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Antimicrobial resistance patterns of colonizing flora on nurses' hands in the neonatal intensive care unit

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Abstract

Background—The Centers for Disease Control and Prevention recommends the use of an alcohol-based handrub for health care worker hand hygiene. The purpose of this study was to examine effects of hand hygiene product and skin condition on the antimicrobial resistance patterns of colonizing hand flora among nurses.

Methods—Colonizing hand flora of 119 nurses working in 2 neonatal intensive care units was compared during a 22-month crossover study using alcohol handrub or antiseptic soap.

Results—Altogether, 1442 isolates from 834 hand cultures (mean, 7 cultures/nurse) were obtained. In 3 of 9 regression analyses modeling for resistant staphylococcal flora, the use of antiseptic soap was a significant predictor of resistance, and nurses with damaged skin were 2.79 times more likely to carry *Staphylococcus warneri* isolates resistant to gentamicin.

Conclusion—Hand hygiene product and skin condition may influence resistance patterns of hand flora of care providers.

The hands of health care workers may serve as reservoirs for organisms causing health care-associated infections, including infections caused by multidrug-resistant strains.^{1,2} In 2002, the Centers for Disease Control and Prevention revised the recommendations for hand hygiene to include the use of alcohol-based products for standard hand hygiene.³ However, the possible effects of hand hygiene products on the skin flora of personnel hands, including antibiotic resistance patterns, have not been extensively studied. We recently reported that the hands of a small number of new graduate nurses initially harbored methicillin-susceptible strains of staphylococci, which were replaced by methicillin-resistant strains within a few months of employment in the 2 study units.⁴ The purposes of this study were to describe the types and antimicrobial resistance patterns of hand flora among nurses working in 2 neonatal intensive care units (NICUs) over a 22-month period and to examine risk

factors, including the type of hand hygiene product, for antimicrobial resistance in the hand flora.

METHODS

Sample and setting

A substudy was conducted of a larger clinical trial in which a crossover design was used to evaluate the impact of 2 hand hygiene products,⁵ a detergent-based antiseptic containing 2% chlorhexidine gluconate (Bactoshield; Steris Corporation, St. Louis, MO) and a 61% alcohol-based handrub (Avagard; 3M HealthCare, St. Paul, MN), on health care-associated infections in neonates. The study was conducted in 2 Manhattan NICUs between March 2001 and January 2003. The 2 NICUs are part of the New York Presbyterian Hospital system: a 43-bed unit (NICU 1) and a 50-bed unit (NICU 2).

All full-time nurses working in these units were asked to volunteer for the study; 119 of 155 nurses (76.8%) agreed to participate. Nurses were selected for this study because they were the primary staff members permanently assigned to the study units and they had the most frequent contact with the neonates.⁶ Throughout the study, all staff on each unit used the same hand hygiene product, including a moisturizer provided by the hospital, and artificial fingernails were prohibited. Nonstened rings were allowed. The study was approved by the institutional review boards of both study sites, and each of the participating nurses signed a consent form. During the 22 study months, the condition of each nurse's hands was assessed monthly, and a hand culture was obtained quarterly, as described below.

Skin assessment

Nurses' skin condition was recorded monthly using 2 assessment tools. First, trained research personnel rated the skin on the hands of the participating nurses under $\times 3$ magnification on a scale of 0 to 5, with 0 being extensive cracking of the skin and widespread redness and 5 being normal skin with no observable irritation. Previous studies have confirmed that the ratings generated from this observer assessment tool were consistent with dermatologist ratings of skin condition and had an interrater reliability of 90% over a range of skin conditions.⁷⁻⁹

The second assessment tool was a self-rated scale with which participating nurses scored the skin on their hands using 4 criteria: appearance, intactness, moisture content, and sensation. The scale ranged from 4 to 28, with healthier skin having a higher score. Previous studies have demonstrated that this self-rating tool was significantly correlated with other measures of skin condition.¹⁰⁻¹³ The observer-rated and self-rated skin condition scores were collected independently of one another.

Hand culturing and species identification

Hand cultures were obtained every 3 months using a modified glove-juice technique.¹⁴ Nurses cleaned their hands with the available product immediately prior to sampling so that we could identify resident rather than transient flora. Their dominant hand was inserted into a sterile polyethylene bag containing 50 mL sampling solution (0.075 mol/L phosphate buffer, pH 7.9, containing 0.1% polysorbate 80 and 0.1% sodium thiosulfate), and the hand was massaged through the bag for 1 minute. The Clinical Microbiology Service of New York-Presbyterian Hospital, Columbia Campus, performed all microbiologic testing. Undiluted, 10- and 100-fold diluted aliquots of sampling solution were plated onto 5% sheep blood agar plates (BBL; Becton Dickinson Microbiology Systems, Cockeysville, MD). Organisms were identified by the MicroScan Walk Away 96 system (Dade Behring Inc,

Newark, DE). Using the clinical laboratory's standard protocols, a colony of each morphologically distinct colony type was selected for identification.

Antimicrobial susceptibility patterns

Antimicrobial susceptibilities were determined by the MicroScan Walk Away 96 system (Dade Behring Inc). In accordance with the Clinical and Laboratory Standards Institute,¹⁵ the isolates were dichotomized as either susceptible or nonsusceptible based on minimum inhibitory concentration values, with isolates determined to be "intermediate" or "resistant" categorized as nonsusceptible. Further analyses were conducted to examine changes in the staphylococcal flora (*Staphylococcus warneri*, *Staphylococcus epidermidis*, *Staphylococcus aureus*) of each nurse during the study period. For each nurse, we assessed changes in susceptibility of their *S epidermidis* isolates to oxacillin, rifampin, and gentamicin at each sampling period. We defined a potentially clinically relevant change in resistance as a change from susceptible to resistant or from resistant to susceptible to 2 or more of these agents.

Data analysis

Initially, χ^2 tests were used to examine differences in the relative proportions of the type of organism (ie, gram negative, gram positive, or fungi) by study unit (NICU 1 or 2) and hand hygiene product in use at the time of the culture (antiseptic soap or alcohol-based rub) as well as the difference in the proportion of isolates susceptible or nonsusceptible to antibiotics during the 2 hand hygiene product periods. Next, logistic regression models were used to examine potential predictors of carrying *S warneri*, *S epidermidis*, or *S aureus* isolates resistant to oxacillin, rifampin, and gentamicin. Models to predict vancomycin resistance were not included because there were too few isolates resistant to vancomycin. Separate regression models were fit for each species and antibiotic combination. In each model, the dependent variable was the presence or absence of resistance, and predictor variables included hand product used (alcohol-based rub or antiseptic soap), number of years worked in the NICU, site (NICU 1 or 2), and observer-rated skin condition. Only observer-rated skin condition was used in the models because observer and self-rating assessments of skin condition were significantly correlated ($P < .01$). A stepwise procedure with a variable entering the model at $P = .1$ and retained in the model at $P = .05$ was used (SAS software; SAS Institute, Cary, NC).

RESULTS

There were 834 hand cultures available for this analysis: 417 during the alcohol-based handrub period and 417 during the antiseptic soap period. An average of 7.0 (range, 1–8) cultures were obtained from each nurse participant (7.1/nurse in NICU 1 and 6.4/nurse in NICU 2). Only 3 of the 119 nurses were male, and the average age of participating nurses was 41.1 years.⁵

Types of flora

During the study period, 1442 isolates were collected from the hands of 119 nurses (Table 1). Most were gram-positive bacteria (88.8%, 1281/1442), 6.6% were gram-negative bacteria (95/1442), and 4.6% were fungi (66/1442). Of all the isolates, 85.7% (1236/1442) were staphylococci, with *S epidermidis* and *S warneri* accounting for 36.3% (524/1442) and 36.1% (520/1442) of isolates, respectively. Because there were no differences in the types of organisms found on the hands of nurses from the 2 units ($P = .13$), the data from the 2 sites were combined for the subsequent multivariate analyses.

Antibiotic susceptibility patterns

The antibiotic susceptibility profiles of staphylococcal species for which 10 isolates were identified during the study period are shown in Table 2. In general, *S epidermidis* and *S warneri* isolates were not susceptible to β -lactam agents, including oxacillin, but were susceptible to quinolone agents.

During the 22-month study period, there were 81.5% (97/119) of nurses from whom *S epidermidis* was isolated more than once, and 70.6% (84/119) of nurses had *S epidermidis* isolated from at least half of the cultures obtained. The antimicrobial susceptibility profiles of *S epidermidis* remained the same in 69.2% (63/91) of nurses from whom more than 1 isolate was identified; in 16.5% (15/91), there was 1 change, and, in 14.3% (13/91), there were 2 or more changes in the antibiotic resistance profile over time. However, changes in the resistance patterns of *S epidermidis* isolates from these 13 nurses had no distinguishable pattern with time.

Hand product, skin condition, and antibiotic resistance

We explored 9 regression models to identify predictors of resistance to oxacillin, rifampin, or gentamicin in *S warneri*, *S epidermidis*, and *S aureus*. In 6 of these models, none of the variables assessed were significant predictors of resistance. In 3 models, the hand product was the only significant predictor of antibiotic resistance; during the period when antiseptic soap was used, there was a significant increase in *S epidermidis* isolates resistant to oxacillin (relative risk, 1.92; 95% confidence intervals (CI): 1.08–3.43) and gentamicin (relative risk, 1.50; 95% CI: 1.00–2.27) and a 7.22 times increased risk of rifampin resistance among *S warneri* isolates (95% CI: 2.97–17.56). Additionally, the relative risk of resistance to gentamicin among *S warneri* isolates from nurses with damaged skin compared with nurses with healthy skin was 2.79 (95% CI: 1.35–5.81; Table 3).

DISCUSSION

To our knowledge, this is the largest longitudinal study of nurses' hand flora conducted to date. New and clinically relevant information was identified regarding the types and antimicrobial resistance patterns of nurses' hand flora, the relationship between hand hygiene product and flora, and the potential role of skin health on antimicrobial resistance of the colonizing flora.

Types and antimicrobial susceptibilities of nurses' hand flora

As expected, *S epidermidis* was the most common organism isolated from nurses' hands. In this sample, however, there was also a higher prevalence of *S warneri* than has been noted in other studies,^{9,16,17} despite the fact that it was not known to be a prevalent organism in the study hospital. This finding is important for 2 reasons. First, it may indicate that the colonizing flora of health care personnel can become modified over time to reflect individual differences by practice site. This was further confirmed by our finding that, although these nurses often retained the same strain of *S epidermidis* during the study period, they also shared a single clone among other each other and among their patients, as previously reported,¹⁸ evidence that this clone was associated with this specific critical care environment.

Our group and others have recently reported decreased susceptibility to vancomycin among neonates.^{19,20} Although *S warneri* is not known to be of major clinical significance, cases of health care-associated infection with this organism have been reported among neonates.²¹⁻²⁴ Hence, this "normal flora" may be emerging as yet another multidrug-resistant opportunistic pathogen among high-risk, low-birth-weight neonates.

The majority of the *S epidermidis* and *S warneri* isolates from the nurses' hands were resistant to antibiotics commonly used on the NICU. In previous studies conducted more than a decade ago, rates of oxacillin resistance among coagulase-negative staphylococci from nurses' hands ranged from 26% to 60%.^{16,23} In our study, 79% of these isolates were resistant to oxacillin. Patient care personnel have been found to be colonized with higher rates of antimicrobial-resistant *S aureus* isolates than nonmedical personnel.²⁵ In contrast with the high rates of oxacillin resistance noted in coagulase-negative staphylococci, generally low rates of methicillin-resistant *S aureus* have been isolated from nurses' hands in previous studies: 1 of 129 (0.8%)²² and 3 of 79 (3.8%) nurses.²⁵ These rates are slightly lower than the rate noted in our study (5/119, 4.2%), although in 1 survey of several hospitals in which MRSA was endemic, the majority of *S aureus* isolates from staff were methicillin resistant.²⁶ Clearly, personnel hands are a potential reservoir of resistance that can be transmitted to patients in high-risk environments.

Factors associated with antimicrobial resistance

We found in this study that antimicrobial resistance was significantly more common for several antibiotics when nurses were using the chlorhexidine-containing soap or when the skin on their hands was damaged. In a 6-year study, Gordin et al²⁷ reported a decrease in the number of health care-associated methicillin-resistant *S aureus* and vancomycin-resistant enterococcus acquired by patients when an alcohol product was used for staff hand hygiene, but we did not examine the rates of clinical infections caused by antibiotic-resistant organisms among neonates in our study.

Although others have reported a higher prevalence of certain potential pathogens such as *S aureus* and gram-negative bacteria among nurses with damaged skin,⁹ this may be the first report of a possible relationship between hand hygiene product, skin condition, and antimicrobial resistance. Irritant contact dermatitis and other skin problems are prevalent among health care providers, and the detergent base in soaps is one of the primary causes.^{7,28}

To our knowledge, there has been no biologic mechanism identified that could explain our observed association between chlorhexidine use and antibiotic resistance. It is possible that the link may be an intermediary one, ie, that the soap is associated with more skin damage and that it is the damaged skin that facilitates the colonization of hands with hospital-associated resistant flora. We have previously reported, in fact, that skin condition was significantly better when the alcohol product as compared with the antiseptic soap was used among these nurses.⁵ An alternative explanation is that microorganisms may develop resistance mechanisms to the chlorhexidine itself, as has been described with triclosan, a bisphenol ingredient used in some antiseptic products.²⁹ The potential association between the antiseptic soap and increased antibiotic resistance clearly needs further study. In the meantime, the Centers for Disease Control and Prevention's recommendations³ regarding use of alcohol hand hygiene products and attention to skin health among staff hands may be important for reducing the possibility that the hands of health care personnel could become reservoirs of antimicrobial resistance.

This study had several limitations that must be considered when assessing the relevance of our findings. First, we only obtained cultures every 3 months and did not sample every nurse participant at every sampling interval, although the average number of samples was 7/nurse. Clearly, this represents only a snapshot of changes in skin flora that might occur over time. Second, only a single colony-forming unit of each morphologic type was speciated from each hand sample. Hence, it is possible that several strains or different clones of any organism, in particular the coagulase-negative staphylococci, were present but not detected. Furthermore, we were unable to study multidrug-resistant, gram-negative bacteria on nurses'

hands because there were too few isolates. Finally, there may have been unmeasured factors that changed during the periods when the 2 hand hygiene products were used that could have affected resistance patterns.

CONCLUSION

In summary, it appears that the normal skin flora of nurses has become increasingly resistant when compared with earlier studies. Furthermore, in this study hand hygiene product and skin condition were associated with antibiotic resistance in the staphylococcal flora; for some antibiotics, an increased risk of resistance was found when nurses used an antiseptic soap rather than an alcohol product and when their skin was damaged. The potential impact of various hand hygiene products on the numbers, types, and resistance patterns of staff hand flora warrants further research.

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Table 1

Gram positive, gram negative, and fungal species isolated from NICU nurses' hands*

Species	Isolates		Nurses	
	n	%	n	% [†]
Gram-positive organisms				
<i>Enterococcus faecalis</i>	20	1.4	18	15.1
<i>Enterococcus faecium</i>	4	0.3	4	3.4
<i>Micrococcus</i> species	10	0.7	9	7.6
<i>Staphylococcus aureus</i>	41	2.8	26	21.8
<i>Staphylococcus auricularis</i>	15	1.0	13	10.9
<i>Staphylococcus capitis</i>	14	0.9	12	10.1
<i>Staphylococcus capitis-ureolyticus</i>	35	2.4	24	20.2
<i>Staphylococcus epidermidis</i>	524	36.3	118	99.2
<i>Staphylococcus haemolyticus</i>	27	1.9	20	16.8
<i>Staphylococcus hominis</i>	27	1.9	22	18.5
<i>Staphylococcus simulans</i>	16	1.1	13	10.9
<i>Staphylococcus warneri</i>	520	36.1	116	97.5
<i>Staphylococcus</i> species (other)	17	1.2	15	12.6
<i>Streptococcus</i> species	3	0.2	3	2.5
Other gram-positive organisms	8	0.6	8	6.7
Subtotal	1281	88.8	421	
Gram-negative organisms				
<i>Acinetobacter baumannii</i>	8	0.6	5	4.2
<i>Acinetobacter lwoffii</i>	17	1.2	16	13.4
<i>Enterobacter cloacae</i>	10	0.7	9	7.6
<i>Enterobacter</i> species (other)	10	0.7	10	8.4
<i>Escherichia coli</i>	3	0.2	3	2.5
<i>Klebsiella oxytoca</i>	10	0.7	9	7.6
<i>Klebsiella pneumoniae</i>	11	0.8	9	7.6
<i>Pseudomonas aeruginosa</i>	2	0.1	2	1.7
<i>Pseudomonas</i> species (other)	7	0.5	7	5.9
<i>Serratia marcescens</i>	4	0.3	4	3.4
Other gram-negative organisms	13	0.9	11	9.2
Subtotal	95	6.6	85	
Other				
<i>Candida albicans</i>	8	0.6	5	4.2
<i>Candida parapsilosis</i>	41	2.8	27	22.7
<i>Candida</i> species (other)	12	0.8	11	9.2
Other	5	0.3	5	4.2
Subtotal	66	4.6	48	
Total	1442	100.0	554	

* N = 119 nurses.

[†] Some nurses carried >1 isolate of a given species, but were only counted once/species. This percentage is the percentage of nurses from whom this organism was isolated at least once.

Table 2

Proportion of staphylococcal isolates susceptible to selected antimicrobial agents

	Total No. isolates	Percentage (number of isolates)			
		Oxacillin	Rifampin	Gentamicin	Levofloxacin
<i>Staphylococcus aureus</i>	41	85.4 (35)	92.7 (38)	85.4 (35)	92.7 (38)
<i>Staphylococcus auricularis</i>	15	53.3 (8)	100 (15)	86.7 (13)	80.0 (12)
<i>Staphylococcus capitis-capitis</i>	14	92.9 (13)	92.9 (13)	85.7 (12)	92.9 (13)
<i>Staphylococcus capitis-ureolyticus</i>	35	82.9 (29)	97.1 (34)	82.9 (29)	94.3 (33)
<i>Staphylococcus epidermidis</i>	524	12 (63)	96.6 (506)	27.5 (144)	93.3 (489)
<i>Staphylococcus haemolyticus</i>	27	19.2 (5)	92.6 (25)	55.6 (15)	66.7 (18)
<i>Staphylococcus hominis</i>	27	48.1 (13)	100 (27)	66.7 (18)	96.2 (25)
<i>Staphylococcus simulans</i>	16	60.0 (9)	100 (16)	75.0 (12)	93.8 (15)
<i>Staphylococcus warneri</i>	520	14.6 (76)	88.6 (459)	21.4 (111)	93.8 (486)

Vancomycin not included in table because most isolates were susceptible.

Table 3
Predictors of resistance in staphylococcal hand flora of nurses, as analyzed by logistic regression *

Organism	Antibiotic (No. isolates tested)	Significant variables	Relative risk (95% CI)
<i>Staphylococcus epidermidis</i>	Oxacillin (n = 478)	Hand product	
		Alcohol	Referent
	Gentamicin (n = 472)	Antiseptic soap	1.92 (1.08–3.43)
		Hand product	
<i>Staphylococcus warneri</i>	Gentamicin (n = 447)	Alcohol	Referent
		Antiseptic soap	1.50 (1.00–2.27)
		Skin Condition	
	Rifampin (n = 463)	Healthy	Referent
		Damaged	2.79 (1.35–5.81)
		Hand product	
		Alcohol	Referent
		Antiseptic soap	7.22 (2.97–17.56)

*. Predictor variables included in each model: hand product, skin condition, years worked on unit, and site (NICU 1 or 2).