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ORIGINAL ARTICLE

Gram-Negative Bacilli in Infants Hospitalized in The Neonatal Intensive Care Unit

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Background. Gram-negative bacilli (GNB) account for a significant burden of infection and colonization in neonatal intensive care units (NICUs), and antibiotic resistance among these pathogens is of increasing concern.

Methods. A prospective cohort study was performed in 4 NICUs between May 2009 and April 2012. The body sites from which GNB were isolated, antimicrobial susceptibilities of the GNB isolated, and antimicrobial therapy were assessed.

Results. Attending neonatologists treated 3.0% (188 of 6184) of eligible infants for GNB infection; 23% of 214 GNB isolates were nonsusceptible to antimicrobial agents, including gentamicin (14.8%), piperacillin-tazobactam (9.9%), third-generation cephalosporin (7.0%), and/or carbapenem agents (4.5%). Gentamicin was the most commonly used antibiotic overall, and much of its use was empiric. However, third-generation cephalosporin agents and cefepime were used more commonly as targeted therapy for identified Gram-negative pathogens.

Conclusions. One-quarter of the GNB isolates were nonsusceptible to ≥1 antibiotic. Antimicrobial stewardship strategies for reducing antimicrobial use in NICUs should be implemented.

Keywords. antimicrobial stewardship; bloodstream infections; healthcare-associated infections; neonate; resistance.

INTRODUCTION

Gram-negative bacilli (GNB) are well-described causes of healthcare-associated infections (HAIs) among infants hospitalized in neonatal intensive care units (NICUs). Infants in NICUs are exposed to antibiotics frequently, and substantial practice variation exists between centers [1]. Antibiotic exposure may be associated with changes in microbial ecology and susceptibility to infection, which can lead to unintended adverse consequences [2]. Prolonged empiric antibiotic treatment has been associated with increased rates of necrotizing enterocolitis (NEC) and death for extremely low-birth-weight infants [3, 4]. Neonates with multidrug-resistant GNB bacteremia are more likely to receive inadequate initial antibiotic therapy and have a higher rate of infectious complications and death [5]. These observations suggest a critical need for the promotion of antimicrobial stewardship and reduction of unnecessary antibiotic use in the NICU population.

The aims of this study were to determine the epidemiology of GNB isolated in the NICU population, assess current patterns of antibiotic resistance, and determine antibiotic use in neonates with GNB infection.

METHODS

Study Design and Sites

A multicenter prospective cohort study was conducted at 4 level III NICUs between May 2009 and April 2012. The 4 study sites were NewYork-Presbyterian Morgan Stanley Children's Hospital at Columbia University Medical Center, NewYork-Presbyterian Komansky Center for Children's Health at Weill Cornell Medical Center, Children's Hospital of Philadelphia, and Christiana Care Health System. The 4 NICUs had 247 beds (50–75 per site) and approximately 4500 annual discharges (830–1400 per site). All of the sites except Christiana underwent antimicrobial stewardship interventions as part of their participation in a multicenter study of antimicrobial stewardship interventions. In addition, Children's Hospital of Philadelphia had an existing antimicrobial stewardship program. None of the programs had an active GNB surveillance program. Three of the 4 sites had an inborn delivery service, and 3 of the 4 sites allowed readmissions from home. The most commonly used empiric therapies for late-onset sepsis were vancomycin and gentamicin (3 sites) and vancomycin and cefepime (1 site). Institutional review board approval

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was obtained from each site, and the requirement for written informed consent was waived.

Patients and Outcomes

Data in this study were derived from a larger study that evaluated interdisciplinary interventions to improve antibiotic use in NICUs. Whether inborn or transferred, included patients were <7 days of age on admission to a study NICU and hospitalized for at least 4 days. Study outcomes in this analysis were GNB isolated from patients ≥4 days of age, defined as the isolation of GNB from a sterile or nonsterile body site with an infection documented in the attending neonatologist's progress notes plus treatment with intravenous antibiotics (for ≥4 days). GNB isolated from a culture of the conjunctiva or from a surveillance culture were excluded. Rates of GNB infections were calculated per 1000 patient-days stratified according to birth weight (BW) categories: ≥2500, 1500–2499, 1000–1499, or <1000 g.

Antimicrobial Susceptibility Testing

Antibiotic-susceptibility testing was performed at each site's clinical microbiology laboratory. Susceptibilities to gentamicin, piperacillin-tazobactam, third-generation cephalosporin agents, and carbapenem agents were defined using the 2010 Clinical and Laboratory Standards Institute breakpoints [6]. Nonsusceptibility criteria included both intermediate and resistant breakpoints.

Antibiotic Use

For every patient in the study, the number of calendar days of therapy with gentamicin, piperacillin-tazobactam, third-generation cephalosporin agents plus cefepime, or carbapenem agents was calculated; in addition, the number of calendar days used to treat identified GNB infections (ie, targeted therapy) was calculated. Examples of nontargeted therapy include brief "rule-out" treatment for late-onset sepsis with negative culture results, treatment of culture-negative sepsis, and treatment without performing cultures or with negative culture results. Calendar days associated with treatment of all stages of NEC were excluded from the analysis of targeted therapy, because although GNB have been implicated as potential NEC-causing pathogens, cultures of samples obtained from an intra-abdominal site are rarely obtained before starting antibiotics, and the results of such blood cultures are frequently negative.

Statistical Analysis

The difference in the proportion of nonsusceptible strains to selected agents according to study site was assessed by using the χ^2 test. We also determined if the relative proportion of targeted versus empiric therapy varied among different antimicrobial agents. Statistical analyses were computed in SAS 9.2 for Windows (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Rates of GNB Isolation

During the study period, approximately 15 000 infants were admitted to 1 of the study NICUs, 6184 of whom fulfilled the inclusion criteria and were enrolled in this study. GNB were isolated from 188 (3.0%) of these 6184 infants (1.298 GNB isolates per 1000 patient-days) (Table 1). In this study population, 22% of the bloodstream infections (BSIs) diagnosed by an attending neonatologist that occurred on day of life 4 or later were associated with GNB, whereas Gram-positive cocci and *Candida* spp. caused 74% and 3%, respectively, of the BSIs. The rates of GNB, Gram-positive organisms, and *Candida* spp. isolated from the blood were 0.588, 1.419, and 0.054 per 1000 patient-days, respectively.

The rate of GNB isolation was highest in infants with a BW of <1000 g and lowest in those with a BW of 1500 to 2499 g (2.090 vs 0.803 GNB isolates per 1000 patient-days, respectively). Among 539 infants with a BW of <1000 g, 78 (14.5%) had a GNB infection diagnosed by an attending neonatologist. Of the 22 infants with >1 GNB infection diagnosed, 4 had a BW of ≥2500, 6 had a BW of 1500 to 2499, 2 had a BW of 1000 to 1499, and 10 had a BW of <1000 g.

Body Sites Diagnosed with a GNB Infection

Urinary tract infections (UTIs) and BSIs were diagnosed most commonly and accounted for 45.3% (n = 97) and 31.3% (n = 67), respectively, of GNB infections diagnosed in this study. Other body sites included the respiratory tract excluding tracheitis (6.1% [n = 13]), surgical sites (5.1% [n = 11]), skin and

Table 1. Number and Rate of Diagnosed GNB Infections According to Birth Weight Category

Birth Weight Category	No. of Infants Diagnosed With GNB Infection/ No. of Infants Enrolled (%)	No. of Diagnosed GNB Infections/Patient-Days	Rate of Diagnosed GNB Infection/1000 Patient-Days
≥2500 g	56/2760 (2.0)	60/48 446	1.238
1500–2499 g	28/2192 (1.3)	37/46 071	0.803
1000–1499 g	26/685 (3.8)	28/28 801	0.972
<1000 g	78/539 (14.5)	89/41 473	2.146
Total	188/6184 (3.0)	214/164 881	1.298

Abbreviation: GNB, gram-negative bacilli.

soft tissue (4.2% [n = 9]), bone and joint (0.5% [n = 1]), abdomen (0.5% [n = 1]), and central nervous system (0.5% [n = 1]). Coinfections that involved concurrent body sites from which GNB were isolated (6.5% [n = 14]) included urosepsis (n = 7), meningitis with bacteremia (n = 2), skin and soft-tissue infections with bacteremia (n = 3), and respiratory infection with a UTI (n = 2).

GNB Species

Overall, 230 GNB (representing 16 species) were isolated. The most common species were *Escherichia coli* (30.4% [n = 70]), *Klebsiella pneumoniae* (23.0% [n = 53]), *Enterobacter cloacae* (17.0% [n = 39]), *Klebsiella oxytoca* (8.7% [n = 20]), and *Pseudomonas aeruginosa* (6.5% [n = 15]). More than 1 GNB species was isolated during 11 diagnosed infections (7 respiratory tract infections, 3 UTIs, and 1 BSI).

Antimicrobial Resistance

Among the 230 isolates, 23% (n = 53) were nonsusceptible to at least 1 of the selected antimicrobial agents (Table 2). Nonsusceptibility to gentamicin was most common, and the rates varied among the sites (0%–21.5%; $P = .04$). The rates of nonsusceptibility to gentamicin were higher at the sites that used gentamicin as empiric therapy for late-onset sepsis (sites 1, 2, and 4). Gentamicin resistance rates did not differ among isolates found in the respiratory tract (3 [13.6%] of 22), blood (13 [17.1%] of 76), and urine (15 [15.3%] of 98). Although the rate of nonsusceptibility to third-generation cephalosporin agents was highest at the site that used cefepime as empiric therapy (site 3), this difference was not significant. Less than 1% of GNB isolates were nonsusceptible to carbapenem agents, and 5.8% were nonsusceptible to >1 antimicrobial agent.

Antibiotic Use

Overall, targeted antibiotic therapy for the treatment of GNB infection accounted for 15.8% (2342 of 14 816) of all calendar days in the antibiotic categories used in this study (Table 3). Gentamicin was used for the highest number of calendar days but had the lowest proportion of targeted use (11.3% [765 of 6788]). Third-generation cephalosporin agents plus cefepime were used for the highest number of targeted calendar days. Carbapenem agents were used infrequently (706 calendar days) but had a relatively higher proportion of targeted use (41.8%). Twenty infants were not receiving empiric therapy when they were diagnosed with GNB infection (15 UTIs, 2 BSIs, 1 respiratory tract infection, and 2 skin and soft-tissue infections).

DISCUSSION

In this large multicenter study for describing GNB isolated from infants hospitalized in an NICU, we found that attending neonatologists diagnosed infections caused by GNB in 3.0% of eligible infants; these GNB infections were associated with 22% of the diagnosed BSIs that occurred on day of life 4 or later. Gentamicin resistance was most common, and its rates varied significantly among the study sites. Gentamicin therapy was used for the highest number of total calendar days, whereas third-generation cephalosporin agents and cefepime were used for the highest number of targeted calendar days.

We found that nearly one-quarter of GNB isolated were nonsusceptible to ≥1 antimicrobial agent commonly used in NICUs. It is unclear if the infants initially acquired resistant GNB or if the GNB developed resistance from antibiotic exposures that occurred during their NICU hospitalization. Nonetheless, understanding local resistance is of paramount importance and facilitates the identification of trends in antibiotic resistance, comparisons of resistance trends among institutions or units, and the selection of empiric antibiotic therapy [7]. National pediatric antibiograms have been published, but NICU-specific antimicrobial resistance patterns may not be similar [8]. Although the creation of NICU-specific antibiograms for some GNB might not be possible because of a limited number of isolates, aggregating GNB species might be useful for examining overall resistance patterns and assessing the safety of empiric

Table 2. Antibiotic Susceptibility of GNB Isolates in This Study

	No. of Nonsusceptible Isolates/No. of Tested Isolates (%)[a]				
Antibiotic Agent	Site 1 (N = 96)	Site 2 (N = 75)	Site 3 (N = 43)	Site 4 (N = 16)	Total (N = 230)[b]
Gentamicin[c]	20/93 (21.5)	11/73 (15.1)	0/41 (0.0)	2/16 (12.5)	33/223 (14.8)
Piperacillin-tazobactam	7/87 (8.0)	3/71 (4.2)	2/33 (6.1)	2/10 (20)	14/201 (7.0)
Third-generation cephalosporin agents or cefepime[d]	10/94 (10.6)	5/73 (6.8)	6/41 (14.6)	1/14 (7.1)	22/222 (9.9)
Carbapenem agents	1/94 (1.1)	0/71 (0.0)	0/38 (0.0)	0/14 (0.0)	1/217 (0.5)
≥2 of these agents	5/94 (5.3)	4/73 (5.6)	2/41 (4.9)	2/16 (12.5)	13/224 (5.8)
≥3 of these agents	1/94 (1.1)	1/73 (1.3)	0/38 (0.0)	0/14 (0.0)	2/219 (0.9)

Abbreviation: GNB, gram-negative bacilli.

[a]Not every isolate underwent antimicrobial susceptibility testing.

[b]Some infections were caused by >1 GNB.

[c]Rates of gentamicin susceptibility were significantly different among the study sites ($P < .05$).

[d]Nonsusceptibility to ceftazidime was used to determine nonsusceptibility of *P aeruginosa* to third-generation cephalosporin agents.

Table 3. Days of Targeted Therapy and Total Antibiotic Use

Antibiotic	No. of Targeted Calendar Days	Total No. of Calendar Days	Percent Targeted Use
Gentamicin	765	6788	11.3
Piperacillin-tazobactam	480	2881	16.7
Third-generation cephalosporin agents or cefepime	803	4441	18.1
Carbapenem agents	295	706	41.8
Total	2343	14816	15.8

therapy by examining the proportion of isolates resistant to initial empiric therapy.

The characterization of antibiotic use in terms of targeted calendar days, in addition to total calendar days of use, might be informative to clinicians, particularly if used with prescriber audits and feedback. Combined with antibiograms, this metric can inform us of whether antibiotic use is driven by actual resistant pathogens, suspected infections caused by such pathogens, or other unrelated factors. Schulman et al [1] demonstrated a 40-fold variation in NICU antibiotic-prescribing practices across 127 NICUs with similar burdens of proven infection and NEC, surgical volumes, and rates of death, which suggests that a substantial portion of antibiotic use is unwarranted. Hence, understanding local antibiotic prescribing in relation to local resistance could inform antimicrobial stewardship efforts.

Decreasing the use of broad-spectrum antibiotics in the NICU population is challenging, because neonatal infections can present with nonspecific findings and can be indistinguishable from conditions with a noninfectious etiology [9]. Nonetheless, opportunities for stewardship exist. Institutional protocols for narrow-spectrum empiric therapy for early-onset sepsis can reduce the emergence of antibiotic resistance [10]. We showed previously that common opportunities for antibiotic stewardship in NICUs include using narrow spectrum antibotic therapy when susceptibilities are known and avoiding prolonged perioperative prophylaxis [11]. For conditions without microbiological data, such as "culture-negative sepsis" and NEC, even a modest reduction in the duration of therapy might decrease antibiotic exposure.

Our study had limitations. It was performed at large tertiary care NICUs, and the results might not be generalizable to other NICU settings. We did not use the National Healthcare Safety Network case definitions for HAIs, including device-associated infections; rather, we considered infections diagnosed by an attending neonatologist. Thus, some of these infections might have represented colonization. Nonetheless, susceptibility patterns of colonizing flora still provide an understanding of the burden of resistance, because colonizing flora can progress to infection [12]. The sites' clinical microbiology laboratories did not provide susceptibility data for all aminoglycoside agents or cefepime. We did not have a sufficient number of isolates to provide susceptibility patterns of GNB isolated from different body sites. We did not measure the potential effects of maternal antibiotic exposure, other medications, breast milk, or probiotics on GNB infections and/or resistance. We did not include infections for which cultures were not performed but might have been caused by GNB (eg, intra-abdominal infections).

In conclusion, in this large multicenter study, we found that 3.0% of all infants and 14.5% of infants with a BW of <1000 g were diagnosed with an infection caused by GNB. Although the rates of antimicrobial resistance to third-generation cephalosporin agents and carbapenem agents were low, the overall rate of resistance was 23%, and the rate of resistance to gentamicin was 14.8%. The reduction of antibiotic use, including the use of antibiotic stewardship strategies for limiting unnecessary treatment, deserves further emphasis. Additional avenues of research include determining the comparative effectiveness of short-course therapy for GNB HAIs, the utility of biomarkers to help discontinue antibiotics more promptly when infection is no longer suspected, and the effect of antimicrobial stewardship programs on infection rates.

Notes

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