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Prevalence and Clinical Relevance of *Staphylococcus warneri* in the Neonatal Intensive Care Unit

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Abstract

Objective—To describe the prevalence of *Staphylococcus warneri* on the hands of nurses and the clinical relevance of this organism among neonates in the neonatal intensive care unit (NICU).

Design—Prospective cohort study that examined the microbial flora on the hands of nurses and clinical isolates recovered from neonates during a 23-month period (March 1, 2001, through January 31, 2003).

Setting—Two high-risk NICUs in New York City.

Participants—All neonates hospitalized in the NICUs for more than 24 hours and all full-time nurses from the same NICUs who volunteered to participate.

Intervention—At baseline and then every 3 months, samples for culture were obtained from each nurse's cleaned dominant hand. Pulsed-field electrophoresis compared *S. warneri* isolates from neonates and staff.

Results—Samples for culture (n = 834) were obtained from the hands of 119 nurses; 520 (44%) of the 1,195 isolates of coagulase-negative staphylococci recovered were identified as *S. warneri.* Of the 647 clinically relevant isolates recovered from neonates, 17 (8%) of the 202 isolates that were identified to species level were *S. warneri.* Pulsed-field electrophoresis revealed a common strain of *S. warneri* that was shared among the nurses and neonates. Furthermore, 117 (23%) of 520 *S. warneri* isolates from nurses' hands had minimum inhibitory concentrations for vancomycin of 4 μg/mL, which indicate decreasing susceptibility.

Conclusions—Our findings that *S. warneri* can be pathogenic in neonates, is a predominant species of coagulase-negative staphylococci cultured from the hands of nurses, and has decreased vancomycin susceptibility underscore the importance of continued surveillance for vancomycin resistance and pathogenicity in pediatric care settings.

Coagulase-negative staphylococci (CoNS) are the most common microorganisms that cause healthcare-associated infections in neonates hospitalized in the neonatal intensive care unit (NICU),[1,2] with *Staphylococcus epidermidis* being the most common species recovered from clinically significant cultures.[3-5] Although not a predominant pathogen, *Staphylococcus warneri* has been recovered from the hands of healthcare workers[6,7] and hand carriage has been linked to the transmission of disease.[8-10] Because of high oxacillin resistance rates among

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CoNS, vancomycin is the antimicrobial agent of choice. However, a trend toward decreased susceptibility to this glycopeptide has been shown among CoNS isolates.[11,12]

Few data are available regarding the prevalence and clinical significance of CoNS species other than *S. epidermidis.* We report on the prevalence of CoNS on the hands of nurses in 2 NICUs, with a focus on the epidemiology and vancomycin susceptibility of *S. warneri.* In addition, the clinical relevance of *S. warneri* among neonates in the same NICUs is explored.

Methods

Sample and Setting

This study was conducted in 2 NICUs that are part of the New York-Presbyterian Hospital in Manhattan in New York City. Data were obtained from all neonates hospitalized in the NICUs for more than 24 hours. Nurse participants were full-time employees who volunteered to participate in a larger clinical trial conducted from March 1, 2001, through January 31, 2003, that examined the relationship between hand hygiene practices and healthcare-associated infections in critically ill neonates.[13] Sixty-one (77%) of 79 nurses from NICU 1, a 43-bed unit, and 58 (76%) of 76 nurses from NICU 2, a 50-bed unit, agreed to participate. Institutional review board approval was obtained from the participating institutions, and each nurse participant provided written consent before data collection. Data from 2,935 neonatal admissions and 119 nurses were included in this study.

Procedures

Patient cultures—Clinical specimens were collected from neonates by NICU staff when clinically indicated. A nurse epidemiologist hired specifically for this study conducted prospective surveillance for infections in neonates in both NICUs. Infections met Centers for Disease Control and Prevention case definitions[14] and included bloodstream infections, pneumonia, conjunctivitis, skin and soft-tissue infections, and infections of the central nervous system. Samples for surveillance cultures were not obtained from infants.

Surveillance cultures from nurses' hands—Samples for culture were collected from the dominant hand of each nurse participant at baseline and every 3 months during the 23-month study. Before sampling, participants cleansed their hands using the hand hygiene product (ie, 2% chlorhexidine gluconate or alcohol-based hand rub) available on the unit. A modified "glove-juice" technique was used for sampling, as described elsewhere.[15]

Microbiologic Procedures

All bacteriologic isolation, identification, antibiotic susceptibility tests, and molecular analyses were performed by the Clinical Microbiology Service of Columbia University Medical Center. As reported elsewhere,[15] undiluted aliquots and diluted aliquots (10-fold and 100-fold) of samples of microbial flora were inoculated onto 5% sheep blood agar (Becton Dickinson Microbiology Systems) for determining total colony counts.

Staphylococcal isolates from cultures were speciated using the MicroScan System (Dade Behring) according to the manufacturer's instructions. We confirmed the ability of the MicroScan System to identify *S. warneri* accurately to species level by 16S ribosomal RNA sequencing in 4 isolates from this study.

Antimicrobial susceptibility testing was performed using the microbroth dilution MicroScan System according to the manufacturer's instructions. CoNS susceptibility to oxacillin was indicated by a minimum inhibitory concentration (MIC) of 0.25 μg/mL or less, and resistance was indicated by an MIC of 0.5 μg/mL or more. We used the current Clinical Laboratory

Standards Institute (formerly National Committee for Clinical Laboratory Standards) guidelines[16] for vancomycin MIC breakpoints of susceptible (4 μg/mL or less), intermediate (8 to 16 μg/mL), and resistant (more than 32 μg/mL). For the purposes of this study, MIC susceptibility subcategories of 2 μg/mL or less and 4 μg/mL were examined to determine the degree of decreased vancomycin resistance among *S. warneri* isolates. The Etest (AB Biodisk North America) was used to confirm vancomycin MICs for a random sample of *S. warneri* isolates ($n = 24$) recovered from nurses' hands. To limit the possibility of error in susceptibility testing, all isolates from both neonates and nurses were tested in real time by the same laboratory technician. Isolates were stored by the clinical microbiology laboratory at -70°C for future analyses. For comparative purposes, the antimicrobial susceptibility patterns of unrelated *S. warneri* strains isolated during the study period from pediatric patients ($n = 20$) and adult patients ($n = 37$) hospitalized outside the NICU in 1 study hospital were analyzed.

Molecular Typing

The clinical isolates of *S. warneri* from neonates with bloodstream infection and *S. warneri* from the hands of nurses who provided care to these infected neonates in the same NICU underwent molecular typing. The isolates from nurses that were selected for molecular typing fulfilled the following criteria: the strain was isolated from a nurse's culture sample within 1 month of a neonatal infection with *S. warneri*, and the *S. warneri* strains from the nurse and infected neonate had 2 or fewer differences in susceptibility to the antimicrobial agents tested. For comparative purposes, *S. warneri* strains from NICU nurses who did not provide nursing care to the infected neonates ($n = 4$), from adults with hospital-acquired infections caused by *S. warneri* ($n = 4$), and from adults in the community with hand carriage of *S. warneri* ($n = 4$) during the same period were also typed.

Bacterial genomic DNA was digested using *Sma*I endo-nuclease, and its genetic fingerprint determined by pulsed-field gel electrophoresis (PFGE) using the GenePath system (BioRad), as described elsewhere.[17] The pattern of DNA restriction fragments was interpreted as indistinguishable, related, closely related, possibly related, or different, according to established criteria.[18]

Results

A total of 647 bacterial isolates were associated with clinical infection in the neonates during the 23-month study, of which 442 (68%) were from neonates in NICU 1 and 205 (32%) from neonates in NICU 2. Two hundred twenty-one (34%) of the isolates were CoNS, and 202 (91%) were identified to the species level (Table 1). Most CoNS-associated neonatal infections were caused by *S. epidermidis* (151 [68%]), followed by *S. warneri* (17 [8%]). The 17 neonatal infections caused by *S. warneri* included 15 bloodstream infections (13 catheter-related, 2 non-catheter related), 1 skin infection, and 1 eye infection.

A total of 834 samples for culture were analyzed from the nurses in NICU 1 ($n = 450$) and NICU 2 ($n = 384$). Of 1,442 bacterial isolates recovered, 1,195 (83%) were CoNS. Most CoNS isolates were *S. epidermidis* (524 [44%]), followed by *S. warneri* (520; 44%). Eleven additional CoNS species were also identified (Table 1).

Antibiotic Susceptibility

The distribution of *S. warneri* MICs for oxacillin and vancomycin is given in Table 2. Most NICU isolates were resistant to oxacillin; 84% of isolates from NICU nurses and 88% of *S. warneri* isolates from neonates had MICs of 0.5 μg/mL or higher.[16] In comparison, lower rates of resistance to oxacillin were found among isolates from pediatric patients (65% of isolates) and adult patients (30% of isolates) hospitalized outside the NICU. Furthermore, 402 (77%)

of the *S. warneri* isolates from nurses' hands had vancomycin MICs of 2 μg/mL or less, and 117 (23%) had vancomycin MICs of 4 μg/mL. Only 1 isolate had intermediate susceptibility to vancomycin (MIC, 8 μg/mL). In contrast, 100% of the *S. warneri* isolates from neonates in the NICU and the isolates from pediatric and adult comparative populations had vancomycin MICs of 2 μg/mL or less.

Molecular Typing

Fifteen *S. warneri* isolates were recovered from 12 neonates with bloodstream infection in the NICU, 13 of which were available for PFGE. Of these isolates from neonates, 4 (31%) were indistinguishable from each other (ie, PFGE-designated clone A), 8 (61%) were related (ie, clones A_1 and A_2), and 1 isolate was unrelated. Eighteen isolates from nurses caring for 6 infected neonates were compared. Overall, 5 (83%) of these 6 neonates had isolates of clone A that were either indistinguishable from or related to isolates from at least 1 of their nurse caregivers. The Figure depicts the PFGE patterns of select isolates from 6 adults (different strains) and 8 study subjects with clone A.

Discussion

CoNS in Hospitalized Neonates

To our knowledge, this is the largest study to examine the prevalence and molecular epidemiology of *S. warneri* among NICU staff and patients. Previous studies have assessed the relative prevalence of *S. warneri* compared with other CoNS species and demonstrated that, after *S. epidermidis*, *S. warneri* is usually the second most common species, responsible for 10%-50% of infections caused by CoNS.[19-21] In a retrospective study at a children's hospital, Buttery and colleagues[20] reported that *S. warneri* was the second most common CoNS species associated with bloodstream infection. They reported no evidence of a single strain of *S. warneri*, but half of the isolates (6 of 12) were oxacillin resistant. In contrast, our susceptibility results show that 88% of the neonates and 84% of the NICU nurses had *S. warneri* strains that were oxacillin resistant. Among the isolates from neonates, no trend toward decreased resistance to vancomycin was seen; 100% of the strains had MICs of less than 2 μg/mL.

In the relatively few reports that describe the molecular typing of *S. warneri* associated with late-onset sepsis, small numbers of isolates have been studied. Kacica et al.[21] demonstrated that 4 *S. warneri* isolates that caused late-onset sepsis were unrelated, whereas Raimondo et al.[4] reported that during an initial 12-month study period, all *S. warneri* isolates (n = 8) that caused late-onset sepsis were unrelated, but during a follow-up 12-month study conducted 3 years later, most *S. warneri* isolates (5 of 7) were related. Our findings are similar in that almost all neonates (12 of 13) infected with *S. warneri* had isolates that were shown to be indistinguishable or related by PFGE.

CoNS Flora on Nurses' Hands

As reported by others, most isolates cultured from the hands of nurses were CoNS[6,7,22,23]; however, the predominance of *S. warneri* has not been previously described. Horn et al.[7] cultured samples from the skin of nurses, physicians, office workers, and students in an acute care hospital and found that 38% of CoNS isolates were *S. epidermidis* and 2% were *S. warneri*. Larson et al.[6] examined the hand flora of 40 nurses and found that 131 (60.6%) of 216 isolates were staphylococcal species, of which 6.8% were *Staphylococcus aureus*, 31.3% were *S. epidermidis*, 21.4% were *Staphylococcus hominis*, 19.1% were *S. warneri*, and 21.4% were other CoNS species. Kloos et al.[24] noted that *S. warneri* was "only occasionally" isolated from the skin of 40 adult participants. Although other studies have reported that NICU nurses

are colonized with strains of CoNS shared with patients,[25-27] to our knowledge none have described a predominant clone of *S. warneri* shared among nurses and neonates.

Several possible explanations for the high prevalence of *S. warneri* on nurses' hands were considered. It is possible that prevalent species change over time or vary by geographic region. Perhaps prevalent species and skin colonization patterns emerge within specific work settings. For example, we noted in this same study that the staphylococcal species and antimicrobial resistance patterns of the hand flora of new graduate nurses changed after a few months of employment in the study unit and more closely resembled the flora of nurses who had been long-term employees.[28]

We also speculate that patterns of antimicrobial use, specifically vancomycin, in neonates may have facilitated *S. warneri* hand carriage. Of interest, we observed a trend toward reduced vancomycin susceptibility (MICs greater than 2 μg/mL) among *S. warneri* isolates recovered from nurses' hands. The increased use of vancomycin in medical centers due to high oxacillin resistance may be responsible for the selection of subpopulations with decreased susceptibility to vancomycin. Center et al.[29] reported that exposure to vancomycin, especially exposure for more than 10 days, and an NICU stay of more than 28 days were significantly associated with bloodstream infections caused by *S. warneri* strains with vancomycin MICs of greater than 2.0 μg/mL. Although none of the isolates that caused neonatal infections in our study were resistant to vancomycin, the decreased susceptibility to vancomycin seen in the *S. warneri* isolates among nurses' hand flora may reflect trends in the strains that colonized the neonates. This speculation is strengthened by the observations that strains were shared among staff and neonates and that hand hygiene was suboptimal during the study.[30] However, there may be other important, as yet unknown, epidemiologic or biologic factors associated with the transmission dynamics of *S. warneri*.

In conclusion, *S. warneri* can be a pathogen in neonates. Our finding that *S. warneri* was the predominant CoNS species cultured from the hands of nurses in the NICU is unique. The decreased vancomycin susceptibility noted among these *S. warneri* strains underscores the need for continued surveillance for vancomycin resistance. Future studies should further explore the dynamics of transmission of antibiotic-resistant flora among NICU patients and staff and the mechanisms of vancomycin resistance. Continued surveillance is needed to assess the potentially changing epidemiology of *S. warneri* to determine whether it is emerging as a healthcare-associated pathogen and whether the decreased susceptibility to vancomycin is contributing to its spread.

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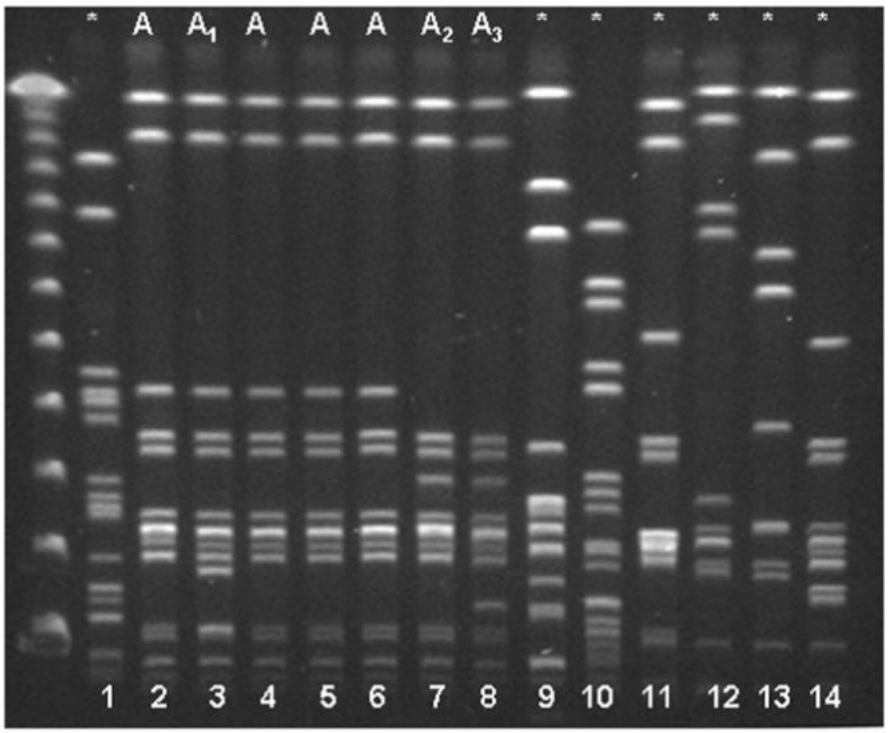

Figure 1.

Pulsed-field gel electrophoresis patterns of *Staphylococcus warneri* isolates. Asterisk indicates unrelated isolates. *Lanes 1-4*, isolates from study neonates; *lanes 5* and *6*, isolates from nurses in neonatal intensive care unit 2; *lanes 7* and *8*, isolates from nurses in neonatal intensive care unit 1; *lanes 9-11*, isolates from adult patients; and *lanes 12-14*, isolates from adults from the community.

Table 1

Distribution of Coagulase-Negative *Staphylococcus* (CoNS) Species From Nurses' Hands and Clinical Infections Among Neonates in 2 Neonatal Intensive Care Units (NICUs)

CoNS Species	No. (%) of isolates		*P*[a]
	From nurses	From neonates	
S. epidermidis	524 (44)	151 (68)	<.001
S. warneri	520 (44)	17 (8)	<.001
S. capitis	49 (4)	6 (3)	.33
S. hominis	27 (2)	16 (7)	<.001
S. haemolyticus	27 (2)	4 (2)	.81
S. simulans	16 (1)	5 (2)	.30
S. auricularis	15 (1)	1 (.5)	.50
Other[b]	17 (2)	2 (1)	.75
Total	1,195	202	...

[a] χ^2 or Fisher exact test.

[b] Includes *S. cohnii* (*n* = 6), *S. lugdunensis* (*n* = 5), *S. saprophyticus* (*n* = 3), *S. schleiferi* (*n* = 1), *S. sciuri* (*n* = 1), and *S. xylosus* (*n* = 3).

Table 2

Vancomycin and Oxacillin Susceptibilities of *Staphylococcus warneri* Isolates From Study Subjects

Cohort	No. of isolates	No. (%) of isolates, by agent and susceptibility category				
		Vancomycin MIC, μg/mL[a]			Oxacillin MIC, μg/mL	
		Susceptible, ≤ 2	Susceptible, 4	Intermediate, 8-16	Susceptible, <0.25	Resistant, >0.5
Nurses in NICU	520	402 (77)	117 (23)	1 (0.2)	81 (16)	438 (84)
Neonates in NICU	17	17 (100)	0	0	2 (12)	15 (88)
Pediatric patients, other units	20	20 (100)	0	0	7 (35)	13 (65)
Adult patients	37	37 (100)	0	0	26 (70)	11 (30)

NOTE. MIC, minimum inhibitory concentration; NICU, neonatal intensive care unit.

[a] The χ^2 was used to compare MICs among the 4 groups ($P < .001$).