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Emergence of Resistant Staphylococci on the Hands of New Graduate Nurses

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Abstract

OBJECTIVE—To describe the aerobic microbial flora on the hands of experienced and new graduate nurses over time.

DESIGN—A prospective cohort design that examined the relationship between duration of employment in an intensive care unit (ICU) and the microbial flora on the hands of experienced and new graduate nurses during a 23-month period.

SETTING—A 50-bed, level III-IV neonatal ICU in New York City.

PARTICIPANTS—Twelve experienced nurses and 9 new graduate nurses working full time in the NICU.

INTERVENTION—One hundred fifty samples were obtained from the clean, dominant hands of the nurses. Cultures were performed at baseline and then quarterly for each experienced and new graduate nurse. Baseline and final cultures of *Staphylococcus epidermidis* were further examined using pulsed-field gel electrophoresis.

RESULTS—At baseline, a significantly larger proportion of the experienced nurses had methicillin-resistant, coagulase-negative staphylococci isolated from their hands compared with the new graduate nurses (95% and 33%, respectively; $P = .0004$). For a second culture, performed 1 to 4 months later, there were no longer significant differences between the two groups (82% and 54%, respectively; $P = .12$). By the last culture, all staphylococcal isolates were methicillin resistant in both groups of nurses; 3 were methicillin-resistant *S. aureus*.

CONCLUSIONS—Colonization with methicillin-resistant staphylococci occurred after brief exposure to the hospital environment, despite the use of antiseptic hand hygiene agents. Furthermore, at final culture, the two groups shared one dominant hospital-acquired strain of *S. epidermidis*.

Among the functions of the normal skin flora, microbial interference as a means of defense against bacterial infection is one of the most important.¹ The flora on the hands of healthcare workers have been shown to vary by work setting and differ from those of non-healthcare workers.² Temperature and moisture,³ as well as the work environment, affect the numbers and types of bacterial flora and yeast found on the hands.^{2,4-6}

To date, there have been no studies that describe or compare the bacterial flora on the hands of experienced nurses versus new nurse graduates. The purpose of this prospective cohort survey was to describe the aerobic microbial flora on the hands of experienced and new graduate nurses over time and to examine the relationship between the duration of employment

in an intensive care setting and the types, quantities, and resistance patterns of microbial flora on the hands.

METHODS

Sample and Setting

Nurses in this study were members of the full-time staff of a 50-bed, level III-IV neonatal intensive care unit (NICU) in New York City who had volunteered to participate in a clinical trial conducted from March 1, 2001, through January 31, 2003, designed to examine the effects of hand hygiene practices on healthcare-associated infections in critically ill neonates. The hand hygiene intervention for all staff during the first year of the study consisted of a mild soap and a waterless, alcohol-based hand antiseptic; in the second year, the intervention was an antiseptic soap containing chlorhexidine gluconate. During this 23-month prospective study, 13 new nursing school graduates were hired. An experienced nurse was defined as a nurse with 10 or more years of full-time nursing experience on the specific NICU. A new graduate nurse was a recent nursing school graduate with 2 or fewer months of full-time nursing experience on the specific NICU.

Procedures

Institutional review board approval was obtained from the participating institution, and each participant provided written consent prior to data collection. At baseline and then on a quarterly basis during a 23-month period, a hand culture was performed for each new and experienced nurse. The second culture was performed 3 months after baseline for the experienced nurses; however, as a result of varying dates of hire, the second culture for the new graduate nurses was performed from 1 to 4 months after baseline. Prior to sampling for cultures, participants cleansed their hands for 10 seconds using the hand hygiene regimen available on the unit (either an antiseptic soap containing 2% chlorhexidine gluconate or a 62% alcohol-based hand antiseptic).

A modified glove-juice technique was used for sampling.⁶ Each participant inserted her dominant hand into a sterile polyethylene bag containing 50 mL of sampling solution (0.075 M phosphate buffer [pH, 7.9] containing 0.1% polysorbate 80 and 0.3% lecithin). This solution neutralizes any residual antiseptic on the skin and facilitates removal of microorganisms by dispersing the macro-colonies into single cells, which were then counted as colony-forming units (CFU). Trained project staff obtained samples for culture by massaging the entire hand through the wall of the sterile bag for 1 minute.

Microbiologic Protocols

The Clinical Microbiology Laboratory of Columbia University Medical Center performed all microbiologic isolation, identification and quantification, and antibiotic susceptibility testing using standard laboratory techniques and six general and selective media. Antimicrobial susceptibility testing of bacteria was conducted using the MicroScan system (Dade Behring, Sacramento, CA) and yeast was identified by Yeast ID (API, Murex, Lenexa, KS) and germ tube formation. Results were reported as resistant, intermediate, or susceptible, following current recommendations of the National Committee for Clinical Laboratory Standards.⁷ For this study, the intermediate and resistant categories were combined, so that organisms were categorized as either susceptible or resistant (non-susceptible).

Molecular Typing

Fourteen nurses had *Staphylococcus epidermidis* isolated from their hands both at baseline and at the final culture in the study. A comparison of the baseline with the last isolates of *S.*

epidermidis for these nurses was done to determine whether there was shared clonality among isolates. Strains were fingerprinted by pulsed-field gel electrophoresis (PFGE) using the GenePath system (Bio-Rad, Hercules, CA) as previously described.⁸ The pattern of DNA restriction fragments was interpreted as indistinguishable, related, closely related, possibly related, and different according to established criteria.⁹

Data Analysis

SAS software (version 8.2 for Windows; SAS Institute, Inc., Cary, NC) was used for data analysis. The mean ages of experienced nurses and new graduate nurses were compared using the *t* test for independent samples. After conversion to log base₁₀, the mean CFU of the two groups were compared using the *t* test. The Wilcoxon rank sum test was used to compare the mean number of different species from the two groups. Chi-square and Fisher's exact tests were used to compare the proportion of staphylococcal isolates that were oxacillin (methicillin) resistant, susceptible, or both and the proportion of enterococcal, gram-negative organisms and yeast recovered or absent in the two groups.

RESULTS

Fifty-eight (75%) of 77 nurses volunteered to participate in the study, with 35 (60%) having 10 or more years of experience on the specific NICU. Six nurse practitioners, 2 part-time nurses, and 2 assistant nurse managers were excluded because they did not meet the criteria of full-time staff nurse; 1 experienced nurse dropped out of the study after 1 culture.

Thirteen (22%) new graduate nurses had 2 or fewer months of experience on the specific NICU. Four were excluded: 3 dropped out of the study after the first culture and 1 enrolled late in the second year. The 9 new nurses were female and either white or Asian. The final sample consisted of 12 experienced nurses who were female and white or Asian and 9 new graduate nurses. Not surprisingly, the new graduate nurses were significantly younger than the experienced nurses (mean age, 24.7 and 42 years, respectively; $P < .0001$).

A total of 150 samples were obtained from the dominant hands of the nurses (91 from the experienced nurses and 59 from the new graduate nurses) during the study. From these, a total of 234 microbial isolates were recovered (154 from the experienced nurses and 80 from the new graduate nurses). The mean number of samples obtained from the experienced and the new graduate nurses was 7.7 (range, 4 to 8) and 6.6 (range, 5 to 8), respectively ($P = .02$). The numbers of organisms on the hands of the experienced nurses and the new graduate nurses were not significantly different (mean log CFU, 4.99 and 4.86, respectively; $P = .34$), but the experienced nurses had significantly more types of isolates per sample than did the new graduate nurses (mean, 1.70 and 1.34 isolates, respectively; $P = .003$).

Table 1 summarizes the microorganisms isolated from the nurses' hands. Eight experienced nurses and 6 new graduate nurses had *S. epidermidis* isolated at baseline. A smaller proportion of staphylococci were isolated from the hands of the experienced nurses than from the hands of the new graduate nurses (88.9% and 97.5%, respectively; $P = .02$). There were no significant differences in the proportion of bacterial isolates between experienced and new graduate nurses for enterococci (1.9% and 1.2%, respectively; $P = 1.0$) or gram-negative bacteria (4.6% and 1.25%, respectively; $P = .2$). However, the experienced nurses had 7 isolates (6 different species) of gram-negative bacteria, whereas the new nurse graduates had only 1 gram-negative isolate, which was found early in the study. One isolate of vancomycin-resistant enterococci (VRE) and one isolate of beta-hemolytic streptococcus, group A, were cultured from the hands of two experienced nurses. Whereas 4.6% (7 of 154) of the isolates from the experienced nurses were *Candida* species, there were no yeast isolated from the hands of the new graduate nurses ($P = .05$).

Eight different species of staphylococci were isolated: *S. epidermidis* (n = 102), *S. warneri* (n = 76), *S. haemolyticus* (n = 8), *S. aureus* (n = 8), *S. capitis* (n = 8), *S. hominis* (n = 4), *S. simulans* (n = 2), and *S. saprophyticus* (n = 2). At the baseline culture, methicillin-resistant, coagulase-negative and coagulase-positive staphylococci were isolated significantly more often from the experienced nurses than from the new graduate nurses (95% and 33%, respectively; $P = .0004$). By the second culture, there were no longer any significant differences between the two groups (82% and 54%, respectively; $P = .12$). For the last culture performed during the study, all staphylococci recovered from the two groups were methicillin resistant. Among the staphylococcal isolates, 3 were methicillin-resistant *S. aureus* (MRSA) and were recovered during the second year of the study: 1 isolate was from an experienced nurse and 2 were from a new nurse graduate.

A comparison of the PFGE results for *S. epidermidis* can be found in Table 2. As noted, all (8 of 8) of the strains isolated from the experienced nurses were methicillin resistant as compared with 2 of 6 strains isolated from the new nurses (Fisher's exact test, $P = .01$). At baseline, 12 (85.7%) of 14 of the nurses had a unique strain of *S. epidermidis*. At the end of the 2-year study, all of the new graduate nurses and 75% (6 of 8) of the experienced nurses had acquired PFGE strains of *S. epidermidis* different from those they had had at baseline. By the final culture, 8 (57%) of 14 isolates of *S. epidermidis* were indistinguishable or closely related (called clone A). The figure is a pulsed-field gel of *S. epidermidis* isolates recovered from 3 new nurse graduates and 2 experienced nurses that depicts the dominant A clone acquired by both groups.

DISCUSSION

In this small cohort, we prospectively examined the types, quantities, and resistance patterns of microorganisms found on the hands of experienced and new graduate nurses. The prevalence of staphylococci recovered from nurses' hands in this study was similar to that reported in previous surveys.^{10,11} Several studies have reported differences in types and microbial resistance of hand flora among nursing personnel when compared with other groups,^{2,10,12-17} but we found only a few studies that described the colonization of new or student nurses during their initial exposure to the hospital environment.

In one study, samples from the nares and hands of 22 nursing students were examined for yeast colonization before and after exposure to the hospital environment. Half of the students had a culture positive for yeast prior to entering a training program. After 62 days of training, yeast was isolated from 15 (68.1%) of the students.⁴ In contrast, there were no isolates of yeast among the new nurses in our study and only 5.2% (8 of 154) of the isolates from the experienced nurses were yeast, all of which were *Candida* species. Strausbaugh et al. reported that 58% of nurses (21 of 36) and 38% of non-nursing personnel (8 of 21) carried *Candida* species on their hands.⁵ This striking difference (ie, the lower prevalence of yeast on nurses' hands in our study) may be due to the fact that in the other studies hands were not washed before culture, whereas in this study hand hygiene was performed immediately before the culture sample was obtained.

In a study published in 1957, nasal colonization with staphylococci was examined among three classes of nursing students (class 1, 42 students; class 2, 9 students; and class 3, 53 students) and a control group (n = 15) of laboratory staff. During an 18-month period, isolates of *S. aureus* (then called *S. pyogenes*) were recovered in large numbers from the nasal swabs of students in classes 1 and 2 with carriage rates that remained relatively constant (30% to 38%). A marked change was noted in the types of strains isolated: 71% were susceptible to all antibiotics at the beginning of training, but only 13% remained susceptible at 18 months.¹² A comparison of these results with our results indicates that despite more than four decades of improved barrier precautions, hand hygiene regimens, and infection control practices, the flora of hospital staff continue to be influenced by the work environment. Of particular concern is

the fact that organisms isolated from the clean hands of nurses quickly acquired antibiotic resistance, becoming an ongoing reservoir for resistant strains.

Several other differences between the flora of experienced and new graduate nurses were also disturbing. Although nurses who had worked in the unit for years did not have higher microbial counts on their hands, they did have more isolates, they were less likely to have *S. epidermidis*, and they were more likely to carry gram-negative bacteria and yeast. Because the experienced nurses were significantly older than the new graduate nurses, these differences could be a function of age, or this could represent an evolution of their normal flora to a microbial population more reflective of organisms prevalent in the hospital environment. Regardless of the reason, the antimicrobial-resistant microorganisms isolated from the hands of experienced nurses pose a risk to patients. Finally, in previously reported research, we found that this same clone A isolated from nurses' hands was associated with clinical infections in 19 (46%) of 41 neonates during the study period, demonstrating transmission of this clone between staff and neonates.¹⁸

Although the reservoir for staphylococcal skin flora is the staff and the patients themselves, the environment is likely to serve as a vehicle for contact spread if it is not properly and frequently cleaned. Coagulase-negative staphylococci have been isolated from stethoscopes; MRSA, enterococci, and gram-negative bacteria from computer keyboards and faucet handles; and methicillin-susceptible and methicillin-resistant *S. aureus* from hospital room door handles, floors, walls, and patient tables.¹⁹⁻²² The fact that enterococcal and staphylococcal isolates, including VRE and MRSA, are able to survive on hospital fabrics and plastic from days to months²³ and that resistant *S. epidermidis* has been isolated from the clothes of 77% of auxiliary nurses, 59% of nurses, and 22% of physicians after close patient contact²⁴ further emphasizes the important role that the hospital environment can play in the transfer of these resistant microorganisms.

This study has several limitations. Most importantly, the sample was limited to nurses in a single NICU and hence the results may not be generalizable to other populations. Additionally, the sample size was small. Compared with the experienced nurses, the new graduate nurses had fewer samples obtained due to their dates of hire. Furthermore, the new graduate nurses were significantly younger than the experienced nurses, which could result in differences in skin health and acquisition of hand flora, although we did not find any detectable difference in skin flora based on age in a previous study.²⁵

In this small case series, we found the following: the staphylococcal flora on the hands of nurses who had worked 10 or more years in the study unit were uniformly resistant to methicillin and more likely to include gram-negative bacteria and yeast; nurses newly exposed to this acute care setting acquired methicillin-resistant strains within a few months of employment; and although new nurses came into the unit with unique strains of *S. epidermidis*, most of the strains were indistinguishable after a year, indicating that hand flora were being shared among staff members.

Acquisition of antibiotic-resistant flora is an occupational hazard for healthcare workers. Clearly, current hand hygiene regimens are not preventing the acquisition of resistant staphylococcal flora among these workers. Such flora serve as a reservoir for transmission of resistant strains to the hospital environment, other staff, patients, and household contacts.^{18, 26} We recommend further research regarding more effective ways to limit the transfer of organisms between and among staff and patients.

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FIGURE.

Pulsed-field gel electrophoresis of *Staphylococcus epidermidis* isolates from the first and last cultures of two experienced nurses and three new graduate nurses. Lane 1 = the lambda marker; lane 2 = first culture for new graduate nurse 1 showing clone B; lane 3 = last culture for new graduate nurse 1 showing clone A; lane 4 = first culture for new graduate nurse 2 showing clone C; lane 5 = last culture for new graduate nurse 2 showing clone A; lane 6 = first culture for new nurse graduate 3 showing clone D; lane 7 = last culture for new graduate nurse 3 showing clone A₃; lane 8 = first culture for experienced nurse 1 showing clone A₃; lane 9 = last culture for experienced nurse 1 showing clone A; lane 10 = first culture for experienced nurse 2 showing clone E; and lane 11 = last culture for experienced nurse 2 showing clone A.

TABLE 1

Microorganisms From the Experienced Nurses and the New Graduate Nurses

Microorganism	No. (%) of Isolates From Experienced Nurses	No. (%) of Isolates From New Graduate Nurses
Coagulase-negative staphylococci	133 (86.3)	74 (92.5)
<i>Staphylococcus aureus</i>	4 (2.6)	4 (5.0)
Enterococci	3 (1.9)	1 (1.25)
Gram-negative bacteria	7 (4.6)	1 (1.25)
<i>Candida</i>	7 (4.6)	0 (0)
Total	154 (100)	80 (100)

TABLE 2

Pulsed-Field Gel Electrophoresis Patterns of *Staphylococcus epidermidis* Isolated at the First and Last Cultures (12 to 22 Months Apart)

Type of Nurse	First Culture	Last Culture
Experienced	C*	J*
Experienced	K*	A*
Experienced	L*	A*
Experienced	A*	A*
Experienced	E*	A*
Experienced	M*	E*
Experienced	N*	A*
Experienced	F*	F*
New	D*	A*
New	G	NR*
New	H	O*
New	B	A*
New	C*	A*
New	H	I*

NR = not recognizable.

* Methicillin resistant.