Risk factors of initial inappropriate antibiotic therapy and the impacts on outcomes of neonates with gram-negative bacteremia

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Abstract

Background Timely appropriate empirical antibiotic plays an important role in critically ill patients with gram-negative bacteremia. However, the relevant data and significant impacts have not been well studied in the neonatal intensive care unit (NICU).

Methods An 8-year (1 January 2007-31 December 2014) cohort study of all NICU patients with gram-negative bacteremia in a tertiary-care medical center was performed. Inadequate empirical antibiotic therapy was defined when a patient didn’t receive any antimicrobial agent to which the causative microorganisms were susceptible within 24 hour of blood culture sampling.

Results Among 376 episodes of Gram-negative bacteremia, 75 (19.9%) received inadequate empirical antibiotic therapy. The cause of inadequate treatment was mostly due to the pathogen resistant to prescribed antibiotics (88.0%), and Pseudomonas aeruginosa (Odds ratio [OR]: 20.8, P < 0.001) and ESBL-producing bacteria (OR: 18.4, P < 0.001) had the highest risk. Previous exposure with 3rd generation cephalosporin was identified as the only independent risk factor (OR: 2.52, 95% CI: 1.18-5.37, P = 0.018). Empirically inadequately treated bacteremias were significantly more likely to have worse outcomes than those with adequate therapy, including more prolonged illness, higher rate of infectious complications (25.3% versus 9.3%, P < 0.001) and overall mortality (22.7% versus 11.0%, P = 0.013).

Conclusions Inadequate empirical antibiotic therapy occurs in one-fifth of Gram-negative bacteremias in the NICU, and is associated with worse outcomes. Further effort to decrease emergence of antibiotic resistance and highly suspicion of
infection by drug-resistant bacteria clinically is important to reduce rates of inadequacy.

Introduction

Bloodstream infection is the major cause of mortality in the neonatal intensive care unit (NICU) after more advanced perinatal care and improved delivery room resuscitation have been achieved in recent years [1, 2]. Although coagulase-negative staphylococci remains the most common pathogen of neonatal bacteremia, there is an increasing trend of Gram-negative bacteremia (GNB) in the NICU [3, 4], especially after long duration of hospitalization, gram-negative bacteria colonization, or underlying gastrointestinal pathology [5–7]. The emergence of antibiotic resistance among GNB is a great concern, since there is limited development of new antibiotics to successfully treat GNB [8–10]. The condition may become especially critical when severe GNB, defined as a fulminant and rapidly devastating sepsis, is encountered.

Appropriate initial antibiotic therapy has been demonstrated as the key independent factor of treatment outcomes in patients with Gram-negative bacteremia in several previous studies [11–14]. However, this issue has not been fully studied in the NICU, except for a small sample size, case-control study found in the literature [15]. GNB in the NICU are well known to potentially cause life-threatening septic shock, especially by multi-drug resistant pathogens or in neonates with underlying chronic comorbidities [4, 16]. Besides, it is worthwhile to examine clinical parameters that can be used as prognostic markers in critically ill neonates, which can facilitate assessment of the GNB course and guide treatment strategies. Therefore, we aimed to investigate the impact of initial inadequate antibiotic therapy on outcomes of
neonates with severe GNB and examine clinical parameters that can be predictors of final adverse outcomes.

Patients and methods

Setting and Study Design

This study was conducted in the NICUs of Chang Gung Memorial Hospital (CGMH), and all neonates hospitalized in the NICUs of Linkou CGMH were enrolled. These NICUs contain a total of three units and has a total capacity of 47 beds equipped with mechanical ventilator and 48 beds with special care nurseries. Since 2003, all neonates admitted to our NICUs were included in a database and followed up until death or hospital discharge. This database contained basic demographics, comorbidities of prematurity, all nosocomial infections, and discharge diagnosis. We conducted a retrospective cohort study using this prospectively collected database. Written consent for this study was not required by the Ethics Committee of CGMH, which approved this research.

All patients between January 2007 and December 2014 with microbiologically documented GNB were enrolled for analysis. Polymicrobial infections were also included if one of the pathogens was Gram-negative organism. Subsequent episodes of bacteremia in study patients within one month from the first episode of Gram-negative bacteremia were excluded due to better outcome analysis. We reviewed the medical records of the neonates with initial inadequate antibiotics and compared them with those who received appropriate initial antimicrobial therapy. The main outcome measures included the infectious complications, early mortality (death within 7 days of bacteremia onset) and overall mortality (death due to any reason within 30 days of bacteremia onset).
Data collection

In addition to all basic demographics and complications of prematurity retrieved from our neonatal database, chart and electronic medical records of patients who met inclusion criteria were reviewed for the presence of the following features: details of clinical features, laboratory data and treatment courses of bacteremias, initial antimicrobial therapy regimen, use of central venous catheter (CVC), total parenteral nutrition (TPN) and/or intrafat, mechanical ventilators, underlying chronic conditions, surgical interventions and receipt of corticosteroid or antibiotics within 30 days prior to bacteremia. Severity of illness was calculated by two investigators (Dr. S.-M.C. and Dr. J.-F.H.) based on the Neonatal Therapeutic Intervention Scoring System (NTISS) [19].

Definitions

Gram-negative bacteremia was defined as identification of gram-negative bacilli in a blood culture specimen. Only clinically significant bacteremias were enrolled, which was defined as at least one positive blood culture together with clinical features compatible with systemic inflammatory response syndrome. The bacteremia onset was defined as the time point of blood culture sampling through peripheral vein or artery, as we never obtained the blood culture from CVC. Inadequate antimicrobial therapy referred to the administration of antimicrobial agents to which the causative microorganisms were resistant in vitro within the first 24 hours of bacteremia onset or lack of administration of an antimicrobial therapy.

In our institution, blood cultures were incubated in the Bactec 9240 system, and antibiotic susceptibility testing was performed by using the disk diffusion method according to the recommendations of the Clinical Laboratory Standards Institute.
Multidrug resistance was defined using previously published criteria [21]. The microbiology laboratory would notify the clinician when a blood culture was positive, and the clinician was responsible to modify the antimicrobial regimens according to subsequent bacterial identification and antimicrobial susceptibility results. Early-onset sepsis and late-onset sepsis were defined as clinical sepsis combined with bacterial growth on a blood culture obtained within the first 72 hours of life and > 72 hours of life, respectively [5, 8]. A bacteremia was classified as community-acquired in this study if the neonate had been discharged from NICU or baby room for at least two days, and the first positive blood culture occurred ≤ 48 hours after hospital admission. Antimicrobial exposure was defined as systemic administration of an antibiotic class for at least 3 days in the preceding 30 days before the onset of bacteremia. All comorbidities of prematurity, including respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), and periventricular leukomalacia (PVL) were based on the latest updated diagnostic criteria in the standard textbook of neonatology [22]. Shock was defined as a mean blood pressure < lower limit according to gestational age that was unresponsive to fluid treatment or required vasoactive agents [23]. Persistent bacteremia was defined as two or more consecutive positive blood cultures with at least 48 hours apart. Infectious complications were defined as any newly infectious focus or persistent organ damage occurred within one week after the onset of bacteremia, but not concurrently.

Statistical analysis

The student’s t test or Mann-Whitney U-test were used to compare continuous
variables depending on values distribution, and $\chi^2$ or Fisher’s exact test was used to compare categorical variables. A two-sided P value of $< 0.05$ was considered significant. In identifying the independent risk factors for mortality, a backward stepwise logistic regression analysis was sued to control for the effects of confounding factors. Variables with a P value of $< 0.05$ in the univariate analysis were candidates for multivariate analysis as well as the main variable of interest (i.e., inappropriate initial antimicrobial therapy). Statistical analyses were performed using SPSS version 15.0 (SPSS®, Chicago, IL).

Results

The epidemiology of Gram-negative bacteremia in the NICU

During the study period, a total of 333 neonates with 395 episodes of Gram-negative bacteremia were identified. 19 of them were the recurrent episodes which occurred within one month after the first episode and were excluded, leaving a total of 333 neonates with 376 episodes of Gram-negative bacteremia for analysis (Table 1).
### Table 1

Bacterial isolates in 333 neonates with 376 episodes of Gram-negative bacteremia

| Microorganism                  | Total case no. (%) | Inadequate treatment, n (% of each type of bacteria) |
|-------------------------------|--------------------|------------------------------------------------------|
| Early-onset sepsis            |                    |                                                      |
| Escherichia coli              | 16 (4.3)           | 2 (12.5)                                             |
| Others                        | 6 (1.6)            | 1 (16.7)                                             |
| Late-onset sepsis             | 354 (94.1)         |                                                      |
| Klebsiella pneumoniae         | 82 (21.8)          | 15 (18.3)                                            |
| Escherichia coli              | 76 (20.2)          | 12 (15.8)                                            |
| Acinetobacter baumannii       | 43 (11.4)          | 4 (9.3)                                              |
| Klebsiella oxytoca            | 39 (10.4)          | 5 (12.8)                                             |
| Enterobacter cloaceae         | 26 (6.9)           | 8 (30.8)                                             |
| Enterobacter aerogenes        | 17 (4.5)           | 4 (23.5)                                             |
| Pseudomonas aeruginosa        | 16 (4.3)           | 12 (75.0)                                            |
| Serratia marcescens           | 10 (2.7)           | 0 (0)                                                |
| Others*                       | 12 (3.2)           | 4 (33.3)                                             |
| Polymicrobial organisms¶      | 33 (8.8)           | 8 (24.2)                                             |
| Total                         | 376 (100)          | 75 (19.9)                                            |
| ESBL producing bacteria&      | 47 (12.5)          | 34 (72.3)                                            |

*Including Citrobacter freundii (3), Stenotrophomonas maltophilia (3), Hafnia alvei (2), Neisseria Meningitidis (2), Chryseobacterium meningoseptium (1) and Flavobacterium (1)

**Indicating two or more microorganisms were recovered from the same blood culture set

&47 ESBL (extended-spectrum β-lactamase) producing bacteria were included in the total 376 episodes, and included K. pneumonia (28), E. coli (9), K. oxytoca (6), and E. cloacae (4)

Most of our cases were nosocomial infections (371 episodes, 98.7%), and only 5 episodes (1.3%) were community-acquired. There were 354 episodes of late-onset sepsis and 22 episodes of early-onset sepsis. The predominant organisms for monomicrobial bacteremias were Klebsiella spp. (n = 122; 32.4%), Escherichia coli (n = 92; 24.5%), Enterobacter spp. (43; 11.4%), and Acinetobacter baumannii (43; 11.4%). Thirty-three episodes of bacteremias were polymicrobial (8.8%). There were 70 (18.6%) multidrug-resistant organisms among the isolates, including 47 extended-spectrum β-lactamase (ESBL) producers.

**The frequency of inadequate antibiotic treatment of Gram-negative bacteremia**

The antibiotics with Gram-negative activity that were most frequently prescribed during the first 24 hours of bacteremias onset were cefotaxime (228; in 60.6% of episodes), gentamicin (51; 13.6%), meropenem (46; 12.2%), ceftazidime (38;
10.1%), and aztreonam (4; 1.1%). In 9 (2.4%) episodes, only monotherapy with vancomycin was prescribed initially. In 348 cases (92.6%), more than one antibiotic were used during this time period.

Seventy-five (19.9%) episodes of Gram-negative bacteremia were treated with inadequate empirical antibiotics. In 66 (88.0%) of these cases, subsequently identified pathogens resistant to the prescribed antibiotics were responsible for the inadequate treatment, including multi-drug resistant strains for 51 (68.0%) isolates. The other 9 (12.0%) cases were due to failure to administer in-vitro susceptible antibiotics within 24 hours of the initial positive blood culture. The median time from bacteremia onset to receive adequate antibiotic treatment in those without initially adequate treatment was 47 hours (range: 29–94 hours). Within 24 hours after notification of antibiotic susceptibility, all patients received adequate antibiotic treatment.

Klebsiella pneumoniae (n = 15, 20.0%) was the most common pathogen treated with initially inadequate antibiotic, followed by E. coli (14, 18.7%) and Pseudomonas aeruginosa (12, 16.0%). However, ESBL-producing bacteria (n = 34, 72.3%, odds ratio [OR]: 18.4, 95% confidence interval [CI]: 9.0-37.7, P < 0.001) and Pseudomonas aeruginosa (n = 12, 75%, OR: 20.8, 95% CI: 5.8–75.3, P < 0.001) had the highest rate of receiving inadequate antibiotics initially (Table 1).

Factors associated with inadequate empirical antibiotic treatment of Gram-negative bacteremia

Between patients receiving initially inadequate and adequate empirical treatment, there were no significant differences in terms of demographics, age at onset of bacteremia, use of CVC, TPN and/or intrafat, mechanical ventilation, and underlying
chronic conditions (Table 2). Initially inadequately treated bacteremias were significantly more likely to be the recurrent episode (37.3% vs. 22.9%, P = 0.018), and significantly more likely to have antibiotic exposure with a 3rd generation cephalosporin (P < 0.001) and vancomycin (P = 0.004) within one month before bacteremia onset than adequately treated bacteremias. In terms of clinical manifestations and laboratory data at onset of bacteremia, there were no significant differences between the two groups, except higher serum C-reactive protein concentration in the bacteremias without adequately treated (median, 78.0 vs. 59.1, P = 0.038). With multivariate logistic regression analysis, only previous antibiotic exposure to a 3rd generation cephalosporin within one month was identified as the independent risk factor (Odds ratio [OR]: 2.52, 95% CI: 1.18–5.37, P = 0.018).

Table 2
Comparison of 376 episodes of Gram-negative bacteremias treated with inadequate versus adequate empirical antibiotic treatment

| Case No.(%) (Total n = 376) | Univariate analysis | Multivariate analysis |
|-----------------------------|---------------------|----------------------|
|                             | Inadequate treatment (n = 75) | Adequate treatment (n = 301) | P value |
| Gestational age (weeks), median (IQR) | 30.0 (27.0–35.0) | 31.0 (27.0–36.0) | 30.0 (27.0–35.0) | 0.192 | - |
| Birth body weight (g), median (IQR) | 1345.0 (900.0-2051.3) | 1600.0 (960.0-2135.0) | 1265.0 (895.0-2020.0) | 0.084 | - |
| Male gender | 191 (50.8) | 35 (46.7) | 156 (51.8) | 0.518 | - |
| Day of bacteremia onset, median (IQR) | 25.0 (13.0-54.0) | 31.0 (13.0-66.0) | 24.0 (13.3-52.8) | 0.136 | - |
| Late-onset sepsis | 354 (94.1) | 72 (96.0) | 282 (93.7) | 0.854 | - |
| Episode sequence of bacteremia | 0.018 | 1 (reference) | 1.41 (0.78-2.58) | |
| 1st episode | 279 (74.2) | 47 (62.7) | 232 (77.1) | |
| Recurrent episode | 97 (25.8) | 28 (37.3) | 69 (22.9) | |
| Underlying chronic conditions | | | | |
| Congenital anomalies | 24 (6.4) | 5 (6.6) | 19 (6.3) | 1.000 | - |
| Neurological sequelae | 57 (15.2) | 12 (16.0) | 45 (15.0) | 0.858 | - |
| Bronchopulmonary dysplasia | 170 (45.2) | 31 (41.3) | 139 (46.1) | 0.517 | - |
| Chronic gastrointestinal | 17 (4.5) | 4 (5.3) | 13 (4.3) | 0.710 | - |
| Pathology                                      | Others   | Use of central venous catheter | On high frequency oscillatory ventilator | Under invasive ventilation (intubation) | Use of total parenteral nutrition/intrafa | Previous operation (within one month) | Use of steroid (within one month) | Antibiotic exposure (within one month) | 3rd generation cephalosporin | Vancomycin or teicoplanin | Carbapenem | Antifungal treatment | Clinical manifestations at bacteremia onset* | Sepsis-induced hypotension | GI bleeding and/or coagulopathy | Disseminated intravascular coagulopathy | NTISS score, median (IQR) | Laboratory data at onset of bacteremia |
|----------------------------------------------|----------|-------------------------------|------------------------------------------|----------------------------------------|------------------------------------------|--------------------------------------|-----------------------------------|------------------------------------------|---------------------------------|-------------------------------|------------|-----------------|------------------------------------------|-------------------------------|-----------------------------|-----------------------------------------------|-----------------|------------------------------------------|
|                                              | 23 (6.1) | 285 (75.8)                   | 37 (9.8)                                 | 182 (48.4)                             | 263 (69.9)                               | 56 (14.9)                          | 13 (34.5)                          | 142 (39.6)                               | 161 (42.8)                     | 149 (39.6)                      | 22 (5.9)  | 12 (3.2)        | Sepsis-induced hypotension             | 96 (25.5)                       | 147 (39.1)                  | 67 (17.8)                                   | 17.0 (13.0–20.0)           | Leukopenia (WBC count < 4000/ul)           |
|                                              |          | 6 (8.0)                      | 59 (78.7)                                | 45 (67.3)                               | 56 (74.7)                                | 10 (13.3)                          | 5 (6.6)                           | 41 (54.7)                                 | 47 (62.7)                      | 29 (38.6)                       | 6 (8.0)   | 5 (6.7)         | GI bleeding and/or coagulopathy         | 74 (24.6)                       | 118 (39.2)                  | 52 (17.3)                                   | 17.0 (12.0–20.0)           | Leukocytosis (WBC count > 20,000/ul)       |
|                                              |          |                              | 17 (5.6)                                 | 30 (10.0)                               | 207 (68.8)                               | 46 (15.3)                          | 8 (2.7)                           | 108 (35.9)                                | 114 (37.9)                     | 118 (39.2)                      | 16 (5.3)  | 7 (2.3)         | Disseminated intravascular coagulopathy |                                |                            |                                           |                               | Leukocyte shift to left**              |
|                                              |          |                              |                                          |                                        |                                         |                                    |                                  |                                       |                                |                               |                               |                   |                              | NTISS score, median (IQR)              |                               |                            |                                             |                               | Anemia (hemoglobin < 11.0 mg/dL)           |
|                                              |          |                              |                                          |                                        |                                         |                                    |                                  |                                       |                                |                               |                               |                   |                              | Laboratory data at onset of bacteremia   |                               |                            |                                             |                               | Thrombocytopenia (platelet < 80,000/ul)    |
|                                              |          |                              |                                          |                                        |                                         |                                    |                                  |                                       |                                |                               |                               |                   |                              | C-reactive protein (mg/dL), median (IQR)  |                               |                            |                                             |                               | Metabolic acidosis                     |
|                                              |          |                              |                                          |                                        |                                         |                                    |                                  |                                       |                                |                               |                               |                   |                              |                                    |                               |                            |                                             |                               |                                    |
|                                              |          |                              |                                          |                                        |                                         |                                    |                                  |                                       |                                |                               |                               |                   |                              |                                    |                               |                            |                                             |                               |                                    |
|                                              |          |                              |                                          |                                        |                                         |                                    |                                  |                                       |                                |                               |                               |                   |                              |                                    |                               |                            |                                             |                               |                                    |

*Within the first 24 hours after blood culture sampling. **Indicating immature WBC ≥ 20% of total WBC

WBC: white blood cell, NTISS: Neonatal therapeutic intervention scoring system, IQR: interquartile range, 95% CI: 95% confidence interval
The outcome of inadequate empirically treated Gram-negative bacteremia

Table 3 showed the comparison of clinical outcomes between the patients with and without initially adequately treated bacteremias. Although the rates of persistent bacteremia were comparable, significantly more patients with inadequately treated bacteremias had prolonged feeding intolerance (> 3 days) and higher severity of illness at the third day of bacteremia (scored by NTISS). Besides, infants with Gram-negative bacteremias but without initially adequate antibiotic therapy had a significantly higher risk of progression to severe sepsis or septic shock (P = 0.002) and infectious complications (p < 0.001).

| Outcome comparisons of Gram-negative bacteremia treated with inadequate antibiotics versus adequate antibiotics |
|--------------------------------------------------|--------------------------------------------------|----------------|
| Persistent bacteremia*                          | Adequate treatment                                | P value       |
| (n = 75)                                         | (n = 301)                                         |               |
| 3 (4.0)                                          | 6 (2.0)                                           | 0.309         |
| Prolonged ileus and/or feeding intolerance (> 3 days) | 30 (40.0)                                        | < 0.001       |
| Progression to septic shock or severe sepsis¶   | 14 (18.7)                                         | 0.002         |
| NTISS scores at the third day of bacteremia, median (IQR) | 17.0 (12.0–20.0)                    | 0.010         |
| Infectious complications#                       | Adequate treatment                                | P value       |
| (n = 75)                                         | (n = 301)                                         |               |
| 19 (25.3)                                        | 28 (9.3)                                          | < 0.001       |
| Major organ damage                              | Adequate treatment                                | P value       |
| (n = 75)                                         | (n = 301)                                         |               |
| 15 (20.0)                                        | 20 (6.6)                                          | 0.001         |
| Newly infectious focus                          | Adequate treatment                                | P value       |
| Early mortality (within 7 days)                 | (n = 75)                                         | (n = 301)     |               |
| 8 (10.7)                                         | 13 (4.3)                                          | 0.046         |
| 9 (12.0)                                         | 23 (7.6)                                          | 0.250         |
| Overall mortality (within 30 days due to any reason) | 17 (22.7)                                        | 0.013         |
| 5 (6.7)                                          | 33 (11.0)                                         | 0.390         |

All data were expressed as number (percentage %), unless indicated otherwise
*Defined as two or more consecutive positive blood cultures with at least 48 hours apart
†Defined as newly infectious focus or major organ damage after bacteremias, but not occurred concomitantly. Some episodes had both newly infectious focus and major organ damage
¶Indicating bacteremias without septic shock as the initial presentation, but progressed to septic shock or severe sepsis 24 hours later
NTISS: Neonatal Therapeutic Intervention Scoring System, IQR: interquartile range

For bacteremias without initial adequate therapy, a total of nine patients died within

7 days, including 5 patients who died within 48 hours after bacteremia onset
without receiving any effective antimicrobial therapy. Infants without initially adequate antibiotic therapy did not have a significantly higher early mortality rate than those with adequately therapy, and the risk of recurrent bacteremia within one month was also comparable (Table 3). However, inadequately treated bacteremias had significantly higher overall mortality rate than adequately treated bacteremias (22.7% vs. 11.0%, \( P = 0.013 \)). This can also be confirmed by log rank test (\( p = 0.051 \)) [figure 1].

**Discussion**

Results from this study indicated that initially inadequate antimicrobial treatment was significantly associated with antibiotic resistant Gram-negative bacteria, mainly *P. aeruginosa* and ESBL producing bacteria. Previous antibiotic exposure to a 3rd generation cephalosporin was identified as the independent risk factor for initially inadequately treated bacteremias before blood culture results and antibiotic susceptibilities were available. The possible explanation could be that broad-spectrum cephalosporin exposure caused selection of drug-resistant bacteria, which further resulted in inadequately treated bacteremias if alternatively more effective antibiotics were not prescribed. The adverse outcomes were the significantly higher rates of infectious complications and overall mortality (within 30 days) than adequately treated patients.

Several factors have been found for inadequate treatment in other settings, e.g. hospital admission in the 90 days prior to the current admission, admission to surgery, nosocomial infection, polymicrobial infection [16, 24, 25], which were not found in the present study. In the present study, we found that antibiotic exposure to broad-spectrum cephalosporin was associated with a significantly higher risk of
infection caused by antibiotic-resistant bacteria, which was consistent with previous reports [26-29]. Although some other broad-spectrum antibiotics, such as carbapenem or fluoroquinolones [28, 30], are also found to cause antibiotic resistance in Gram-negative bacteria, these antibiotics were much less prescribed in our cohort. We found initially inadequately treated Gram-negative bacteremias seemed inevitable since it was difficult to differentiating non-resistant bacteremia from drug-resistant bacteremias by both the clinical presentations and laboratory data. Some other factors may exist and explain why these bacteremias were inadequately treated, including initial partial response of these inadequately treated bacteremias masked the requirement of antibiotic modification, or clinically worsening progression has been ignored by the clinicians.

The appropriateness of initial antibiotics depends on the empirical antibiotic prescribed and the local antibiotic susceptibility patterns. It seemed reasonable that the lower the rate of drug resistant organisms in the NICU, the lower the rate of initially inadequate antimicrobial therapy. Therefore, the issue how to reduce antimicrobial resistance in the ICU [31, 32] is important but not fully studied in the neonatal settings. For example, in contrast to previous studies that S. marcescens and A. baumannii causing bacteremias were multidrug resistant and associated with high mortality or morbidity in the NICU [33, 34], all of the S. marcescens and most of the A. baumannii isolates in our cohort were susceptible to aminoglycoside or cefotaxime, so they were not difficult to treat. The significantly higher mortality of inadequately treated Gram-negative bacteremias may partially explained by the pathogens themselves, and P. aeruginosa and ESBL-producing bacteria, the major causative organisms of this study, have been found to be more likely to cause fulminant illness or early mortality [35, 36].
In the present study, Gram-negative bacteremias without initially adequate antimicrobial therapy had a significantly higher risk of overall mortality, prolonged illness, progression to severe sepsis and infectious complications but not early mortality within seven days of onset. It seemed that in neonates, Gram-negative bacteremias without initially adequate antimicrobial therapy did not cause early mortality within seven days of onset but they caused a prolonged illness, major organs damage, infectious complications and then led to clinical deterioration, which subsequently resulted in final in-hospital mortality.

There are some limitations in this study. This study was observational and not randomized; therefore the choices of initial empirical antibiotic depended on attending physicians instead of the researchers. We failed to account for some important confounders that might influence the decision of attending physician in modifying antibiotics, such as the clinical progression on the first day of bacteremia. Besides, the data were derived from a single center; therefore our conclusions are necessarily limited in generalizability to other settings and institutions.

Conclusion

In conclusion, initial inappropriate antibiotic therapy in neonates with gram-negative bacteremia is associated with a significant higher risk of overall mortality and infectious complications. Early identification of neonatal sepsis caused by antibiotic resistant pathogens, such as ESBL producing gram-negative bacilli or Pseudomonas spp, may reduce the rate of inadequate antibiotic treatment, which can potentially improve the treatment outcomes.
Abbreviations
CI: confidence interval; CSF: cerebrospinal fluid; CVC: central venous catheter;
CGMH: Chang Gung Memorial Hospital; EOD: early-onset disease; ESBL: extended-spectrum β-lactamase; GNB: gram-negative bacteremia; GBS: Group B streptococcus;
LOD: late-onset disease; NTISS: Neonatal Therapeutic Intervention Scoring System;
OR: odds ratio; TPN: total parenteral nutrition

Declarations

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Availability of data and materials
The datasets used/or analyzed during the current study available from the corresponding author on reasonable request.

Authors’ contributions
Conceptualization: SMC, MHT, JFH. Data collection and verification: MYL, MHT, SMC, HRH, MCC, RHF. Formal analysis: CCL, MHT, SMC. Funding acquisition: JFH.
Investigation: CCL, MHT, MCC. Methodology: JFH, MYL, SMC, HRH. Supervision: MHT.
Writing—original drift: SMC and MHT. Writing—review & editing: MHT. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate
This study was approved by the institutional review board of Chang Gung Memorial Hospital, with a waiver of informed consent because all patients records and information were anonymized and de-identified prior to analysis.

Declaration

Consent for publication: not applicable

Competing interests

The authors declare that they have no competing interests

References

1. Gowda H, Norton R, White A, Kandasamy Y. Late-onset neonatal sepsis-A 10-year review from North Queensland, Australia. Pediatr Infect Dis J 2017;36(9):883-8.
2. Ouchenir L, Renaud C, Khan S, Bitnun A, Boisvert AA, McDonald J, et al. The epidemiology, management, and outcomes of bacterial meningitis in infants. Pediatrics 2017;140(1):e20170476.
3. Mohsen L, Ramy N, Saied D, Akmal D, Salama N, Abdel Haleim MM, et al. Emerging antimicrobial resistance in early and late-onset neonatal sepsis. Antimicrob Resist Infect Control 2017;6:63.
4. Tsai MH, Chu SM, Hsu JF, Lien R, Huang HR, Chiang MC, et al. Risk factors and outcomes for multidrug-resistant Gram-negative bacteremia in the NICU. Pediatrics 2014;133(2):e322-9.
5. Folgori L, Tersigni C, Hsia Y, Kortsalioudaki C, Heath P, Sharland M, et al. The relationship between Gram-negative colonization and bloodstream infections in neonates: a systemic review and meta-analysis. Clin Microbiol Infect 2018;24(3):251-7.
6. Wu IH, Tsai MH, Lai MY, Hsu LF, Chiang MC, Lien R, et al. Incidence, clinical
features, and implications on outcomes of neonatal late-onset sepsis with concurrent infectious focus. BMC Infect Dis 2017;17(1):465.

7. Hsu JF, Chu SM, Huang YC, Lien R, Huang HR, Lee CW, et al. Predictors of clinical and microbiological treatment failure in neonatal bloodstream infections. Clin Microbiol Infect 2015;21(5):482.e9-17.

8. Investigators of the Delhi Neonatal Infection Society (DeNIS) collaboration. Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centers in Delhi, India: a cohort study. Lancet Glob Health 2016;4(10):e752-60.

9. Dharmapalan D, Shet A, Yewale V, Sharland M. High reported rates of antimicrobial resistance in India neonatal and pediatric bloodstream infections. J Pediatric Infect Dis Soc 2017;6(3):e62-e68.

10. Gkentzi D, Kortsalioudaki C, Cailes BC, Zaoutis T, Kopsidas J, Tsolia M, et al. Epidemiology of infections and antimicrobial use in Greek Neonatal Units. Arch Dis Child Fetal Neonatal Ed. 2019;104(3):F293-F297.

11. Lacy MK, Stryjewski ME, Wang W, Hardin TC, Nogid B, Luke DR, et al. Telavancin hospital-acquired pneumonia trials: impact of Gram-negative infections and inadequate Gram-negative coverage on clinical efficacy and all-cause mortality. Clin Infect Dis 2015;61(Suppl 2):S87-93.

12. Fitzpatrick JM, Biswas JS, Edgeworth JD, Islam J, Jenkins N, Judge R, et al. Gram-negative bacteremia: a multi-center prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. Clin Microbiol Infect 2016;22(3):244-51.

13. Martoletti M, Giannella M, Lewis R, Caraceni P, Tedeschi S, Paul M, et al. A prospective multicentre study of the epidemiology and outcomes of bloodstream
infection in cirrhotic patients. Clin Microbiol Infect 2018;24(5):546.e1-e8.

14. Wang W, Jiang T, Zhang W, Li C, Chen J, Xiang D, et al. Predictors of mortality in bloodstream infections caused by multidrug-resistant gram-negative bacteria: 4 years of collection. Am J Infect Control. 2017;45(1):59-64.

15. Apisarnthanarak A, Holzmann-Pazgal G, Hamvas A et al. Antimicrobial use and the influence of inadequate empiric antimicrobial therapy on the outcomes of nosocomial bloodstream infections in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2004; 25: 735-41.

16. Tsai MH, Hsu JF, Chu SM, Lien R, Huang HR, Chiang MC, et al. Incidence, clinical characteristics and risk factors for adverse outcome in neonates with late-onset sepsis. Pediatr Infect Dis J 2014;33(1):e7-e13.

17. Sotto A, Lefrant JY, Fabbro-Peray P et al. Evaluation of antimicrobial therapy management of 120 consecutive patients with secondary peritonitis. J Antimicrob Chemother 2002; 50: 569-76.

18. Valles J, Rello J, Ochagavia A et al. Community-acquired bloodstream infection in critically ill adult patients: impact of shock and inappropriate antibiotic therapy on survival. Chest 2003; 123: 1615-24.

19. Gray JE, Richardson DK, McCormick MC et al. Neonatal therapeutic intervention scoring system: a therapy-based severity-of-illness index. Pediatrics 1992; 90: 561-7.

20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement M100-S22. CLSI, Wayne, PA, USA, 2012.

21. Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for
interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18: 268-81.

22. Avery's Diseases of the Newborn, 8th Edition. H. William Taeusch, Roberta A. Ballard, and Christine A. Gleason 2006.

23. Kermorvant-Duchemin E, Laborie S, Rabilloud M et al. Outcome and prognostic factors in neonates with septic shock. Pediatr Crit Care Med 2008; 9: 186 –91.

24. Harbarth S, Garbino J, Pugin J et al. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. Am J Med 2003; 115: 529-35.

25. McDonald JR, Friedman ND, Stout JE et al. Risk factors for ineffective therapy in patients with bloodstream infection. Arch Intern Med 2005; 165: 308-13.

26. Le J, Nguyen T, Okamoto M et al. Impact of empiric antibiotic use on the development of infection caused by extended-spectrum beta-lactamase bacteria in a neonatal intensive care unit. Pediatr Infect Dis J 2008; 27: 314-8.

27. Linkin DR, Fishman NO, Patel JB et al. Risk factors for extended-spectrum beta-lactamase-producing Enterobacteriaceae in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2004; 25: 781-3.

28. Ong DS, Jongerden IP, Buiting AG, et al. Antibiotic exposure and resistance development in Pseudomonas aeruginosa and Enterobacter species in intensive care unit. Crit Care Med 2011; 39: 2458-63.

29. Ye Y, Li JB, Ye DQ, Jiang ZJ. Enterobacter bacteremia: clinical features, risk factors for multiresistance and mortality in a Chinese University Hospital. Infection 2006; 34: 252-7.

30. Fraser A, Paul M, Almanasreh N et al. Benefit of appropriate empirical antibiotic treatment: thirty-day mortality and duration of hospital stay. Am J Med 2006; 119:
31. Marra AR, de Almeida SM, Correa L et al. The effect of limiting antimicrobial therapy duration on antimicrobial resistance in the critical care setting. *Am J Infect Control* 2009; 37: 204-9.

32. Cook PP, Catrou PG, Christie JD et al. Reduction in broad-spectrum antimicrobial use associated with no improvement in hospital antibiogram. *J Antimicrob Chemother* 2004; 53: 853-9.

33. Maltezou HC, Tryfinopoulou K, Katerelos P et al. Consecutive *Serratia marcescens* multiclonal outbreaks in a neonatal intensive care unit. *Am J Infect Control* 2012; 40: 637-42.

34. Simmonds A, Munoz J, Aguero-Rosenfeld M et al. Outbreak of *Acinetobacter* infection in extremely low birth weight neonates. *Pediatr Infect Dis J* 2009; 28: 210-4.

35. Makhoul IR, Sujov P, Smolkin T et al. Pathogen-specific early mortality in very low birth weight infants with late-onset sepsis: a national survey. *Clin Infect Dis* 2005; 40: 218-24.

36. Abdel-Hady H, Hawas S, El-Daker M, El-Kady R. Extended-spectrum betalactamase producing *Klebsiella pneumoniae* in neonatal intensive care unit. *J Perinatol* 2008; 28: 685-90.

**Figures**
Figure 1

Survival following onset of gram-negative sepsis from neonates in the NICU. The I

Declarations

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Availability of data and materials
The datasets used/or analyzed during the current study available from the corresponding author on reasonable request.

Authors’ contributions

Conceptualization: SMC, MHT, JFH. Data collection and verification: MYL, MHT, SMC, HRH, MCC, RHF. Formal analysis: CCL, MHT, SMC. Funding acquisition: JFH. Investigation: CCL, MHT, MCC. Methodology: JFH, MYL, SMC, HRH. Supervision: MHT. Writing—original drift: SMC and MHT. Writing—review & editing: MHT. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

This study was approved by the institutional review board of Chang Gung Memorial Hospital, with a waiver of informed consent because all patients records and information were anonymized and de-identified prior to analysis.

Declaration

Consent for publication: not applicable

Competing interests

The authors declare that they have no competing interests

References

1. Gowda H, Norton R, White A, Kandasamy Y. Late-onset neonatal sepsis-A 10-year review from North Queensland, Australia. Pediatr Infect Dis J 2017;36(9):883-8.
2. Ouchenir L, Renaud C, Khan S, Bitnun A, Boisvert AA, McDonald J, et al. The epidemiology, management, and outcomes of bacterial meningitis in infants. Pediatrics 2017;140(1):e20170476.
3. Mohsen L, Ramy N, Saied D, Akmal D, Salama N, Abdel Haleim MM, et al. Emerging antimicrobial resistance in early and late-onset neonatal sepsis. Antimicrob Resist Infect
4. Tsai MH, Chu SM, Hsu JF, Lien R, Huang HR, Chiang MC, et al. Risk factors and outcomes for multidrug-resistant Gram-negative bacteremia in the NICU. Pediatrics 2014;133(2):e322-9.

5. Folgori L, Tersigni C, Hsia Y, Kortsalioudaki C, Heath P, Sharland M, et al. The relationship between Gram-negative colonization and bloodstream infections in neonates: a systemic review and meta-analysis. Clin Microbiol Infect 2018;24(3):251-7.

6. Wu IH, Tsai MH, Lai MY, Hsu LF, Chiang MC, Lien R, et al. Incidence, clinical features, and implications on outcomes of neonatal late-onset sepsis with concurrent infectious focus. BMC Infect Dis 2017;17(1):465.

7. Hsu JF, Chu SM, Huang YC, Lien R, Huang HR, Lee CW, et al. Predictors of clinical and microbiological treatment failure in neonatal bloodstream infections. Clin Microbiol Infect 2015;21(5):482.e9-17.

8. Investigators of the Delhi Neonatal Infection Society (DeNIS) collaboration. Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centers in Delhi, India: a cohort study. Lancet Glob Health 2016;4(10):e752-60.

9. Dharmapalan D, Shet A, Yewale V, Sharland M. High reported rates of antimicrobial resistance in India neonatal and pediatric bloodstream infections. J Pediatric Infect Dis Soc 2017;6(3):e62-e68.

10. Gkentzi D, Kortsalioudaki C, Cailes BC, Zaoutis T, Kopsidas J, Tsolia M, et al. Epidemiology of infections and antimicrobial use in Greek Neonatal Units. Arch Dis Child Fetal Neonatal Ed. 2019;104(3):F293-F297.

11. Lacy MK, Stryjewski ME, Wang W, Hardin TC, Nogid B, Luke DR, et al. Telavancin hospital-acquired pneumonia trials: impact of Gram-negative infections and inadequate Gram-negative coverage on clinical efficacy and all-cause mortality. Clin Infect Dis 2015;61(Suppl 2):S87-93.

12. Fitzpatrick JM, Biswas JS, Edgeworth JD, Islam J, Jenkins N, Judge R, et al. Gram-negative
bacteremia: a multi-center prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. Clin Microbiol Infect 2016;22(3):244-51.

13. Martoletti M, Giannella M, Lewis R, Caraceni P, Tedeschi S, Paul M, et al. A prospective multicentre study of the epidemiology and outcomes of bloodstream infection in cirrhotic patients. Clin Microbiol Infect 2018;24(5):546.e1-e8.

14. Wang W, Jiang T, Zhang W, Li C, Chen J, Xiang D, et al. Predictors of mortality in bloodstream infections caused by multidrug-resistant gram-negative bacteria: 4 years of collection. Am J Infect Control. 2017;45(1):59-64.

15. Apisarnthanarak A, Holzmann-Pazgal G, Hamvas A et al. Antimicrobial use and the influence of inadequate empiric antimicrobial therapy on the outcomes of nosocomial bloodstream infections in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2004; 25: 735-41.

16. Tsai MH, Hsu JF, Chu SM, Lien R, Huang HR, Chiang MC, et al. Incidence, clinical characteristics and risk factors for adverse outcome in neonates with late-onset sepsis. Pediatr Infect Dis J 2014;33(1):e7-e13.

17. Sotto A, Lefrant JY, Fabbro-Peray P et al. Evaluation of antimicrobial therapy management of 120 consecutive patients with secondary peritonitis. J Antimicrob Chemother 2002; 50: 569-76.

18. Valles J, Rello J, Ochagavia A et al. Community-acquired bloodstream infection in critically ill adult patients: impact of shock and inappropriate antibiotic therapy on survival. Chest 2003; 123: 1615-24.

19. Gray JE, Richardson DK, McCormick MC et al. Neonatal therapeutic intervention scoring system: a therapy-based severity-of-illness index. Pediatrics 1992; 90: 561-7.

20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement M100-S22. CLSI, Wayne, PA,
USA, 2012.

21. Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18: 268-81.

22. Avery's Diseases of the Newborn, 8th Edition. H. William Taeusch, Roberta A. Ballard, and Christine A. Gleason 2006.

23. Kermorvant-Duchemin E, Laborie S, Rabilloud M et al. Outcome and prognostic factors in neonates with septic shock. Pediatr Crit Care Med 2008; 9: 186 –91.

24. Harbarth S, Garbino J, Pugin J et al. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. Am J Med 2003; 115: 529-35.

25. McDonald JR, Friedman ND, Stout JE et al. Risk factors for ineffective therapy in patients with bloodstream infection. Arch Intern Med 2005; 165: 308-13.

26. Le J, Nguyen T, Okamoto M et al. Impact of empiric antibiotic use on the development of infection caused by extended-spectrum beta-lactamase bacteria in a neonatal intensive care unit. Pediatr Infect Dis J 2008; 27: 314-8.

27. Linkin DR, Fishman NO, Patel JB et al. Risk factors for extended-spectrum beta-lactamase-producing Enterobacteriaceae in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2004; 25: 781-3.

28. Ong DS, Jongerden IP, Buiting AG, et al. Antibiotic exposure and resistance development in Pseudomonas aeruginosa and Enterobacter species in intensive care unit. Crit Care Med 2011; 39: 2458-63.

29. Ye Y, Li JB, Ye DQ, Jiang ZJ. Enterobacter bacteremia: clinical features, risk factors for multiresistance and mortality in a Chinese University Hospital. Infection 2006; 34: 252-7.

30. Fraser A, Paul M, Almanasreh N et al. Benefit of appropriate empirical antibiotic treatment:
30. Thirty-day mortality and duration of hospital stay. *Am J Med* 2006; 119: 970-6.

31. Marra AR, de Almeida SM, Correa L et al. The effect of limiting antimicrobial therapy duration on antimicrobial resistance in the critical care setting. *Am J Infect Control* 2009; 37: 204-9.

32. Cook PP, Catrou PG, Christie JD et al. Reduction in broad-spectrum antimicrobial use associated with no improvement in hospital antibiogram. *J Antimicrob Chemother* 2004; 53: 853-9.

33. Maltezou HC, Tryfinopoulou K, Katerelos P et al. Consecutive *Serratia marcescens* multiclone outbreaks in a neonatal intensive care unit. *Am J Infect Control* 2012; 40: 637-42.

34. Simmonds A, Munoz J, Aguero-Rosenfeld M et al. Outbreak of *Acinetobacter* infection in extremely low birth weight neonates. *Pediatr Infect Dis J* 2009; 28: 210-4.

35. Makhoul IR, Sujov P, Smolkin T et al. Pathogen-specific early mortality in very low birth weight infants with late-onset sepsis: a national survey. *Clin Infect Dis* 2005; 40: 218-24.

36. Abdel-Hady H, Hawas S, EI-Daker M, EI-Kady R. Extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in neonatal intensive care unit. *J Perinatol* 2008; 28: 685-90.

**Figures**
Figure 1

Survival following onset of gram-negative sepsis from neonates in the NICU. The Kaplan-Meier graph shows the survival rate over days after onset of gram-negative sepsis, stratified by appropriate antibiotic treatment within 24 hours vs. delay in appropriate antibiotic use.