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### Key Words

bacteremia;  
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## Neonatal Bacteremia in a Neonatal Intensive Care Unit: Analysis of Causative Organisms and Antimicrobial Susceptibility

**Background.** Infections cause significant mortality and morbidity in neonates, especially the premature ones. Although there are various antibiotics can be used to combat neonatal infection, resistant strains have subsequently emerged. In an epidemiological survey, we analyzed bacterial isolates and their antibiotic susceptibilities for cases of bacteremia in a neonatal intensive care unit (NICU) of a teaching hospital.

**Methods.** From November 1999 to October 2001, 623 neonates admitted to the NICU were enrolled. The incidence and incidence density of bacteremia, morbidity and mortality of sepsis, as well as antibiotic susceptibility, were investigated.

**Results.** Totally, 754 blood cultures were done on 623 patients. Fifty-eight patients experienced 85 episodes of bacteremia, with 87 isolates cultured. The incidence of bacteremia in our NICU was 9.31% (58/623) with an incidence density of 10.98/1000 patient-days. The overall mortality rate was 7.22%. The case fatality rate of bacteremia was 20.7% (12/58). The bacterial pathogens encountered, in order of frequency, were coagulase-negative *Staphylococcus* (29%), *Staphylococcus aureus* (22%), and *Enterobacter cloacae* (17%). All of the gram-positive bacteria were susceptible to vancomycin, while the gram-negative bacteria were susceptible to imipenem, amikacin and ciprofloxacin. Oxacillin-resistant *S. epidermidis*, oxacillin-resistant *S. aureus*, and multi-drug resistant enterobacteriae were the leading microorganisms causing bacteremia in our NICU.

**Conclusions.** It is an endless struggle to combat neonatal infection. Periodic evaluation of bacterial antibiotic susceptibility is necessary. More judicious selection of antibiotics and rotating antibiotic regimens should be kept in mind to reduce the resurgence of multidrug resistant strains.

Sepsis is a severe problem for neonates. It always results in mortality or prolonged hospitalization,<sup>1,2</sup> and the control over nosocomial infections has been a formidable challenge for a long period of time.<sup>2-4</sup> Recently, the Neonatal Intensive Care Unit (NICU) of the Taipei Veterans General Hospital (VGH-Taipei) encountered outbreaks of severe bacteremia. In order to understand the epidemiology of neonatal sepsis in the NICU, we retrospectively collected data on bacteremia to analyze its microbiology and determine the antibiotic susceptibilities of the

causative organisms.

### METHODS

#### Study population

From November 1999 to October 2001, all neonates admitted to the NICU of VGH-Taipei were recruited as the cohort. There were totally 623 infants, including 324 boys and 299 girls, accounting for 7,742 patient-days. Babies born in VGH-Taipei accounted for 412 cases, and

those born outside accounted for 211 cases.

### Data collection

For patients with positive blood cultures, data collection included gestational age, gender, birth body weight, APGAR score, clinical diagnosis on admission, length of hospitalization, the pathogenic bacteria of positive cultures, and their antibiotic susceptibilities. Blood cultures were obtained when sepsis was suspected. Around 0.5 ml of blood was drawn in sterile conditions from peripheral punctures. Blood was injected into Baxtar blood culture bottles for incubation in BACTEC 9240 at 35.5 °C. After bacterial growth was detected, the microorganisms were transferred to culture media for further identification. A Baxter Microscan AutoSCAN-4 was used to analyze the species and antibiotic sensitivities.

Gram staining and disc diffusion methods were performed if the results were controversial or if a rapid report was required. If there was no bacterial growth after 7 days of incubation, the culture was reported to be negative. Antibiotic sensitivity tests for gram-positive bacteria included ampicillin, ampicillin/sulbactam, penicillin, oxacillin, clindamycin, cefazolin, cefotaxime, vancomycin and ciprofloxacin. For the gram-negative bacteria, the antibiotics tested were ampicillin, ampicillin/sulbactam, piperacillin, gentamycin, amikacin, cefazolin, cefuroxime, ceftazidime, ceftriaxone, imipenem and ciprofloxacin.

The incidence of bacteremia was defined as patients of the study cohort with positive cultures. The bacteremia incidence density was defined as positive blood culture episodes/1,000 patient-days. The overall mortality rate was defined as the number of deaths among the total number of patients. The case fatality rate was defined as the number of deaths among patients with bacteremia. Non-parametric data are expressed as the median and range.

## RESULTS

### Patient population

Totally, 754 blood cultures were done on 623 patients. There were 58 patients with 85 episodes of bacteremia caused by 87 isolates. The demographic data of patients with bacteremia are given in Table 1. The overall inci-

**Table 1 Demographics of the study group**

	n = 58
Male / Female	31 / 27
GA (wks)	32.3 ± 5.6 (22 - 42)
BBW (g)	1770 ± 982 (520-3774)
A/S*	
1 min	5 (0-8)
5 min	7 (1-9)
LOS(d)*	
NICU	37.5 (1-248)
Hospital	52.5 (1-248)
Time of positive blood culture	
After admission (d)*	19 (1-188)
Admission diagnosis	
Prematurity	37
CHD	6
Sepsis	3
Operation	1
Miscellaneous	11

A/S = Apgar Score ; BBW=birth body weight ; CHD = congenital heart disease; LOS = length of stay ; NICU = neonatal intensive care unit. \*median (range).

dence of bacteremia was 9.31% (58/623), with an infection incidence density of 10.98/1000 patient-days (85/7742). The distribution of bacteremia in neonates of normal birth weight (birth body weight [BBW] > 2,501 g), low birth weight ([LBW] BBW 1,501-2,500 g), very low birth weight ([VLBW] BBW 1,001-1,500 g), and extremely low birth weight ([ELBW] BBW < 1000 g) was 25.9%, 17.6%, 21.2%, and 35.3%, respectively. The overall NICU mortality rate was 7.22% (45/623).

The case fatality rate in patients with positive blood culture was 20.7% (12/58). Three patients expired within 3 days of onset of sepsis. Among the cases who died, 5 patients had a birth body weight less than 1000 g, while the remaining 7 had severe underlying diseases that included complicated congenital heart disease (n = 3), congenital megacystis-microcolon-intestinal hypoperistalsis syndrome (n = 1), congenital diaphragmatic hernia (n = 1), severe perinatal asphyxia (n = 1), and chronic diarrhea with failure to thrive (n = 1).

### Distribution of bacteremia

Table 2 shows the bacteria isolated from our patients. Of the 87 isolates, 55% (48/87) were gram-positive cocci, 38% (33/87) were gram-negative bacilli, and 7% (6/87) were yeast. In the gram-positive cocci, coagulase-

**Table 2 Distribution of microorganisms of positive blood cultures within 3 days of admission and those after**

Bacterial species	≤ 3 days (n = 7)	> 3 days (n = 80)	Total (%) (n = 87)
Gram positive cocci	5	43	48 (55%)
CONS	2	23	25 (29%)
<i>Staphylococcus aureus</i>	1	18	19 (22%)
GBS	2	0	2 (2%)
<i>Enterococcus faecalis</i>	0	2	2 (2%)
Gram negative bacilli	2	31	33 (38%)
<i>Enterobacter</i> species	0	18	18 (21%)
<i>Enterobacter cloacae</i>	0	15	15 (17%)
<i>Enterobacter sakazakii</i>	0	2	2 (2%)
<i>Enterobacter aerogenes</i>	0	1	1 (1%)
<i>Klebsiella pneumoniae</i>	2	8	10 (11%)
<i>Acinetobacter baumannii</i>	0	4	4 (5%)
<i>Serratia marcescens</i>	0	1	1 (1%)
Fungi	0	6	6 (7%)

CONS = coagulase negative *Staphylococcus*; GBS = group B *Streptococcus*

negative *Staphylococcus* (CONS) was the leading microorganism, followed by *Staphylococcus aureus*. In the gram-negative bacilli, *Enterobacter* was most often encountered, followed by *Klebsiella*, *Acinetobacter* and *Serratia*. Six patients had positive cultures within 3 days of admission. The remaining 52 patients showed positive blood cultures more than 3 days after admission.

Forty-three patients had 1 episode of positive blood culture, 8 had 2 episodes, 5 had 3 episodes, 1 had 5 episodes, and 1 patient had 8 episodes. The patient who had 5 episodes of bacteremia was a premature, extremely low birth weight infant (BBW 654 g) with a long hospitalization of 121 days. The patient who had 8 episodes of bacteremia had a birth weight of 538 g and was hospitalized for 248 days. Two specimens grew multiple microorganisms in the same bottle. One was *K. pneumoniae* and *Enterococcus faecalis*, while the other was *Acinetobacter baumannii* and CONS.

There were 6 patients who experienced 7 episodes of bacteremia within 72 hours of admission: 2 episodes of CONS, 1 episode of *S. aureus*, 2 of group B *Streptococcus* (GBS), and 2 of *K. pneumoniae*. Four of them were within 72 hours of birth, and had bacteremia caused by gram-positive cocci (2 GBS, 1 CONS, and 1 *S. aureus*). For the 2 patients with GBS bacteremia, 1 was a case of meconium aspiration syndrome, and the other had a sei-

zure 2 days after birth. The patient with CONS bacteremia was noted to have maternal fever and suspected amnionitis before delivery. Maternal cultures were negative possibly due to the administration of antibiotics. The patient with *S. aureus* bacteremia had a congenital diaphragmatic hernia, and was treated with multiple invasive management, which might have led to the bacterial invasion. The remaining 2 patients were 3 days older on admission. The patient with 2 positive cultures of *K. pneumoniae* septicemia had chronic diarrhea. Two cultures were obtained within 24 hours at different times, and grew the same bacteria with the same antibiotic sensitivities. This indicated that the antibiotics administered did not sterilize the blood. Therefore, strictly speaking, the 2 instances in this case represented the same episode.

There were 52 patients with 80 episodes of bacteremia 3 days or longer after admission. For gram-positive bacteremia, CONS was still the leading microorganism, followed by *S. aureus* and *E. faecalis*. For gram-negative bacilli, *Enterobacter* spp., *K. pneumoniae*, *A. baumannii* and *Serratia marcescens* were noted. For *Enterobacter* spp., *E. cloacae* accounted for 15 cases, followed by 2 cases of *E. sakazakii*, and 1 case of *E. aerogenes*.

### Antibiotic susceptibilities

Table 3 shows the antibiotic susceptibilities of gram-positive bacteria. GBS was sensitive to most of the antibiotics, including ampicillin, penicillin and oxacillin. Ninety-five percent (18/19) of the cases with *S. aureus* was oxacillin-resistant. Strains resistant to vancomycin were not found in the study cohort. Table 4 shows the antibiotic susceptibilities of gram-negative bacteria. For *E. cloacae*, only 1 strain was sensitive to multiple antibiotics. Others were only sensitive to imipenem (15/15), meropenem (2/2) or ciprofloxacin (12/12). *E. sakazakii* and *E. aerogenes* were sensitive to amikacin, imipenem, and ciprofloxacin.

Three patients experienced 4 episodes of *Acinetobacter* septicemia. For the patient with 2 episodes of *Acinetobacter* septicemia, the intervening interval was 3 months, and the bacterial pathogens of the second episode were sensitive to imipenem only. In another premature baby who expired due to *Acinetobacter* sepsis, multi-drug resistance was noted, including amikacin,

**Table 3. Antibiotic susceptibilities of gram-positive organisms**

Antibiotics	Bacterial species <i>Staphylococcus aureus</i>		Coagulase negative <i>Staphylococcus</i>		Group B <i>Streptococcus</i>		<i>Enterococcus faecalis</i>
	age of patients						
	≤ 3 days old n = 1	> 3 days old n = 18	≤ 3 days old n = 1	> 3 days old n = 24	≤ 3 days old n = 2	> 3 days old n = 2	
Ampicillin	0	0	0	0	2	2	
Ampicillin/Sulbactam	0	1	N/A	0	1 (n = 1)	1 (n = 1)	
Penicillin	0	0	0	0	2	2	
Oxacillin	0	1	0	1	2	0	
Clindamycin	0	1	0	4	2	0	
Cefazolin	0	1	0	0	2	0	
Cefotaxime	0	1	0	0	1	N/A	
Vancomycin	1	18	1	24	2	2	
Ciprofloxacin	N/A	3 (n = 15)	N/A	12 (n = 18)	N/A	1	

N/A = not available.

imipenem and 3rd-generation cephalosporins. No antibiotic was effective against that strain at that time. *K. pneumoniae* was 100% (10/10) sensitive to imipenem, followed by amikacin (8/10), cephalosporins (5/10), gentamicin (5/10), and piperacillin (5/10). It was also 100% (7/7) sensitive to ciprofloxacin, although only 7 samples were tested. *S. marcescens* was sensitive to most antibiotics except cefazolin, ampicillin/sulbactam and cefuroxime.

## DISCUSSION

The overall incidences of bacteremia in NICUs have ranged from 1.9% to 30.4%.<sup>5-12</sup> In Taiwan, Lin *et al* re-

ported a sepsis rate of 10.2% in a NICU,<sup>5</sup> which is similar to our result of 9.31%. Case fatality rates from septicemia in NICUs have ranged from 9% to 44.2%.<sup>8,9</sup> In our NICU, it was 20.7% (12/58). Many studies have reported CONS to be the most common bacteria in nosocomial infections,<sup>5-16</sup> and our data showed the same results. For bacteremia that occurred within 3 days of birth, GBS was the leading microorganism, whereas no gram-negative bacteria were found. These results are also similar to those of other reports.<sup>3</sup> Some studies have shown that *E. coli* was the most common bacteria of early onset bacteremia.<sup>6,12</sup> However, we found no *E. coli* bacteremia during our study period since early onset neonatal bacteremia cases was few in our hospital. The occurrence of CONS in early onset bacteremia was also reported.<sup>12</sup> In

**Table 4. Antibiotic susceptibilities of gram-negative organisms**

Antibiotics	Bacterial species		<i>Enterobacter</i> spp.		
	<i>E. cloacae</i> n = 15	<i>E. Sakazakii</i> n = 2	<i>E. aerogenes</i> n = 1	<i>A. baumannii</i> n = 4	<i>K. pneumoniae</i> n = 10
Ampicillin	0	0	0	0	0
Ampicillin/Sulbactam	0	0	0	N/A	0
Piperacillin	1	0	0	0	4
Gentamicin	1	0	0	1	5
Amikacin	5	2	1	2	8
Cefazolin	0	0	0	0	5
Cefuroxime	0	0	0	1	5
Ceftazidime	0	0	0	1	6
Ceftriaxone	1	1	1	1	5
Imipenem	15	1	1	3	10
Ciprofloxacin	12 (n = 12)	2	1	N/A	7 (n = 7)

N/A = not available.

our studies, CONS was not only cultured from patients more than 3 days after admission, but was also found in patients within 3 days of admission.

In this study, all of the gram-negative bacilli were cultured from patients who had stayed in the hospital for more than 3 days. According to previous studies, most strains of *E. cloacae* were sensitive to aminoglycosides, whereas *E. aerogenes* was more sensitive to tetracycline, colistin and chloramphenicol.<sup>17, 18</sup> However, our data revealed that in most cases *E. cloacae* was resistant to aminoglycosides but sensitive to imipenem, meropenem and ciprofloxacin. Since 1983, production of extended spectrum beta-lactamases (ESBLs) or AmpC gene beta-lactamases has been found in many gram-negative bacteria such as *K. pneumoniae*, *E. coli*, *Klebsiella oxytoca*, *Salmonella spp*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Enterobacteriaceae*.<sup>19</sup> The occurrence of multi-drug resistant *E. cloacae* in our NICU might result from these two mechanisms that were against most 3rd-generation cephalosporins in the current usage. However, further analysis should be done for confirmation.

In contrast, for *E. aerogenes*, aminoglycosides and 3rd-generation cephalosporins were still effective. While, *Acinetobacter* bacteremia was reported to be increase, in NICU patients<sup>20,21</sup> the resurgence of multi-drug resistant strains is an important issue. Strains resistant to imipenem are reported to reach 12.9%.<sup>22</sup> Aminoglycoside combined with imipenem, a beta-lactamase inhibitor, or quinolones, was synergistic *in vitro* against such a strain.<sup>23</sup> Multi-drug resistant *Acinetobacter* was also noted in our NICU. Although this was a sporadic case, careful monitoring is mandatory. Fungemia accounted for 7% of sepsis in our series (n = 6). However, in other series, it has been as high as 24.2% and was the second most common microorganism.<sup>5</sup> Treatment with amphotericin B was successful in 5 patients. One patient expired due to fungemia with septic shock.

In our NICU, prophylactic antibiotics were often prescribed for high-risk newborns, with ampicillin plus aminoglycosides serving as the empiric ones. They were often discontinued 3 to 7 days later if patients were in stable condition and blood cultures turned negative. However, this combination therapy seems effective for GBS infections only. Since oxacillin-resistant *S. aureus* (ORSA), oxacillin-resistant *S. epidermidis* (ORSE), and

multi-drug resistant *enterobacteriae* are the prevalent microorganisms in our NICU, the role of prophylactic antibiotics seems questionable. The discovery of multidrug-resistant *Enterobacteriae* and *Acinetobacter* has stimulated a more careful and judicious use of antimicrobial agents, especially because a resurgence in strains resistant to 3rd-generation cephalosporins, imipenem or to quinolones will occur in the future. Prolonged use of antibiotics increases the occurrence of fungemia. Judicious selection of antibiotics for adequate durations should always be kept in mind.

Invasive procedures, empiric antibiotics, hyperalimentation, prolonged hospitalization, and extremely low birth weight per se, increase the risk of infection.<sup>16,24</sup> To control infections, prolonged use of broad-spectrum antibiotics is often encountered, which leads to the resurgence of multidrug-resistant organisms.<sup>24</sup> Therefore, preventive antibiotics should be used as little as possible, while therapeutic antibiotics should be specific and used as short period of time as possible. The combined use of various antibiotics should likewise be judicious. In conditions wherein the use of antibiotics is necessary, rotating antibiotic regimens has been suggested and may be a way to solve this problem.<sup>25</sup>

In conclusion, different NICUs have different epidemiologies of nosocomial infections.<sup>2-4</sup> Collection of up-to-date data is mandatory for appropriate use of antibiotics. To select antibiotics conscientiously according to susceptibility tests is very important, and strategies to avoid the resurgence of multidrug-resistant strains should be established.

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