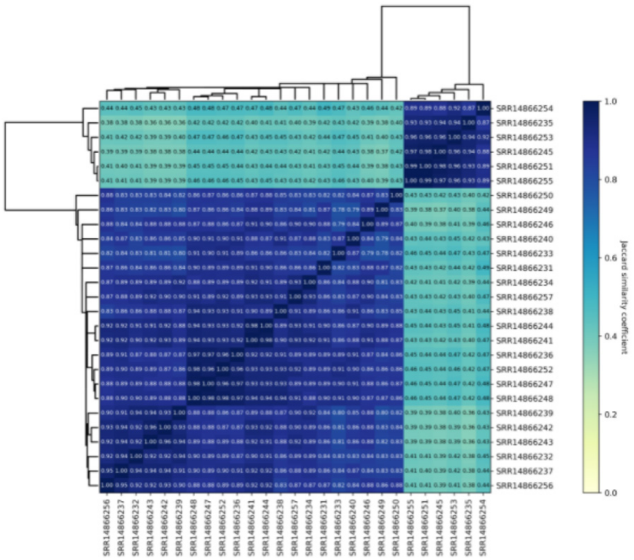


Figure 2: Isolate clustering with the SRST2 app—Analysis of WGS data clusters similar isolates (dark blue) based on detected alleles across multiple databases.

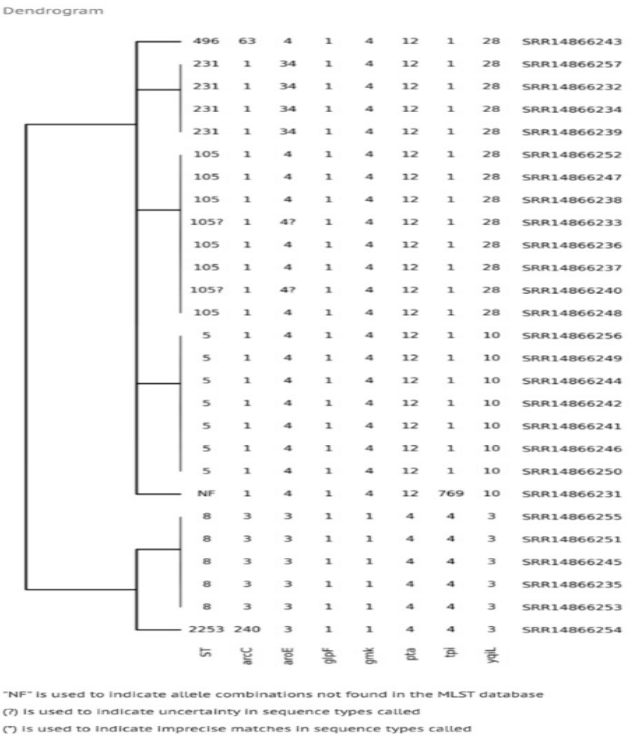
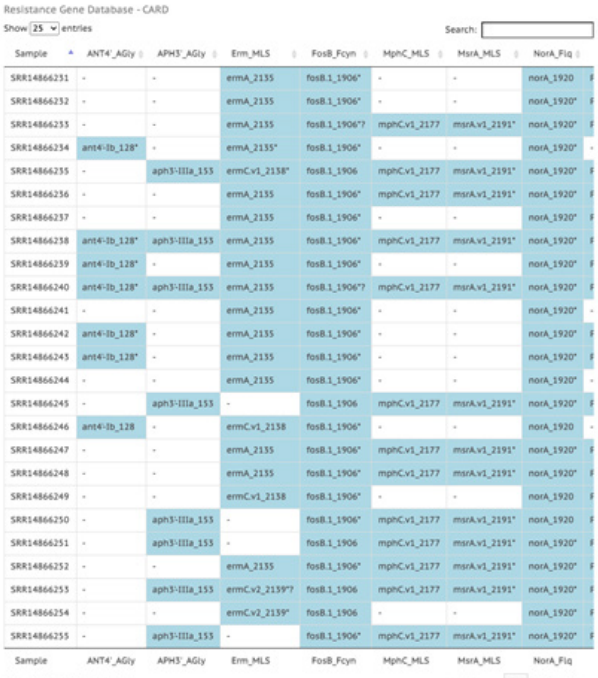



Figure 3: MLST allele profiles calculated from WGS—The SRST2 app clusters isolates based on number of shared alleles.


Resistance Gene Database - CARD

Show 25 entries Search:

| Sample | ANT4'_AGly | APH3'_AGly | Erm_MLS | FosB_Fcyn | MphC_MLS | MsrA_MLS | NorA_Fiq |
|---|---|---|---|---|---|---|---|
| SRR14866231 | - | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920 |
| SRR14866232 | - | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866233 | - | - | ermA_2135 | fosB.1_1906"? | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866234 | ant4-Ib_128" | - | ermA_2135" | fosB.1_1906" | - | - | norA_1920" |
| SRR14866235 | - | aph3-IIIa_155 | ermC.v1_2138" | fosB.1_1906 | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866236 | - | - | ermA_2135 | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866237 | - | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866238 | ant4-Ib_128" | aph3-IIIa_155 | ermA_2135 | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866239 | ant4-Ib_128" | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866240 | ant4-Ib_128" | aph3-IIIa_155 | ermA_2135 | fosB.1_1906"? | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866241 | - | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866242 | ant4-Ib_128" | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866243 | ant4-Ib_128" | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866244 | - | - | ermA_2135 | fosB.1_1906" | - | - | norA_1920" |
| SRR14866245 | - | aph3-IIIa_155 | - | fosB.1_1906 | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866246 | ant4-Ib_128 | - | ermC.v1_2138 | fosB.1_1906" | - | - | norA_1920 |
| SRR14866247 | - | - | ermA_2135 | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866248 | - | - | ermA_2135 | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866249 | - | - | ermC.v1_2138 | fosB.1_1906" | - | - | norA_1920 |
| SRR14866250 | - | aph3-IIIa_155 | - | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920 |
| SRR14866251 | - | aph3-IIIa_155 | - | fosB.1_1906 | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866252 | - | - | ermA_2135 | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866253 | - | aph3-IIIa_155 | ermC.v2_2139"? | fosB.1_1906 | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| SRR14866254 | - | - | ermC.v2_2139" | fosB.1_1906 | - | - | norA_1920" |
| SRR14866255 | - | aph3-IIIa_155 | - | fosB.1_1906" | mphC.v1_2177 | msrA.v1_2191" | norA_1920" |
| Sample | ANT4'_AGly | APH3'_AGly | Erm_MLS | FosB_Fcyn | MphC_MLS | MsrA_MLS | NorA_Fiq |

Figure 4: Resistance genes report—The SRST2 app includes an interactive table that indicates which alleles from CARD are detected in each isolate.

## Summary

HAIs are a major health care concern, contributing to high patient morbidity and mortality and increased health care costs. Using an NGS workflow that features data analysis with the SRST2 app, it is possible to detect the presence of genes linked to clinically relevant phenotypes, including virulence genes, AMR genes, or serotype determinants. With fast Illumina library prep and the MiSeq System, bacterial genomes can be fully sequenced and analyzed within two days, enabling timely HAI surveillance.

## Learn more

SRST2 BaseSpace app

## References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