Molecular Epidemiology of Gram-Negative Bacilli from Infected Neonates and Health Care Workers’ Hands in Neonatal Intensive Care Units

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(See the editorial commentary by Henderson on pages 1688–9)

We sought to characterize the molecular epidemiology of gram-negative bacilli (GNB) causing infections in infants and associated with carriage on nurses’ hands after hand hygiene was performed. From March 2001 to January 2003, GNB caused 192 (34%) of 562 hospital-acquired infections in the 2 participating neonatal intensive care units (NICUs) and were isolated from the hands of 45 (38%) of 119 nurses. Five species—Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens and Enterobacter cloacae, all of which were typed by pulsed-field gel electrophoresis—caused 169 (88%) of 192 of GNB infections. Overall, 58% of infections were caused by unique strains not cultured from other infants or nurses, and 31% of infections were part of unrecognized molecular clusters. In contrast, only 9% of strains that caused infections were cultured from nurses’ hands. These data suggest that practices in addition to hand hygiene are needed to prevent horizontal transmission of GNB in the NICU.

Health care–associated infections among infants in neonatal intensive care units (NICUs) are associated with substantial morbidity and mortality, and infection rates have been reported to be as high as 25% [1]. Data from the Pediatric Prevention Network of the Centers for Disease Control and Prevention [2] and the National Nosocomial Infection Surveillance system [3] identified bloodstream infections (BSIs) and pneumonia as the most common health care–associated infections in this population. Although gram-positive cocci are the most common pathogens in NICU patients, morbidity and mortality rates are highest for sepsis caused by gram-negative pathogens and yeast [4]. Recent studies have indicated that the incidence of gram-negative bacterial infections in NICUs may be increasing [5].

Outbreaks of infections caused by gram-negative bacilli are well recognized [6, 7]. There have been fewer recent attempts, however, to prospectively characterize the epidemiology of endemic health care–associated infections due to gram-negative bacteria in the absence of a recognized outbreak [8, 9]. The relative importance of health care workers in the horizontal transmission of gram-negative pathogens remains to be elucidated.

To further address the epidemiology of gram-negative pathogens among NICU patients, we used molecular typing to identify clones shared among infants and to determine if the hands of nurses carried the same strains as those causing infections in the infants. In doing so, we sought to determine the relative frequency of potential horizontal transmission between patients and health care workers in the NICU.
SUBJECTS AND METHODS

Study sites. This study was part of a larger clinical trial conducted from March 2001 through January 2003 to examine the effects of hand hygiene practices on health care–associated infections in critically ill neonates. The study was performed in 2 level III-IV NICUs, NICU 1 (43 beds) and NICU 2 (50 beds), which are part of New York Presbyterian Hospital in New York City. All neonates hospitalized for at least 48 h, and all consenting nursing staff were included in the study.

Procedures. Surveillance for health care–associated infections, as defined by the NNIS system and adapted for neonates, was performed prospectively by a trained surveillance officer [10]. Sources of data included laboratory, radiology, and pharmacy records; patient medical records; information from the physician and nursing staffs; and direct observation of neonates. Cultures of blood, respiratory tract, conjunctiva, skin and soft-tissue, and CNS specimens were performed by the NICU staff, as clinically indicated. Surveillance cultures of the hands of nurses were done quarterly immediately after hand hygiene was performed, as described elsewhere [11]. Participants were not told the results of their hand cultures. The institutional review boards of both institutions approved this study, and participating nurses signed consent forms.

Species and strain selection. The 5 most frequent gram-negative bacilli causing neonatal infections during the study period were Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, and Enterobacter cloacae. Thus, these 5 species were chosen for molecular typing. PFGE was performed on all available isolates associated with infections in infants, with the exception of multiple isolates of the same species obtained from the same infant within a 3-week period that exhibited differences in susceptibility to ≥2 antimicrobial agents. Similarly, molecular typing was performed on isolates of these 5 species carried on the hands of nurses.

Molecular typing. For PFGE analysis, strains were inoculated into 5 mL of nutrient broth and incubated for 3 h at 37°C with shaking to attain exponential growth. Agarose plugs were prepared from the cultures, and the bacterial cells were lysed within the plugs. The extracted genomic DNA was digested by the following restriction endonucleases: SpeI for E. cloacae, P. aeruginosa, and S. marcescens DNA; XbaI for K. pneumoniae DNA; and NotI for E. coli DNA. Restriction DNA fragments were separated by PFGE with the Chef Mapper system (Bio-Rad). A cluster analysis was performed on the PFGE patterns, and match similarity was calculated using the Molecular Analyst Fingerprinting Plus software (Bio-Rad) to determine strain relatedness [12]. A dendogram was generated using the unweighted pair-group method with arithmetic averages and Dice coefficients for each organism [13]. We defined strains to be genetically related when the level of strain similarity was ≥85%, using previously established criteria [14].

Data analysis. Infant and nurse data were entered into a statistical software program (Stata, version 8; Stata). We identified molecular clusters as ≥2 related isolates (as defined above) that were shared between ≥2 infants or between ≥1 infant and ≥1 nurse. Clusters were further categorized by whether they had been previously identified by epidemiologic or clinical means or had not been previously recognized. Categorical variable proportions were compared using Fisher’s exact test or Pearson’s χ² test. The 2-tailed significance level, α, was <.05.

RESULTS

Isolates associated with infections. Overall, 2935 infants were enrolled in the study. During the study period, 192 (34%) of 562 infections were caused by gram-negative bacilli, of which 169 (88%) of 192 were caused by 1 of the 5 most common species. The number and sites of infections caused by each of these species are shown in table 1. Conjunctivitis and BSI were the most common infections, each representing 42% of infections diagnosed during the study period. The majority of isolates—119 (70%) of 169—were available for typing.

Isolates associated with hand carriage. Overall, 119 (77%) of 155 eligible nurses agreed to participate in the study and had ≥1 hand culture performed. Three nurses from NICU 2 withdrew from the study (each after having 1 positive hand culture result). Hand cultures were performed a mean of 7.4 times/nurse in NICU 1 and a mean of 6.6 times/nurse in NICU 2. Gram-negative bacilli were isolated at least once from the hands of 45 (38%) of 119 nurses. Among these 45 nurses, 20 (30 cultures) harbored ≥1 of the 5 species of interest (table 1); 14 had 1 positive hand culture result, and 6 had ≥1 positive hand culture result, including 3 nurses who had the same species isolated on 2 occasions. Overall, 26 (87%) of 30 isolates were available for typing.

As shown in table 1, K. pneumoniae was the most frequent pathogen isolated from infants (31%) and the most common organism isolated from the hands of nurses (37%). E. cloacae was the only species isolated more frequently from the hands of infants (33%) than from infants (11%) (P = .001, by Pearson’s χ² test). The prevalence of the other species did not differ significantly between nurses and infants.

Molecular typing. The majority of clones were unique and were not shared among infants, among nurses, or between infants and nurses (figure 1). Of the 119 infant isolates available for typing, 69 (58%) of 119 were a unique strain. Similarly, of the 26 isolates typed from the hands of nurses, 17 (66%) of 26 were unique to a single nurse and were not shared with any
Table 1. Gram-negative bacilli associated with nurse hand carriage and infant infections.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolates from nurse hands</th>
<th>Isolates from infants</th>
<th>P*</th>
<th>Site of infant infections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bloodstream</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>11 (37)</td>
<td>53 (31)</td>
<td>.5</td>
<td>31 (58)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3 (10)</td>
<td>39 (23)</td>
<td>.1</td>
<td>17 (44)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2 (7)</td>
<td>33 (20)</td>
<td>.1</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>4 (13)</td>
<td>26 (15)</td>
<td>1</td>
<td>9 (35)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>10 (33)</td>
<td>18 (11)</td>
<td>.001</td>
<td>9 (50)</td>
</tr>
<tr>
<td>Overall</td>
<td>30 (100)</td>
<td>169 (100)</td>
<td>...</td>
<td>71 (42)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of isolates.

* Comparison of the difference in proportion of infant and nurse hand isolates using Pearson’s χ² analysis or Fisher’s exact test.

infants or other nurses. Only 9% of typed strains from infants were also isolated from the hands of nurses. In this small sample, *K. pneumoniae* and *S. marcescens* strains were significantly more likely to be shared between infants and nurses than were *P. aeruginosa*, *E. coli*, or *E. cloacae* strains (*P* = .002, by Fisher’s exact test). In addition, 5 (19%) of 26 isolates cultured from nurses’ hands were shared with other nurses, 3 of which were *E. cloacae* (figure 2) and 2 of which were *S. marcescens*.

The recognized and unrecognized molecular clusters of infant infections are shown in table 2. Overall, 31% of infant infections occurred in previously unrecognized clusters. There were 6 clusters of *K. pneumoniae* infections, 1 of which involved 6 infants and a nurse in NICU 1 in 2001, as reported elsewhere [15]. Results of PFGE of selected *K. pneumoniae* strains are shown in figure 3. Molecular typing of *S. marcescens* revealed 2 clusters, as reported elsewhere [16], and a previously unrecognized cluster involving 4 infants. Of the remaining 3 species—*E. coli*, *P. aeruginosa*, and *E. cloacae*—there were 2–4 previously unrecognized clusters per species. There were 4 instances of shared clones between infants hospitalized in NICU 1 and those in NICU 2. Similarly, 1 clone was shared between an infant in NICU 1 and a nurse in NICU 2. There was no known epidemiologic link to account for these occurrences.

**DISCUSSION**

Health care–associated infections have followed a cyclic pattern during the 20th century. Gram-positive cocci, particularly streptococci and *Staphylococcus aureus*, were the most common cause of hospital-acquired infection in the first half of the century, followed by the emergence of gram-negative bacilli, such as *K. pneumoniae* and Enterobacteriaceae, in the 1970s [17–19]. With the development of antimicrobial agents effective against gram-negative pathogens, gram-positive organisms became the most common cause of infection with the problematic emergence of methicillin-resistant *S. aureus* [20] and vancomycin-resistant enterococci [21]. Gram-negative pathogens, however, remain problematic from the 1980s to the present. In the NNIS report (1986–1994), although 75% of bloodstream infections were due to gram-positive cocci, ~18% of health care–associated infections in NICUs were due to gram-negative bacilli [22]. From 1998 through 2000, gram-negative organisms accounted for 17.6% of late-onset sepsis in neonates with very low birth weight [4], and, in a national point prevalence survey conducted in August 1999, 32% of nosocomial infections were due to gram-negative pathogens [2].

This is the largest prospective study to examine the potential for horizontal transmission of gram-negative bacilli in infants in the NICU using molecular typing techniques. Over one-half (58%) of the infections in our study were caused by unique clones, whereas 42% of clones were shared between infants. This included 31% of infant infections due to molecular clusters that were unrecognized during the study period by the health care workers treating the infants.

Previous studies have shown that the majority of infants with
gastrointestinal colonization with gram-negative bacilli harbor unique clones; only 12%–21% of clones were reported to be shared between infants [8, 9]. Although we also found that the majority of infections due to gram-negative bacilli were caused by unique clones, we found that a substantially higher percentage of clones (42%) were shared among infants than had been previously reported [8, 9]. In contrast with these previous reports that focused on colonization with gram-negative bacilli, our study examined infections due to gram-negative bacilli. This difference may account for the higher percentage of shared clones noted in our study; clones causing infection are most likely more virulent and may have a greater propensity for horizontal transmission. Furthermore, we speculate that the increased use of antimicrobial agents, which exerts selective pressure on neonatal flora that results in resistant organisms, may have contributed to the increased likelihood of shared clones. As evidence, the study site had an increased prevalence of extended spectrum β-lactamase–producing K. pneumoniae [15], as well as an increased prevalence of overall antimicrobial resistance in the infant isolates, compared with those associated with hand carriage [23]. Only 9% of the strains recovered from infants had the same

Table 2. Previously recognized and unrecognized molecular clusters of infant infections.

<table>
<thead>
<tr>
<th>Species</th>
<th>Unique clones, no. (%) of isolates (n = 119)</th>
<th>Cluster group (no. of isolates from infants)</th>
<th>Recognized</th>
<th>Unrecognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>15 (13)</td>
<td>A (6)</td>
<td>B (2), C (2), D (6), E (2), and F (1)²</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>22 (18)</td>
<td>0</td>
<td>A (2), B (3), C (2), and D (1)²</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15 (13)</td>
<td>0</td>
<td>A (2), B (2), C (2), and D (2)</td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>8 (6.7)</td>
<td>A (4) and B (3)²</td>
<td>C (4)</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloaceae</td>
<td>9 (7.6)</td>
<td>0</td>
<td>A (2) and B (2)</td>
<td></td>
</tr>
<tr>
<td>Overall, no. (%) of isolates</td>
<td>69 (58)</td>
<td>13 (11)</td>
<td>37 (31)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Not all isolates were available for typing.
² Shared with a nurse.
Figure 3. PFGE of Klebsiella pneumoniae. Lane 1, λ marker; lanes 2 and 3, unique clone isolated from 1 nurse on 2 separate occasions; lanes 4 and 5, clone shared between an infant and a nurse from neonatal intensive care unit (NICU) 1; lane 6, unique clone from an infant in NICU 1; lanes 7 and 8, unique clone isolated from 1 infant on 2 separate occasions; lanes 9 and 10, unique clone isolated from 1 infant on 2 separate occasions; lane 11, unique clone from a nurse in NICU 2.

type as those isolated from the hands of nurses. *K. pneumoniae*, previously known to be commonly isolated from the hands of nurses [24, 25], was the most prevalent gram-negative bacilli cultured from nurses’ hands and was associated with the most clinical infections in the infants. Our study indicated a potentially higher probability of cross-transmission with certain gram-negative bacilli, such as with *K. pneumoniae* and *S. marcescens*, via the hands of health care workers. For others, such as *E. coli* and *P. aeruginosa*, that do not survive well on the skin [26], environmental reservoirs are more likely.

This study had several limitations. First, 30% of infant samples were unavailable for molecular typing. Second, only 77% of the nursing staff enrolled in the study. Third, we did not assess environmental reservoirs or hand carriage by other staff members. In addition, because nurses may transiently carry organisms on their hands, more frequent culturing may have isolated a greater number of shared strains, or we may have found more shared strains had we cultured hands before rather than after hand hygiene. However, the practice of hand hygiene is unlikely to impact the relative proportion of gram-negative flora [27, 28]. Finally, although there were episodes of infection involving shared clones between NICU 1 and NICU 2, we have no evident epidemiologic link for these occurrences. It is unclear whether this is because of a lack of epidemiologic data or a limitation of the discriminatory power of our molecular typing method.

In conclusion, this study used molecular typing to expand previous observational data on the epidemiology of horizontal transmission of endemic gram-negative bacteria in the NICU. Almost one-third of the infections were part of unrecognized molecular clusters. In addition, although there was some sharing of clones between neonates and nurses, this appeared to be less common with gram-negative bacilli than with gram-positive cocci; molecular typing of gram-positive pathogens such as *S. epidermidis* has demonstrated a predominance of shared clones among infants [29, 30]. This is not surprising, because gram-positive cocci more commonly colonize the skin. There appeared to be a greater risk of horizontal transmission via hands with certain gram-negative bacteria (*K. pneumoniae* and *S. marcescens*), despite the fact that cultures of nurses’ hands were done after hand hygiene was performed. This suggests that, although proper hand hygiene practices are clearly important [31], hand hygiene alone may not be sufficient to prevent horizontal transmission of gram-negative pathogens seen in the NICU. Further studies examining other potential reservoirs and risk factors are needed.
References


