Risk Factors and Outcomes for Multidrug-Resistant Gram-Negative Bacteremia in the NICU

WHAT’S KNOWN ON THIS SUBJECT: There is a perception that Gram-negative bacilli (GNB) bloodstream infection is increasing in the NICU, and those infections caused by a multidrug-resistant (MDR) strain are a growing threat to hospitalized patients.

WHAT THIS STUDY ADDS: Exposure to broad-spectrum antibiotics is the most important risk factor for MDR GNB bacteremia, which is associated with higher mortality. Neonates with risk factors for bacteremia caused by a MDR GNB strain may benefit from empirical antimicrobial therapy with carbapenem.

abstract

OBJECTIVES: To assess the risk factors antibiotic therapy and outcomes of multidrug-resistant (MDR) Gram-negative bacilli (GNB) bacteremia in NICU patients.

METHODS: Episodes of MDR GNB bacteremia were compared with a non-MDR GNB bacteremia group in an 8-year cohort study.

RESULTS: Of 1106 bacteremias, 393 (35.5%) were caused by GNB. Seventy (18.6%) were caused by an MDR strain. The most frequent mechanism of resistance was extended-spectrum β-lactamase production (67.1%), mainly by Klebsiella pneumoniae (59.6%). Previous antibiotic exposure to third-generation cephalosporin (odds ratio [OR]: 5.97; 95% confidence interval [CI]: 2.37–15.08; P = .001) and carbapenem (OR: 3.60; 95% CI: 1.26–10.29; P = .017) and underlying renal disease (OR: 7.08; 95% CI: 1.74–28.83; P = .006) were identified as independent risk factors for MDR GNB acquisition. Patients with MDR GNB bacteremia more likely received inadequate initial antibiotic therapy (72.9% vs 7.8%; P < .001) had higher rates of infectious complication (21.4% vs 10.5%; P = .011) and overall case fatality + rate (28.8% vs 10.5%; P < .001). Independent risk factors for overall mortality were presence of infectious complications after bacteremia (OR: 3.16; 95% CI: 1.41–7.08; P = .005) and underlying secondary pulmonary hypertension with or without cor pulmonale (OR: 6.19; 95% CI: 1.88–20.31; P = .003).

CONCLUSIONS: MDR GNB accounted for 18.6% of all neonatal GNB bacteremia in the NICU, especially in those with previous broad-spectrum antibiotic therapy and underlying renal disease. The most frequent mechanism of resistance was extended-spectrum β-lactamase (ESBL) production. Neonates with MDR GNB were more likely to develop infectious complications, which were independently associated with a higher overall case-fatality rate. Pediatrics 2014;133:e322–e329

AUTHORS: Ming-Horng Tsai, MD,a,b,c Shih-Ming Chu, MD,b,d Jen-Fu Hsu, MD,b,d Reyin Lien, MD,b,d Hsuan-Rong Huang, MD,b,d Ming-Chou Chiang, MD,b,d Ren-Huei Fu, MD, PhD,b,d Chiang-Wen Lee, MD,c and Yhu-Chering Huang, MD, PhD,b,e

aDivision of Neonatology and Pediatric Hematology/Oncology, Department of Pediatrics, Chang Gung Memorial Hospital, Yunlin, Taiwan; bCollege of Medicine, Chang Gung University, Taoyuan, Taiwan; cChang Gung University of Science and Technology, Chiayi, Taiwan; and Divisions of dPediatric Neonatology and dPediatric Infectious Disease, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan

KEY WORDS: Gram-negative bacilli, nosocomial infection, bacteremia, extended-spectrum β-lactamase—producing bacteria, mortality

ABBREVIATIONS
CI—confidence interval
CLSI—Clinical Laboratory Standards Institute
ESBL—extended-spectrum β-lactamase
GNB—Gram-negative bacilli
MDR—multidrug-resistant
OR—odds ratio

Dr Tsai conceptualized and designed the study and drafted the initial manuscript; Drs Chu and Hsu cared for the patients and collected and verified the data; Dr Lien cared for the patients and approved the agreement of interstitial review board; Dr Huang cared for the patients and carried out the initial analyses; Drs Chiang and Fu cared for the patients and helped with data verification; Dr Lee designed the data collection instruments and coordinated and supervised data collection; Dr Huang critically reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

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Address correspondence to Yhu-Chering Huang, MD, PhD, Division of Pediatric Infection Disease, Department of Pediatrics, Chang Gung Memorial Hospital, 5, Fu-Shing St, Kwei-Shan, Taoyuan 333, Taiwan. E-mail: ychuang@adm.cgmh.org.tw

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Bacteremia is a common complication of neonates with a long duration of hospitalization in the NICU and is associated with additional costs, morbidity, and long-term adverse outcomes.12 Although the most common pathogen in the NICU is Gram-negative bacilli (GNB) has increased in the past decades,3 and GNB bacteremia is often associated with a higher mortality rate.5–6 The emergence of multidrug resistance (MDR) among these organisms deserves particular concern, because treatment options of antimicrobial agents for an MDR strain are often limited and inappropriate initial antibiotics will predispose these neonates to an especially high risk of severe sepsis and a poor outcome.6,7

When a preliminary blood culture reveals growth of a GNB species, clinicians require additional guidance in deciding whether the patient can be treated safely with first-line antimicrobial agents. Most current studies have focused on the molecular epidemiology and possible transmission route of MDR GNB in NICU patients,6–11 and clinical data with regard to MDR GNB have only been available in small case series or on a single outbreak.12,13 Understanding the characteristics that differentiate critically ill neonates at risk of infection due to MDR GNB from those caused by a non-MDR strain will assist clinicians with an early treatment decision for GNB bacteremia. We therefore conducted this study to assess the incidence of, risk factors for acquisition, antibiotic therapy for, and outcomes of MDR GNB bacteremia in NICU patients.

METHODS
Setting, Patients, and Study Design
This study was carried out in the NICU of Chang Gung Memorial Hospital, a university-affiliated teaching hospital in northern Taiwan. The NICU contained a total capacity of 49 beds equipped with ventilator and 58-beds of special care nurseries. All infants <34 to 35 weeks’ completed gestation, with a birth weight <2 kg or >5 kg, or with any clinical signs of respiratory distress or cardiovascular, gastrointestinal, or neurologic problems requiring surgical or intensive treatment were eligible to admission in our NICU. From January 2004 to December 2011, all hospitalized neonates with at least 1 episode of bacteremia caused by GNB were included in the study. A neonatology specialist recorded all basic information on inpatient admission, including demographic characteristics, brief hospital course, all nosocomial infections, and discharge diagnosis every weekday beginning before January 2004. This prospectively collected neonatal database contained microbiologic databases, and all neonates corresponding to the study definition were retrieved from this database.

To identify risk factors for MDR GNB infection, all enrolled patients were divided into 2 groups: patients with bacteremia due to an MDR GNB and those with bacteremia due to a non-MDR GNB. If multiple episodes of GNB bacteremia occurred in an individual during the study period, the subsequent episode that occurred <1 month after the previous episode of GNB bacteremia was excluded from the analysis. We also compared patients who died with those who survived to determine the independent risk factors for mortality.

More detailed information, including the presence of a central venous catheter, use of mechanical ventilation and total parenteral nutrition, antimicrobial therapy in the 30 days preceding infection, and treatment courses for bacteremia, were retrospectively reviewed from the medical records. Severity of illness was evaluated at the onset of each episode of bacteremia by using the Neonatal Therapeutic Intervention Scoring System.14 Outborn infants who had been hospitalized in another hospital for >2 weeks and those whose detailed hospital courses were missing or unavailable were excluded from analysis. All recorded data describing the bacteremia episodes were reviewed by 2 investigators (S.-M.C. and J.-F.H.) for face validity. This study was approved by the institutional review board of Chang Gung Memorial Hospital, with a waiver of informed consent.

Definitions
Criteria from the Centers for Disease Control and Prevention were applied to define neonatal bacteremia.15 Patients were considered to have an MDR infection in the following situations: (1) extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae, (2) microorganisms with intrinsic resistance mechanisms such as Stenotrophomonas maltophilia, and (3) MDR strains including Pseudomonas aeruginosa and Acinetobacter baumannii. MDR strains were defined as those resistant to at least 1 agent in ≥3 of the following antimicrobial categories: carbenapens (imipenem and meropenem), penicillins (piperacillin, ticarcillin, and piperacillin/tazobactam), broad-spectrum cephalosporins (cefazidime and cefepime), monobactams (aztreonam), aminoglycosides, and fluorquinolones.16 In cases of polymicrobial bacteremia, which was defined as >1 microorganism identified from a single set of blood culture,17 the episode was defined as an MDR GNB case if 1 of the isolates was an MDR GNB strain.

All comorbidities of prematurity, including respiratory distress syndrome, intraventricular hemorrhage, bronchopulmonary dysplasia, necrotizing enterocolitis, and periventricular leukomalacia, were defined on the basis of the latest updated diagnostic criteria in the standard textbook of neonatology.18 Shock was defined as a mean blood pressure
 Assignment was carried out by using CLSI diffusion method, and categorical as-
patterns were determined according to logic methods. Antibiotic susceptibility 
formed by using standard microbiologic methods. 

In our NICU, empirical antibiotics were prescribed for the coverage of both Gram-positive and Gram-negative or-
ganisms, usually oxacillin or vanco-
mycin plus cefotaxime or gentamicin, 
one late-onset sepsis was suspected. Antimicrobial regimens were modified at the attending physician’s discretion, mostly according to the results and antibiotic susceptibility patterns of blood cultures. The identification of all causative microorganisms was performed by using standard microbiologic methods. Antibiotic susceptibility patterns were determined according to methods recommended by the National Committee for Clinical Laboratory Standards Institute (CLSI) for disk diffusion method, and categorical as-
signment was carried out by using CLSI breakpoints. The following agents were tested: ertapenem, imipenem, meropenem, 
doxycycline, ceftazidime, cefotaxime, aztreonam, piperacillin/tazobactam, 
amoxicillin/clavulanate, ciprofloxacin, levofloxacin, gentamicin, amikacin, and 
flomoxef. ESBL production was screened and confirmed in all isolates with a pro-
file suggestive of resistance by performing a double-disc synergy test according to CLSI guidelines. 
The presence of blaTEM, blaSHV, blaOXA, and 
blaCTX-M genes was investigated by poly-
merase chain reaction amplification, as previously described. 
The molecular characterization of ESBL GNB was typed by infrequent-restriction-site polymerase chain reaction, and restriction patterns were analyzed by applying previously established criteria. 

Statistical Analysis
Categorical variables were compared by using the χ² test or Fisher’s exact test; 
odd ratios (ORs) and 95% confidence intervals (CIs) were calculated. Contin-
uous variables were compared by the Mann-Whitney U test and the t test, 
depending on the distributions. Multi-
variate logistic regression analysis of factors potentially associated with MDR GNB acquisition and mortality included all statistically significant variables with P < .1 in univariate analysis, gender and gestational age, and all clinically 
important variables, whether or not they were statistically significant. Mea-
ures of goodness-of-fit were obtained to assess the performance of the models. 
The analysis was performed by us-

| TABLE 1 | MDR GNB and non-MDR GNB Pathogens That Cause Bacteremia in the NICU |
|-------------------------------|-------------------|-------------------|
| Organism                      | MDR GNB (n = 70), n (%) | Non-MDR GNB (n = 306), n (%) |
| Klebsiella pneumoniae         | 22 (31.4)          | 68 (22.2)         |
| Klebsiella oxytoca            | 5 (7.1)            | 31 (10.1)         |
| Escherichia coli              | 12 (17.1)          | 79 (25.8)         |
| Enterobacter cloaca           | 5 (7.1)            | 21 (6.9)          |
| Enterobacter aerogenes        | 0 (0)              | 16 (5.2)          |
| Pseudomonas aeruginosa        | 9 (12.9)           | 7 (2.3)           |
| Acinetobacter baumannii       | 3 (4.3)            | 39 (12.7)         |
| Serratia marcescens           | 0 (0)              | 10 (3.3)          |
| Citrobacter freundii          | 0 (0)              | 3 (1.0)           |
| Stenotrophomonas maltophilia  | 5 (4.3)            | 0 (0)             |
| Hafnia alvei                  | 0 (0)              | 2 (0.7)           |
| Neisseria meningitidis        | 0 (0)              | 2 (0.7)           |
| Chryseobacterium meningoseptum | 2 (2.6)           | 0 (0)             |
| Flavobacterium                | 0 (0)              | 1 (0.3)           |
| Morganella morganii           | 0 (0)              | 1 (0.3)           |
| Polymicrobial microorganisms  | 9 (12.9)           | 26 (8.5)          |

RESULTS
During the study period, a total of 1106 episodes of bacteremia were recorded. 
Three hundred ninety-three (35.5%) of them were caused by GNB in a total of 333 neonates. Seventeen episodes of GNB bacteremia were excluded from the analysis because they occurred <1 month after the previous episode of GNB bacteremia. Of the 376 episodes of GNB bacteremia enrolled into analyses, 70 (18.6%) were caused by an MDR strain. In the case and control groups, there were 70 episodes in 61 patients and 306 episodes in 278 patients (9 and 22 of these patients, respectively, experi-
enced >1 episode of GNB bacteremia). There were 6 patients with multiple episodes of GNB bacteremia caused by both an MDR and a non-MDR strain. 

Of the 70 episodes of MDR GNB bacteremia (Table 1), 47 (67.1%) were ESBL-producing bacteria, including Klebsiella pneumoniae (n = 28; 59.6%),
**Escherichia coli** (n = 9; 19.1%), **Klebsiella oxytoca** (n = 6; 12.8%), and **Enterobacter cloaceae** (n = 4; 10.6%). Rates of resistance to non-β-lactam antibiotics among ESBL-Enterobacteriaceae were as follows: amikacin, 59.6% (28 of 47); gentamicin, 72.3% (34 of 47); ciprofloxacin, 12.8% (6 of 47); levofloxacin, 35.6% (16 of 45); and cotrimoxazole, 66.7% (14 of 21). All 47 isolates were susceptible to imipenem, but 4 (8.5%) of them were resistant to ertapenem. The ESBLs were characterized in 38 available isolates as follows: 20 from the SHV family, 13 from the CTX-M family, and 5 from the combined CTX-M family (2 CTX-M3+SHV1, 1 CTX-M14+DHA-1, and 2 CTX-M27+CMY-2). The molecular typing of all ESBL-Enterobacteriaceae strains identified 29 different pulsed-field gel electrophoresis (PFGE) patterns, with the most common PFGE profile E (total of 4 cases) isolated during an epidemic in 2009. Four non-ESBL-producing isolates, including 3 **E. coli** and 1 **E. cloaceae** cases were found to be resistant to monobactams, aminoglycosides, and broad-spectrum cephalosporins. The remaining MDR GNB non-Enterobacteriaceae were, in order of frequency, as follows: **P. aeruginosa** (n = 9), **A. baumannii** (n = 5), **S. maltophilia** (n = 5), and **Chryseobacterium mениngosepticum** (n = 2). Two cases of A baumannii were of a pandrug-resistant strain. All other A baumannii and P aeruginosa were resistant to most β-lactam antibiotics but were susceptible to carbapenems. S maltophilia strains were susceptible only to cotrimoxazole and ciprofloxacin. C meningosepticum isolates were susceptible only to ciprofloxacin and piperacillin/tazobactam. Among the above isolates, 9 (12.9%) were the polymicrobial bacteremia episodes. The GNB isolates in the non-MDR GNB group are summarized in Table 1. Baseline and demographic characteristics of the neonates with MDR GNB and non-MDR GNB bacteremia are shown in Table 2. Variables including birth weight, gestational age, gender, perinatal history, and most underlying chronic conditions were similar between these 2 groups. The presence of underlying neurologic sequelae, renal disease, previous episode of bacteremia, use of total parenteral nutrition and/or intralipid, central venous catheter, and antibiotic therapy with several classes of antibiotics within 1 month before bacteremia were more frequently found among the MDR GNB group. In addition, infants in the MDR GNB group had a significantly higher rate of being outborn than those in the non-MDR GNB group (P = .009). After applying a logistic regression model (Table 3), the only independent risk factors for MDR GNB acquisition were

### Table 2: Baseline and Demographic Characteristics of all Episodes of GNB Bacteremia in the Neonatal Intensive Care Unit Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MDR GNB (n = 70)</th>
<th>Non-MDR GNB (n = 306)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth body weight, median (IQR), g</td>
<td>1340.0 (887–2050.0)</td>
<td>1345.0 (900–2047.5)</td>
<td>.809</td>
</tr>
<tr>
<td>Gestational age, median (IQR), wk</td>
<td>29.0 (26.0–36.0)</td>
<td>30.5 (27.0–35.0)</td>
<td>.544</td>
</tr>
<tr>
<td>Gender (male/female), n (%)</td>
<td>35/35 (50.0/50.0)</td>
<td>156/150 (51.0/49.0)</td>
<td>.969</td>
</tr>
<tr>
<td>Inborn/outborn, n (%)</td>
<td>37/33 (52.9/47.1)</td>
<td>210/96 (68.6/31.4)</td>
<td>.009</td>
</tr>
<tr>
<td>Age at onset of bacteremia, median (IQR), d</td>
<td>31.5 (14.0–66.3)</td>
<td>24.0 (13.0–50.5)</td>
<td>.070</td>
</tr>
<tr>
<td>Perinatal history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean/natural vaginal delivery</td>
<td>40/30 (57.1/42.9)</td>
<td>183/125 (59.8/40.2)</td>
<td>.610</td>
</tr>
<tr>
<td>Premature rupture of membrane</td>
<td>13 (18.6)</td>
<td>57 (18.6)</td>
<td>.991</td>
</tr>
<tr>
<td>Maternal fever and/or chorioamnionitis</td>
<td>1 (1.4)</td>
<td>9 (2.9)</td>
<td>.697</td>
</tr>
<tr>
<td>Low Apgar score at 5 min (≤7)</td>
<td>38 (54.5)</td>
<td>130 (42.5)</td>
<td>.073</td>
</tr>
<tr>
<td>Congenital infection and/or early-onset sepsis</td>
<td>2 (2.9)</td>
<td>20 (6.5)</td>
<td>.395</td>
</tr>
<tr>
<td>Underlying chronic conditions, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital anomalies</td>
<td>8 (11.4)</td>
<td>16 (5.2)</td>
<td>.057</td>
</tr>
<tr>
<td>Neurologic sequelae, congenital or acquired</td>
<td>16 (22.9)</td>
<td>41 (13.4)</td>
<td>.048</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>4 (5.7)</td>
<td>19 (6.2)</td>
<td>.871</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td>36 (51.4)</td>
<td>134 (43.8)</td>
<td>.247</td>
</tr>
<tr>
<td>Pulmonary hypertension and/or cor pulmonale</td>
<td>5 (7.1)</td>
<td>10 (3.3)</td>
<td>.168</td>
</tr>
<tr>
<td>Congenital gastrointestinal tract pathology</td>
<td>4 (5.7)</td>
<td>18 (4.9)</td>
<td>.607</td>
</tr>
<tr>
<td>Gastrointestinal sequelae</td>
<td>3 (4.3)</td>
<td>14 (4.6)</td>
<td>.605</td>
</tr>
<tr>
<td>Renal disease</td>
<td>9 (12.9)</td>
<td>4 (1.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hematologic disease</td>
<td>0 (0)</td>
<td>3 (1.0)</td>
<td>.538</td>
</tr>
<tr>
<td>Previous surgery (within 1 month), n (%)</td>
<td>10 (14.3)</td>
<td>45 (14.7)</td>
<td>.940</td>
</tr>
<tr>
<td>Use of corticosteroids (within 1 week), n (%)</td>
<td>11 (15.7)</td>
<td>29 (9.5)</td>
<td>.127</td>
</tr>
<tr>
<td>Previous antibiotic exposure (within 1 month), n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third-generation cephalosporin</td>
<td>53 (75.7)</td>
<td>108 (35.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vancomycin or teicoplanin</td>
<td>40 (57.1)</td>
<td>103 (33.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>12 (17.1)</td>
<td>10 (3.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Monobactam</td>
<td>5 (7.1)</td>
<td>10 (3.3)</td>
<td>.135</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>41 (58.6)</td>
<td>159 (52.0)</td>
<td>.317</td>
</tr>
<tr>
<td>Antifungal drugs</td>
<td>5 (7.1)</td>
<td>7 (2.3)</td>
<td>.037</td>
</tr>
<tr>
<td>Antianaerobic antibiotics (metronidazole)</td>
<td>11 (15.7)</td>
<td>18 (5.9)</td>
<td>.005</td>
</tr>
<tr>
<td>On high-frequency oscillatory ventilator, n (%)</td>
<td>11 (15.7)</td>
<td>26 (8.5)</td>
<td>.067</td>
</tr>
<tr>
<td>Invasive mechanical ventilation (within 1 week), n (%)</td>
<td>36 (51.4)</td>
<td>146 (47.7)</td>
<td>.575</td>
</tr>
<tr>
<td>Use of total parenteral nutrition and/or intralipid, n (%)</td>
<td>56 (80.0)</td>
<td>207 (67.8)</td>
<td>.042</td>
</tr>
<tr>
<td>Use of central venous catheter, n (%)</td>
<td>61 (87.1)</td>
<td>224 (73.2)</td>
<td>.014</td>
</tr>
<tr>
<td>Previous episode of bacteremia, n (%)</td>
<td>25 (35.7)</td>
<td>72 (23.5)</td>
<td>.036</td>
</tr>
</tbody>
</table>

IQR, interquartile range.

a Including the presence at onset of late-onset sepsis; some patients had >1 underlying chronic complex condition.
b Including all neonates with documented and undocumented syndrome, chromosomal anomalies, and genetic and metabolic diseases; simple cleft palate and polydactyly were not included.
c Including congenital complicated heart disease, cyanotic heart disease, and acyanotic heart disease with heart failure signs.
d Including short bowel syndrome, gastrointestinal pseudo-obstruction, adhesion ileus, and chronic malnutrition.
e Including congenital nephrotic syndrome, immunoglobulin A nephropathy, and renal failure requiring hemodialysis.
Previous antibiotic exposure to third-generation cephalosporin (OR: 5.97; 95% CI: 2.37–15.08; P < .001) and carbapenem (OR: 3.60; 95% CI: 1.26–10.29; P = .017) and presence of underlying renal disease (OR: 7.08; 95% CI: 1.74–28.83; P = .006). The goodness-of-fit test of Hosmer and Lemeshow revealed good agreement between observed and predicted values of the model (P = .73).

Clinical manifestations, antibiotic treatment, and patient outcomes are summarized in Table 4. Infants with MDR GNB bacteremia had a significantly higher rate of septic shock than those with non-MDR GNB bacteremia (40% vs 22.2%; P = .002). Otherwise, no significant differences in terms of clinical manifestations were found. All of the patients received empirical antibiotics within a few hours after blood culture collection, and there were no significant differences between groups regarding the most frequently used antibiotic type, except that the most-used broad-spectrum antibiotic combination, namely carbapenem plus vancomycin or teicoplanin, were more frequently prescribed in the MDR GNB group (18.6% vs 9.2%; P = .023). Infants with MDR GNB bacteremia more frequently received inadequate initial empirical antibiotic treatment when compared with the susceptible control group, and the time to adequate antibiotic therapy was also longer (42.7 ± 16.8 versus 4.5 ± 10.8 hours; P < .001).

Although the duration of mechanical ventilation and hospitalization was comparable between the 2 groups, bacteremia due to MDR GNB was associated with a poorer outcome and with significantly higher rates of infectious complication, early case fatality, and overall case fatality.

The results of univariate and multivariate analyses of factors potentially associated with overall case fatality are summarized in Table 5. The mean gestational age of neonates with GNB bacteremia who died within 30 days was relatively lower than those who survived (29.7 ± 5.5 vs 31.1 ± 4.7 days; P = .098). After adjustment, independent risk factors for mortality were presence of infectious complications after bacteremia (OR: 3.16; 95% CI: 1.41–7.08; P = .005) and underlying secondary pulmonary hypertension with/without cor pulmonale (OR: 6.19; 95% CI: 1.88–20.31; P = .003). The goodness-of-fit Hosmer and Lemeshow test revealed good agreement between observed and predicted values of the model (P = .43).

Because our study extended over a relatively long period of time, the study subjects were split into those before 2007 and those after 2008 to determine if the practices within our NICU may have changed and confounded the results. However, the results shown in Tables 2–5 were still consistent over time (data not shown).

**DISCUSSION**

In this study, we found that MDR GNB bacteremia was not uncommon in the NICU and accounted for nearly one-fifth of all episodes of GNB bacteremia. This study reveals that acquisition of MDR GNB bacteremia is not associated with extremely low birth weight, being born extremely preterm, perinatal complications, or most underlying chronic conditions, but is associated with underlying renal disease and previous antibiotic exposure to third-generation cephalosporin and carbapenem. The most common mechanism of antimicrobial resistance in the NICU was ESBL production. In addition, neonates with MDR GNB were more likely to receive inappropriate antibiotics and develop infectious complications, which were independently associated with a higher overall case-fatality rate.

Previous studies have identified very low birth weight (<1000 g), being born extremely preterm, and prolonged exposure to antimicrobial agents as the independent risk factors associated with resistant Enterobacteriaceae infection in critically ill neonates. However, some of these associations were not observed in this study, and we further identified antibiotic exposure of third-generation cephalosporin and carbapenem within 1 month before GNB bacteremia as the independent
Empirical antibiotic treatment, n (%)  
Combination therapy 64 (91.4) 284 (92.8)  
  β-lactam + aminoglycoside 5 (7.1) 25 (8.2) NS  
  β-lactam + third-generation cephalosporin 14 (20.0) 66 (21.8) NS  
  Glycopeptide + aminoglycoside 1 (1.4) 11 (3.4) NS  
  Glycopeptide + third-generation cephalosporin 28 (41.4) 133 (43.5) NS  
  Glycopeptide + carbapenem 13 (18.6) 28 (9.2) .023  
  Above combination + antianaerobes (metronidazole) 2 (2.9) 21 (6.9) NS  
Monotherapy 6 (8.6) 22 (7.2) NS  
  Third-generation cephalosporin 2 (2.9) 12 (3.9) NS  
  Carbapenem 2 (2.9) 3 (1.0) NS  
  Glycopeptide 2 (2.9) 7 (2.3) NS  
Inadequate initial empirical antibiotic therapy\(^a\), n (%) 51 (72.9) 24 (7.8) <.001  
Time to adequate antibiotic therapy >48 h, n (%) 24 (34.3) 18 (5.9) <.001  
Outcome, n (%)  
Infectious complications\(^b\) 15 (21.4) 32 (10.5) .111  
Recurrent bacteremia within one month\(^c\) 6 (8.5) 32 (10.4) .520  
Early case-fatality rate (7 d) 12 (17.1) 22 (7.2) .009  
Overall case-fatality rate (50 d) 20 (28.6) 32 (10.5) <.001  
Duration of mechanical ventilation, median (IQR), d 44.0 (10.0−65.6) 34.0 (3.0−70.5) .423  
Duration of hospitalization, median (IQR), d 78.5 (42.0−122.8) 75.0 (41.0−114.0) .789  

dIC, disseminated intravascular coagulopathy; IQR, interquartile range; NS, nonsignificant; NTISS, Neonatal Therapeutic Intervention Scoring System.  
\(^a\) Indicates patients who did not receive any antimicrobial agent to which the causative microorganisms were susceptible within 24 h of blood culture collection.  
\(^b\) Defined as a newly infectious focus or persistent organ dysfunction occurring within 1 week and directly related to bacteremia but not concurrent with the onset of bacteremia.  
\(^c\) Includes both Gram-positive and Gram-negative bacteremia.

risk factor. These diverse results can be explained by the different definitions of a resistant GNB isolate, different study designs, different empirical antibiotic policies, and different inclusion criteria (nosocomial infection instead of bacteremia in the other studies). Previous antibiotic therapy has been recognized to be significantly related to bacterial resistance development, but all issues regarding appropriate control group selection, case definition, description of previous antibiotic exposure, and adjustment for confounding factors should be refined. Therefore, a prospective study should be conducted to investigate the impact of specific antibiotic type, treatment duration or total dosage in grams, and interval on the development of neonatal MDR GNB and the colonization and risk of subsequent bacteremia.

In the current study, we found significantly higher rates of infectious complications and early and overall case fatality for the infants with MDR GNB bacteremia, which is consistent with previous reports. The durations of mechanical ventilation and hospitalization were comparable between the MDR GNB group and the controls, but these results may be masked by the higher proportions of neonates in the MDR GNB group who died within 30 days after the onset of bacteremia. Although inappropriate empirical antibiotic therapy has been related to higher mortality in GNB bacteremia, we were unable to establish a direct association between increased mortality in neonates with MDR GNB bacteremia and a delay in appropriate antibiotics. We found initial inappropriate antibiotic therapy to be significantly related to overall mortality (OR: 2.35; 95% CI: 1.23−4.51; P = .010) and infectious complications (OR: 3.27; 95% CI: 1.71−6.23; P < .001) but not early case-fatality rate. On the basis of the current study, we suspected that although most neonates with MDR GNB bacteremia can survive the first week even after receiving inappropriate antimicrobial therapy initially, some of them would have a significantly higher risk of progressively clinical deterioration, infectious complications, or an additional episode of nosocomial infection, which may lead to an adverse outcome.

According to the clinical presentations and laboratory findings (data not shown) in the current study, infants with GNB bacteremia caused by an MDR strain could not be differentiated from those with GNB bacteremia caused by a non-MDR strain until the results of antimicrobial susceptibility were available. Although a significantly higher rate of septic shock was noted in infants with MDR GNB bacteremia, not all of these cases were due to initial inappropriate antibiotics. Clinical outcomes of these neonates were not only affected by the initial empirical antibiotic therapy but also by the pathogens and host factors such as underlying conditions or immunity of the patients, concomitant infectious focus, and retaining or removal of infected catheters or endotracheal tubes.
Molecular analysis of ESBL-producing strains in the current study showed uncommon, horizontal patient-to-patient transmission in our NICU, because most of these ESBL-producing strains were different strains. These results were compatible with our finding that broad-spectrum antibiotic selection was highly associated with MDR GNB bacteremia in our NICU. However, several clonal strains of ESBL-producing *K. pneumoniae* and *E. coli* were identified over a period of 2 to 8 months, suggesting their prolonged existence in the NICU environment or long-term colonization. Therefore, both infection control measures and applying new empirical antibiotic policies, especially decreased usage of broad-spectrum cephalosporin, may help to reduce the incidence of MDR GNB colonization and outbreak.35,36 Our results suggested that initial antimicrobial regimens could be based on previous antibiotic use, and local surveillance data and local NICU antibiograms are recommended for the optimization of initial antibiotic choice.37,38

There were some limitations to this study. Because our data source was only a single institution, there might be different epidemics in other centers, although a large cohort of bacteremias were studied in this research. Because our cases of MDR GNB included many varieties of both Enterobacteriaceae and non-Enterobacteriaceae, the influences of different types of microorganisms on clinical manifestations and outcomes were not studied.

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