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ORIGINAL RESEARCH

# WGS Analysis of *Staphylococcus warneri* Outbreak in a Neonatal Intensive Care Unit

Xiang Zhang[1,*], Yu Zhou[2,3,*], Lu Fu[4,*], Lu Zhou[5], Xiangjun Cheng[6], Wei Zhang[2,3], Zhongming Tan[5]

[1]Department of Infection Management, The First Affiliated Hospital with Nanjing Medical University, Jiangsu Province Hospital, Nanjing, Jiangsu Province, People's Republic of China; [2]Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu Province, People's Republic of China; [3]Sanya Institute of Nanjing Agricultural University, Sanya, Hainan Province, People's Republic of China; [4]Department of Nosocomial Infection Management, Affiliated Maternity and Child Health Care Hospital of Nantong University, Nantong, Jiangsu Province, People's Republic of China; [5]NHC Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu Province, People's Republic of China; [6]Department of Laboratory Medicine, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing, Jiangsu Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhongming Tan, NHC Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention, No. 172 Jiangsu Road, Gulou District, Nanjing, Jiangsu Province, 210009, People's Republic of China, Tel +862583759339, Email jstzm@jscdc.cn; Wei Zhang, Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, No. 1 Tongwei Road, Nanjing, Jiangsu Province, 210095, People's Republic of China, Tel +8625843965517, Email vszw@njau.edu.cn



**Purpose:** *Staphylococcus warneri* is an opportunistic pathogen responsible for hospital-acquired infections (HAIs). The aim of this study was to describe an outbreak caused by *S. warneri* infection in a neonatal intensive care unit (NICU) and provide investigation, prevention and control strategies for this outbreak.

**Methods:** We conducted an epidemiological investigation of the NICU *S. warneri* outbreak, involving seven neonates, staff, and environmental screening, to identify the source of infection. WGS analyses were performed on *S. warneri* isolates, including species identification, core genome single-nucleotide polymorphism (cgSNP) analysis, pan-genome analysis, and genetic characterization assessment of the prevalence of specific antibiotic resistance and virulence genes.

**Results:** Eight *S. warneri* strains were isolated from this outbreak, with seven from neonates and one from environment. Six clinical cases within three days in 2021 were linked to one strain isolated from environmental samples; isolates varied by 0–69 SNPs and were confirmed to be from an outbreak through WGS. Multiple infection prevention measures were implemented, including comprehensive environmental disinfection and stringent protocols, and all affected neonates were transferred to the isolation wards. Following these interventions, no further cases of *S. warneri* infections were observed. Furthermore, pan-genome analysis results suggested that in human *S. warneri* may exhibit host specificity.

**Conclusion:** The investigation has revealed that the outbreak was linked to the milk preparation workbench by the WGS. It is recommended that there be a stronger focus on environmental disinfection management in order to raise awareness, improve identification, and prevention of healthcare-associated infections that are associated with the hospital environment.

**Keywords:** *Staphylococcus warneri*, neonatal intensive care unit, whole-genome sequencing, pan-genome

## Introduction

Coagulase-negative *Staphylococcus* (CoNS) is commonly found on the skin and on mucosal surfaces of humans and animals.[1] It encompasses several species including *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus hominis, and Staphylococcus caprae*. Among these, *S. epidermidis* has been extensively studied. *S. warneri* is another member of the CoNS group and has been implicated in a variety of human infections such as native mitral valve congenital infections,[2] artificial valve endocarditis,[3] septic arthritis,[4] and urinary tract infections.[5] Notably, *S. warneri* and *Prevotella bivia*, are commonly associated with balanoposthitis-associated disease.[6]

Coagulase-positive *Staphylococcus* (CoPS), such as *Staphylococcus aureus*, was previously considered a significant pathogen. However, in recent years, CoNS has become increasingly associated with various infections in humans,

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particularly in immunocompromised patients and neonates.[3,7,8] Its high prevalence suggests that it plays a role in the development of diseases. Nonetheless, compared to other CoNS, *S. warneri* has received relatively limited attention, with studies primarily focusing on isolating this bacterium and documenting its associated symptoms. The sources and transmission routes of *S. warneri* infections in humans remain unclear. Therefore, it is imperative to conduct further research on the epidemiology, host specificity, type, virulence, and drug resistance of *S. warneri* strains isolated from different hosts. The increasing rate of CoNS isolation has revealed the presence of more virulence factors in related strains. Similar to other pathogenic staphylococci, *S. warneri* possesses numerous virulence factors, including adhesins, exoenzymes, capsules, iron uptake systems, and biofilms. Biofilm formation is a common and influential virulence factor.[7] Biofilm formation enhances the ability of bacteria to resist antibiotics, exacerbating the problem of antibiotic resistance.

Newborns admitted to the neonatal intensive care unit (NICU) are at high risk of morbidity and mortality due to their low birth weight, premature gestational age, complex care requirements, and prolonged hospitalization. Therefore, outbreaks of bacterial infections are common in NICU. Whole-genome sequencing (WGS) and analysis offer a high-throughput approach for identifying and characterizing bacterial clones, analyzing virulence and drug resistance, and providing insight into these uncommon and understudied infections.[9] The use of WGS in the clinical diagnostic has increased in recent years. More laboratories are favoring to use of single nucleotide polymorphism (SNP) analysis for epidemiological investigation and tracking of nosocomial outbreaks. From December 2020 to February 2021, our neonatal unit detected seven newborns infected with *S. warneri*. An investigation was conducted by the Department of Infection Management to determine whether this outbreak originated from a single common point source.

# Materials and Methods

## Outbreak Recognition

The initial confirmed case occurred on December 25, 2020, when a 28-day-old neonate presented with respiratory distress and positive for blood culture. Subsequently, between February 19 and February 21, 2021, six additional neonates were diagnosed with neonatal sepsis.[10] In response, from 20 to 22 February 2021, an epidemiological investigation was immediately conducted by the infection management department to determine the source of the infection and implement infection control measures.

## Case Definition

Suspected case: If any of the following criteria are present: 1) abnormal clinical manifestations, 2) mother has chorioamniotic infection, 3) Preterm PROM≥18h.

Clinically diagnosed case-patient: Individuals who exhibit clinical abnormalities and meet any of the following criteria: 1) ≥2 positive non-specific blood tests, 2) cerebrospinal fluid tests show changes indicative of suppurative meningitis, 3) DNA from the pathogen was detected in the blood.

Confirmed case: Individuals with clinical abnormalities and a positive blood culture or cerebrospinal fluid culture (or culture of other sterile cavity fluid).

### Epidemiological Investigation

According to the guidelines of control of healthcare associated infection outbreak (WS/T 524 −2016),[11] swabs were collected from the staff, high-contact surfaces, and the environment in order to identify the source of infection. Including the hands, nasal and forehead of the personnel who may be related to the infection. Additionally, samples were taken from the newborn's surrounding environment, including the contact surfaces such as the heating tank, infusion pump and ventilator. The humidification water from the warm tank, pool and faucet was also sampled, along with milk dispensing room, which including workbench, equipment, and supplies surface, residual milk, pool and faucet. Lastly, samples were taken from the tabletop and air of clean bench used for preparing intravenous drugs.

The skin, nose swab and surface samples were collected by dipping sterile swabs into stroke-physiological saline solution. The remaining milk (0.5mL) was extracted using a 1 mL sterile syringe. The humidified water (100 mL) from the warm tank was extracted using a 50mL sterile syringe. For the air sample of the clean bench, the sedimentation

method was used.[12] Five pieces of Columbia blood agar were evenly distributed on a clean bench while working for 30 minutes, then sent to the microbiology laboratory for culture.

### Microbiological Methods

Before the investigation, blood samples of seven neonatal infections were collected by NICU. Operating procedures of blood culture for clinical microbiology laboratory (WS/T 503–2017)[13] was used for isolated. The water sample (50 mL) was centrifuged at 12,000 × g for 30 min and removed the supernatant.[14] An aliquot of 20 μL of each enrichment and environmental swabs were plated on columbia blood agar (BD Diagnostic Systems, Beijing, China) at 35 ± 1 °C for 24h-48h. Each batch of blood cultures includes blank control to ensure accurate detection of true infections. Positive cultures were identified as *S. warneri* using a microflex LT/SH smart MALDI-TOF MS system (Bruker Daltonik, Bremen, Germany) and Vitek GP, Vitek2 automated system (bioMérieaux, Paris, France).[15,16] Drug susceptibility testing was determined using the VITEK 2 automated system. Quality control (QC) was performed by Clinical and Laboratory Standards Institute (CLSI) recommended QC reference strains (*S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212). All QC results were within published acceptable ranges. The results were interpreted using SIR (sensitive, intermediate, resistance) according to CLSI M100-S32 guidelines.[17]

### WGS, Assembly, and Annotation

Genomic DNA was extracted from the cultured strains using a FastPure Bacteria DNA Isolation Mini Kit (Vazyme, Nanjing, China) according to the manufacturer's instructions. The DNA of the eight *S. warneri* strains was sequenced using an MGI2000 Genome Analyzer System according to the manufacturer's specifications at BGI-Wuhan using whole genomic shotgun libraries. Sequence data were assembled using SPAdes v. 3.14.0[18] and genome annotation was performed using Prokka[19] software.

### Genomic Analysis

SNP and genomic analyses included multilocus sequence typing (MLST), pan-genome analysis, and genotypic assessment of the prevalence of specific antibiotic resistance, virulence, and other relevant genes. The core-genome SNP (cgSNP) analysis with higher resolution was conducted to assess the phylogenetic relationships. The Harvest suite,[20] a software package including Parsnp tool, was used to generate cgSNP and the reference genome of *S. warneri* GD01. Parsnp v. 1.2 was used to conduct core-genome alignments with – x option to remove recombination based on defaulted parameters of evolutionary model of generalized time-reversible (GTR) and 1000 bootstrap resamples.

MLST (https://github.com/tseemann/mlst) scans contig files against traditional PubMLST[21] typing schemes. MLST software was bundled with traditional MLST databases. Pan-genome analysis was performed using Roary.[22] In total, 98 genomes of *S. warneri* (eight genomes from this study and 90 different hosts from the NCBI RefSeq database, Supplementary Table S1) were obtained to define the core genome and perform phylogenomic analysis.

Virulence factor genes were identified by aligning bacterial genomic sequences against the reference sequences in the Virulence Factors Database (VFDB)[23] using CLC Genomics WorkBench software (version 22.0, Qiagen Bioinformatics, Aarhus, Denmark). ABRicate (https://github.com/tseemann/abricate) was used for the mass screening of contigs for antimicrobial resistance genes.[24] The query sequences were identified with ≥80% identity and ≥70% coverage.

## Results

### Outbreak Epidemiology and Response

There were seven NICU *S. warneri* infection cases; the first case (*S. warneri*-21-1) occurred in 2020 followed by six additional cases (*S. warneri*-21-2 to *S. warneri*-21-7) 2 months later. *S. warneri* infection was not associated with death. In response, the hospital promptly implemented stringent control measures, including comprehensive environmental disinfection and transfer of all affected neonates to isolation wards. Environmental samples were collected from the hospital to test for *S. warneri*. A total of 195 samples were collected from high-touch surfaces (weighing scales, workbench, stethoscopes, and computer keyboards), equipment (refrigerators, incubators, humidifiers, trolleys, baths, and ventilators), water outlets (faucets and sinks), staff hands, nares, and the forehead. One *S. warneri* strain (*S. warneri*-21-8) was isolated from a milk preparation workbench.

Subsequent risk assessments indicated that the surface of the workbench has been linked to this outbreak. The rigorous environmental disinfection and prevention measures included comprehensive disinfection of the environment using a 500mg/L chlorine-containing disinfectant (ebest, Nanjing, China) and the transfer of all affected neonates to isolation wards. Moreover, stringent protocols have been established to ensure the quality of cleaning processes. The frequency of surface disinfection has been increased from 2 times a day to 3 times a day, now including bedside monitors, ventilators in the ward, workbenches, supplies, sinks, and faucets in the milk and solution preparation room. Nurses working in the milk or liquid preparation room were assigned only to perform milk or liquid preparation work and were not involved in other tasks. Additionally, reinforcement was provided during hand hygiene training, with supervisors conducting daily inspections. There have been no reported cases of new *S. warneri* infections. Through the combination of penicillin sodium, piperacillin sulbactam, meropenem, and vancomycin, all seven cases were successfully recovered and were subsequently discharged from the hospital.

## Strains and WGS

WGS was performed for eight isolates (Table 1), which generated 654,332–3,032,674 reads with an average coverage ranging from 26 to 123-fold. A matrix was constructed based on extracted SNPs in the core genome of 8 isolates using Parsnp (Table 2). Apparently the *S.warneri*-21-1 strain isolated from a sporadic case, either belonging to a different sequence type or having >625 SNP differences (>5 times the maximum SNP distance observed between outbreak isolates) within the same sequence type as the outbreak strain. Six strains (*S.warneri*-21-2 - *S.warneri*-21-7) isolated from newborns in 2021 exhibited 0–35 SNPs among them. According to the criteria for outbreak-related strains: ≤10 SNPs,[14,25,26] it is likely that the six cases were infected by the same source. One strain (*S.warneri*-21-8) isolated from a surface had 11–69 SNPs compared to the six strains. These seven strains are closely related and have been confirmed to be from an outbreak through WGS. Since *S. warneri* does not have a complete MLST system, sequence typing is impossible.

Virulence factor analysis results (Figure 1) revealed the virulence factor profiles of the 98 *S. warneri* strains, all had *hld* gene encoding exotoxin, Three human reference strains (003857215.1, 007667785.1 and 007666705.1) had nutritional/metabolic factor *isdE* and one (001071965.1) had effector delivery system-related virulence factor *esxA*.

The drug-resistant phenotype (Table 3) revealed that all eight *S. warneri* strains exhibited resistance to five antibiotics: clindamycin, cefoxitin, erythromycin, oxacillin, and penicillin. Furthermore, three strains (*S. warneri*-21-1, 2, and 7) displayed a unique resistance to trimethoprim-sulfamethoxazole. We predicted 10 resistance genes associated with seven classes of antibiotics, two of which (*bleO* and *mecA*) were identified in eight *S. warneri* strains, and a notable difference was observed between *S. warneri-21-1* and the other seven strains (Figure 1). A total of 23 resistance genes were identified across the 98 strains, falling into 16 categories based on their antibiotic target. Most strains exhibited good concordance between their resistance genotypes and phenotypes. Resistance genes were predicted in 55 strains, of

**Table 1** Genome Summary of 8 S. Warneri Strains

| Strains ID | Isolation soucre | Genome size (Mb) | No. of CDSs | No. of scaffolds | Contig N50 (bp) | G+C content (%) | Total no. of genes | NCBI Genome accession No. |
|---|---|---|---|---|---|---|---|---|
| S. warneri-21-1 | Neonat | 2.47 | 2364 | 35 | 206,179 | 32.6 | 2432 | JALGYS000000000 |
| S. warneri-21-2 | Neonat | 2.62 | 2563 | 44 | 331,461 | 32.5 | 2635 | JALGYR000000000 |
| S. warneri-21-3 | Neonat | 2.57 | 2487 | 40 | 318,227 | 32.5 | 2551 | JALGYQ000000000 |
| S. warneri-21-4 | Neonat | 2.57 | 2476 | 49 | 318,173 | 32.5 | 2517 | JALGYP000000000 |
| S. warneri-21-5 | Neonat | 2.57 | 2489 | 43 | 318,279 | 32.5 | 2546 | JALGYO000000000 |
| S. warneri-21-6 | Neonat | 2.55 | 2457 | 42 | 318,280 | 32.5 | 2525 | JALGYN000000000 |
| S. warneri-21-7 | Neonat | 2.58 | 2492 | 42 | 318,277 | 32.5 | 2556 | JALGYM000000000 |
| S. warneri-21-8 | Environment | 2.62 | 2567 | 46 | 331,461 | 32.5 | 2634 | JALGYL000000000 |

**Abbreviation**: CDSs: coding sequences.

**Table 2** The cgSNP Analysis of 8 S. Warneri Strains

| Strains | S.warneri-21-1 | S.warneri-21-2 | S.warneri-21-3 | S.warneri-21-4 | S.warneri-21-5 | S.warneri-21-6 | S.warneri-21-7 | S.warneri-21-8 |
|---|---|---|---|---|---|---|---|---|
| S.warneri-21-1 | 0 | 45,083 | 45,083 | 45,080 | 45,084 | 45,084 | 45,084 | 45,085 |
| S.warneri-21-2 | 45,083 | 0 | 0 | 13 | 1 | 1 | 1 | 11 |
| S.warneri-21-3 | 45,083 | 0 | 0 | 13 | 1 | 1 | 1 | 11 |
| S.warneri-21-4 | 45,080 | 13 | 13 | 0 | 14 | 14 | 14 | 16 |
| S.warneri-21-5 | 45,084 | 1 | 1 | 14 | 0 | 1 | 35 | 69 |
| S.warneri-21-6 | 45,084 | 1 | 1 | 14 | 1 | 0 | 2 | 12 |
| S.warneri-21-7 | 45,084 | 1 | 1 | 14 | 35 | 2 | 0 | 12 |
| S.warneri-21-8 | 45,085 | 11 | 11 | 16 | 69 | 12 | 12 | 0 |

**Abbreviation**: cgSNP: core genome single-nucleotide polymorphism.

which 87% were human. The classification of drug resistance genes showed that β-lactamase antibiotic was the most resistant, followed by aminoglycoside antibiotic and streptogramin antibiotic.

## Pan-Genome Analysis

To observe the phylogenetic evolutionary relationship of *S. warneri* at the whole-genome level, a pan-genome analysis was performed. Pan-genome analysis identified 3237 genes represented in eight *S. warneri* strains. The core genome consisted of 1726 genes (shared by >99% of the strains). The soft-core, shell, and cloud genomes comprised 0, 841, and 670, respectively (Figure 2A). The phylogenetic tree (Figure 2B) showed that six strains isolated from neonates (*S. warneri*-21-2–7) and one from the environmental swab (*S. warneri*-21-8) in 2021 exhibited high homology among themselves and no homology with the *S. warneri* strain isolated in 2020 (*S. warneri*-21-1). This observation aligns with the presence-absence matrix of core and accessory genes, where the core genes of *S. warneri*-21-2 to *S. warneri*-21-8 displayed a higher level of conservation and were notably distinct from those of *S. warneri*-21-1.

In the phylogenetic tree of *S. warneri* strains from various hosts (Figure 1), the strains isolated in 2020 (*S. warneri*-21-1) and 2021 (*S. warneri*-21-2 to *S. warneri*-21-8) were located on separate branches, suggesting a relatively distant phylogenetic relationship between the two groups. However, both branches belong to the human *S. warneri* strain group.

# Discussion

We report an *S. warneri* outbreak in the NICU involving seven neonates. Although the initial source is unclear, epidemiological and genomic assessments revealed significant evidence, and one *S. warneri* strain was isolated from a milk preparation room workbench. It is more common for the solution preparation process to be related to bacteremia. The investigation showed that the outbreak was linked to the milk preparation workbench. However, since the milk preparation nurse also participated in newborn nursing work, it is possible that the nurse brought the *S. warneri* strain to the milk preparation room after nursing. Although neonatal gastrointestinal infections can cause bacteremia, the newborn did not display obvious diarrhea symptoms, so stool was not collected for culture during the investigation. Through WGS analysis, we found that the first case was unrelated to the outbreak.

Considering the predominant presence of *S. warneri* on the skin and mucosal surface, it cannot be ruled out whether the hands or other skin of nurses or other staff were contaminated or colonized by this bacterium, subsequently leading to contamination of the milk mixing table surfaces and milk and causing this outbreak of infection. Although we did not isolate *S. warneri* from the hands of the nurses during our investigation, it is possible that this outbreak prompted increased attention to hand hygiene.

CoNS bacteria have highly variable pathogenic potential, especially in hospital-acquired infections.[8] Although the role of *S. epidermidis* has been extensively studied in the past,[27–29] the relative importance of *S. warneri* has received relatively little attention. However, recent studies have suggested that *S. warneri* is emerging as a notable pathogenic

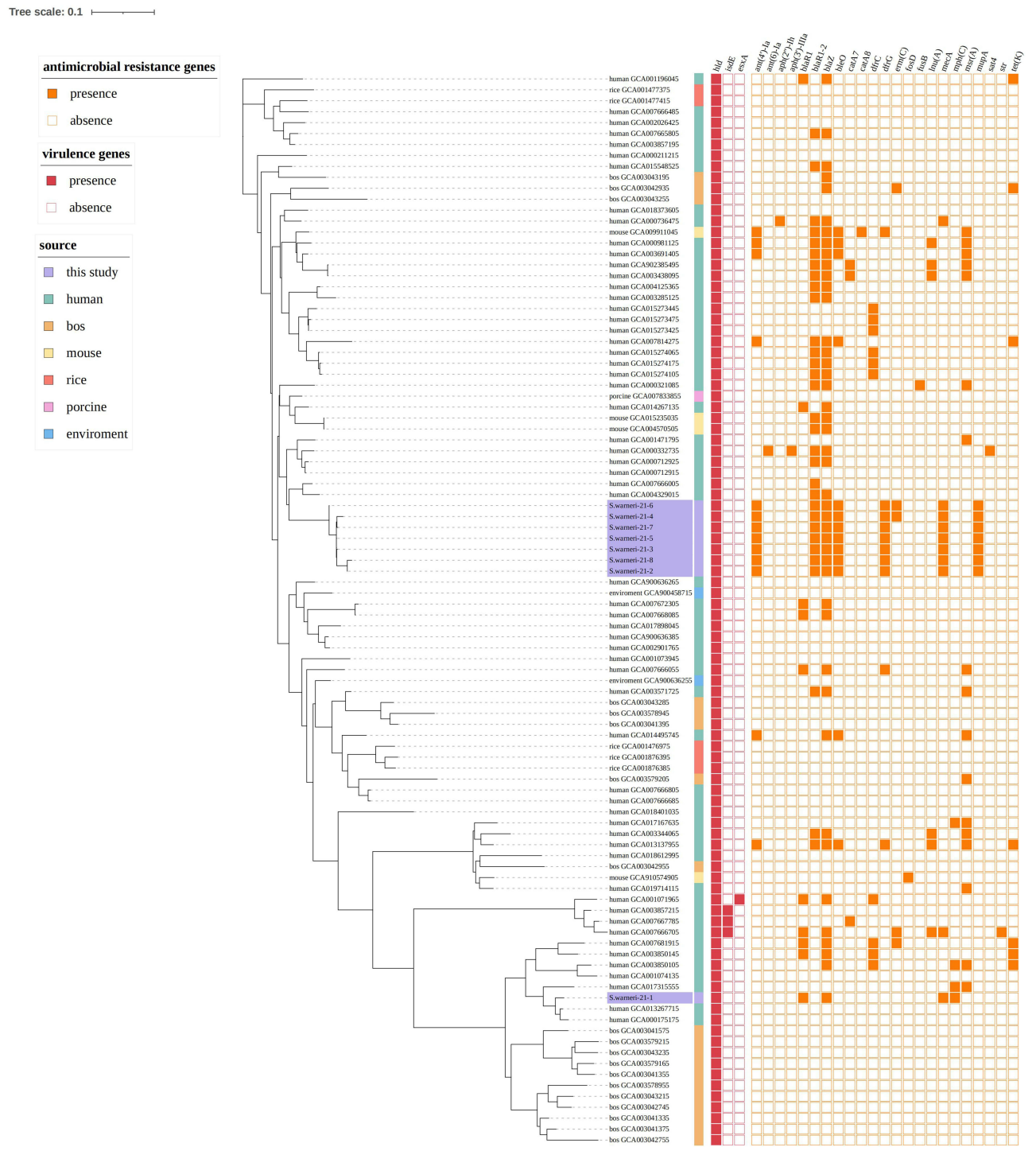


**Figure 1** Complex pan-genome phylogenetic tree illustrating the distribution of antimicrobial resistance genes and virulence genes in 98 *S. warneri* from different hosts.

bacterium, drawing increased attention in the realm of public health concerns.[30] The NICU is a unique setting in which low birth weight, prematurity, and the use of invasive devices contribute to the onset of infection and the spread of outbreak events. During an outbreak, it is crucial to characterize the genetic relationships between isolates expeditiously. WGS offers a high-throughput approach for tracing strains and analyzing virulence and drug resistance. Accurate identification and typing of bacterial isolates has traditionally relied on tools such as pulse-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and multiple-locus variable number tandem repeat analysis (MLVA).

**Table 3** The Drug Resistance Phenotype of 8 S. Warneri Strains

| Strains | VA | CLI | LZD | RIF | NIT | TET | SXT | CFX | QDA | LVX | GEN | TGC | CIP | ERY | OXA | MXF | ICLI | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. warneri-21-1 | S | R | S | S | S | S | R | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-2 | S | R | S | S | S | S | R | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-3 | S | R | S | S | S | S | S | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-4 | S | R | S | S | S | S | S | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-5 | S | R | S | S | S | S | S | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-6 | S | R | S | S | S | S | S | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-7 | S | R | S | S | S | S | R | R | S | S | S | S | S | R | R | S | S | R |
| S. warneri-21-8 | S | R | S | S | S | S | S | R | S | S | S | S | S | R | R | S | S | R |

**Abbreviations**: VA, vancomycin; CLI, clindamycin; LZD, lnezolid; RIF, rifampicin; NIT, nitrofurantoin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; CFX, cefoxitin; QDA, quinupristin-dalfoprisdn; LVX, levofloxacin; GEN, gentamicin; TGC, tigecycline; CIP, ciprofloxacin; ERY, erythromycin; OXA, oxacillin; MXF, moxifloxacin; ICLI, induction of clindamycin; PEN, penicillin. Green represents absence and red represents presence.



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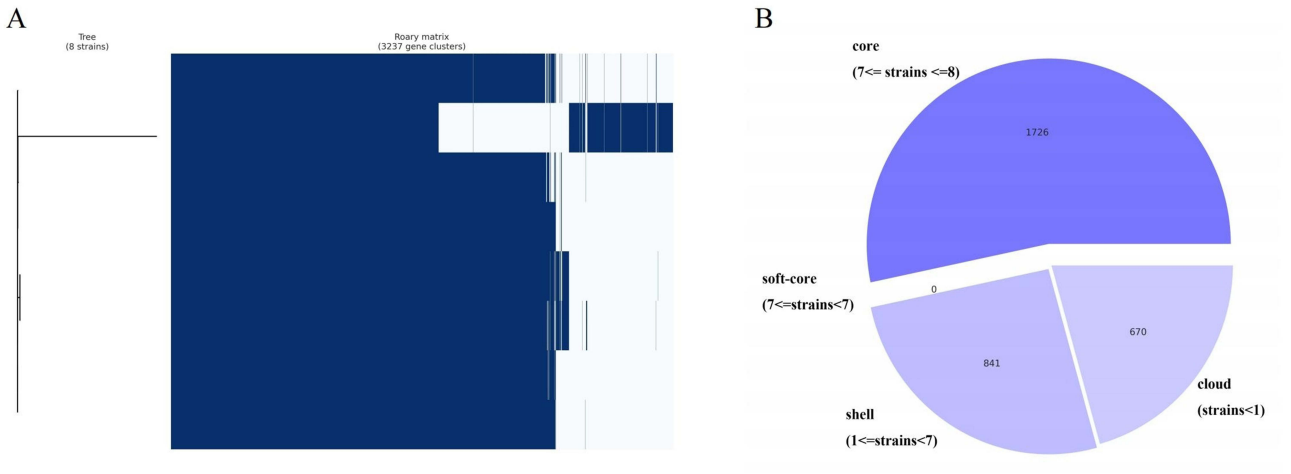


**Figure 2** Pan-genome analysis of 8 *S. warneri* strains. (**A**) Whole-genome phylogenetic tree and a matrix with the presence and absence of core and accessory genes. (**B**) Pie chart depicting the numbers of core-, soft core-, shell- and cloud genomes.

WGS-based strain analysis has been increasingly used in epidemiological studies of bacterial pathogens both for public health and hospital infection control. Establishing phylogenetic relationships among clinical strains is indispensable for hospitals to formulate effective infection control measures and to prevent outbreaks. In this study, SNP analysis revealed a close relationship among the seven isolates from 2021, clearly distinguishing them from the neonatal isolate in 2020.

Clinical infections caused by *S. warneri* were reported in 1984.[31] However, the genetic basis of virulence factors of this emerging pathogen remains poorly understood. Despite being similar to *S. aureus, S. warneri* exhibits a more limited array of virulence factors and has received less research attention than the major CoPS pathogens. In our study, analysis of virulence factors revealed striking similarities among *S. warneri* strains from different hosts and area, all had *hld* gene encoding exotoxin. The higher rates of *hlg* and *hld* in *S. aureus* strains isolated from infections may have a role in pathogenesis.[32] Further evidence is required to establish the significance of the *hld* gene as a virulence factor in infections caused by *S. warneri*. Three human reference strains had nutritional/metabolic factor *isd*E and one reference human strain had effector delivery system-related virulence factor *esx*A, indicating that strains from human had higher virulence than other strains. Compared to the virulence factors of *S. aureus*, those of *S. warneri* are few.

Since the emergence of penicillin- and methicillin-resistant staphylococci, these bacteria have become global public health concerns. Analysis of drug resistance 98 genomes across the world found that, 55 strains precarried antibiotic resistance genes, *blaR1* and *blaZ*, which are responsible for beta-lactam resistance, were detected in most strains. Of these, 87% were human strains, indicating that human *S. warneri* was highly resistant, which is also consistent with actual clinical infections, and bacteria isolated in hospitals tend to have stronger antibiotic adaptability due to antibiotic pressure selection.

In addition, when compared to strains from other regions, the strains isolated in this study specifically contained *mupA* gene, which revealed the regional epidemic characteristics and also reflected the drug use in hospitals to a certain extent. Eight *S. warneri* isolates carried *mecA* gene but were sensitive to vancomycin, indicating that vancomycin remains an effective treatment option for methicillin-resistant *S. warneri* infections.

Notably, the trimethoprim resistance gene *dfrG* was identified in *S. warneri*-21-2 to *S. warneri*-21-8, but was absent in *S. warneri*-21-1. However, only *S. warneri*-21-1, 2, and 7 displayed resistance to trimethoprim-sulfamethoxazole, suggesting the possibility of horizontal gene transfer or mutation. The macrolide resistance gene *erm(C)* encodes 23S ribosomal ribonucleic acid methylase, which methylates the ribosomal target sites of bacteria, reducing the binding of macrolide antibiotics to ribosomes and causing bacterial drug resistance. Consequently, all strains demonstrated resistance to erythromycin.

Pan-genome analysis has gained prominence as an additional tool for typing bacterial genomes, by clustering strains based on the presence or absence of accessory genes. Combining this analysis with variations in core, accessory, and

regulatory genome regions provides a super-resolution view of the bacterial population epidemiology. Pan-genome analysis of the eight *S. warneri* isolates (Figure 2A) clearly indicated that the isolate from 2020 (*S. warneri*-21-1) was not closely related to the other isolates from 2021 (*S. warneri*-21-2 to *S. warneri*-21-8), suggesting that the 2021 infection outbreak was not linked to *S. warneri*-21-1. Core genes shared by all isolates, usually were conserved genes, and other genes were accessory genes, which are often highly variable. A large number of core genes indicated small genomic differences. The core genes preferentially have basic physiological and biological functions, while the function of accessory genes is involved in the process of genetic evolution or adaptation to new environments.[33] Furthermore, the high homology observed between *S. warneri*-21-2 and *S. warneri*-21-8 suggests that neonatal infections may have been caused by a strain present in the hospital environment. This study highlighted the transmission of CoNS clones between hospital environments, particularly *S. warneri* isolates.[8] Therefore, rigorous disinfection of the hospital environment, hand hygiene, and aseptic practices are crucial.

*S. warneri* is a zoonotic pathogen with a broad host range, not limited to infecting humans but also poses a threat to various hosts, including dogs (causing meningoencephalitis) and cattle (resulting in abortion). It is a significant risk factor for intramammary infection (IMI) caused by coagulase-negative Staphylococcus spp.[34] The phylogenetic tree of *S. warneri* strains from different hosts revealed that the eight *S. warneri* isolates exhibited homology only with human *S. warneri* strains. Interestingly, we observed relative affinity between the strains isolated from humans. According to NCBI *S. warneri* genome data, human strains accounted for the largest proportion, followed by strains from *Bos taurus* and *Capra aegagrus hircus*. This suggests that the primary susceptible host of *S. warneri* is humans and that human *S. warneri* strains display a high degree of host specificity. Although both belong to gram-positive Staphylococcus, some lineages of *S. aureus* exhibit a broader host range, such as MRSA ST398,[35] which colonizes pigs and can cause infections in humans.[36] Other studies have highlighted the host shifts in *S. aureus* lineages from humans to chickens and cows.[37,38] These findings suggest that the host range of pathogens can evolve and change dynamically. Determining whether the host specificity of *S. warneri* undergoes these changes requires long-term surveillance.

The initial confirmed case of this outbreak was reported on December 25, 2020. Six additional cases were diagnosed within three days, two months later. In response, the hospital's infection management department immediately initiated investigations and implemented necessary measures. A comprehensive range of samples was collected to ensure the accuracy and reliability in the investigation. Additionally, comprehensive environmental disinfection and transfer of all affected neonates to isolation wards were deemed necessary. Although the initial source remains unclear, effective implementation of multiple measures is crucial to prevent new case*s* of *S. warneri* infection.

## Conclusion

Here, we describe an NICU *S. warneri* outbreak. We sequenced and analyzed eight *S. warneri* strains isolated from seven neonates and one from a hospital environment using cgSNP analysis, genomic characterization, and pan-genome analysis. The investigation revealed that the outbreak was linked to the milk preparation workbench. After implementing multiple preventive measures and control strategies, including rigorous environmental disinfection, hand hygiene practices, and quarantine protocols, no new cases of *S. warneri* infection were reported.

## Ethical Approval

According to the guidelines of control of healthcare associated infection outbreak (WS/T 524 −2016) [12], the samples used in this study were collected as part of normative investigation activities. The study received ethical approval on 2021, which complies with the Declaration of Helsinki, from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (2021-SR-579).

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## Disclosure

The authors report no conflicts of interest in this work

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