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Original Article

Multidrug-resistant Gram-negative bacilli sepsis from a neonatal intensive care unit: a case-case-control study

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Abstract

Introduction: To identify the risks and outcomes for multidrug-resistant Gram-negative bacilli (MDRGNB) sepsis in neonates.
Methodology: This was a retrospective case-case-control study between 1991 and 2016. The control group was selected from the same source records of all neonates with clinical or suspected sepsis but not culture-proven.
Results: The numbers of patients in the MDRGNB sepsis, non-MDRGNB sepsis, and control groups were 157, 88, and 218, respectively. MDRGNB sepsis was significantly associated with outborn infants [adjusted odds ratio (aOR) 2.08; p = 0.003] and infants who had a neurologic sequela (aOR 11.58; p = 0.04), lower gestational age (p = 0.03) or previous aminoglycoside use (aOR 2.43; p < 0.001) compared with the control group. Non-MDRGNB sepsis was associated with outborn infants (aOR 2.63; p < 0.001), and infants who had neurologic sequelae (aOR 48.25; p = 0.001) and previous cephalosporin use (aOR 6.28; p < 0.001) or cefoperazone plus sulbactam use (aOR 6.48; p = 0.02) compared with the control group. Case fatality (OR 3.63; p < 0.001) and septic shock (OR 12.81; p < 0.001) rates, length of stay (p < 0.001), and daily hospital costs (p = 0.01) were higher in the MDRGNB sepsis group than in the control group.
Conclusions: Smaller preterm neonate with previous aminoglycoside use had a higher MDRGNB than non-MDRGNB sepsis compared with the control group. Intervention to reduce MDRGNB sepsis in the NICU is cost-effective.

Key words: bacteremia; Gram-negative bacteria; meningitis; multiple drug resistance; neonatal sepsis; neonate.

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Introduction

Both neonates and preterm infants are more susceptible to sepsis (bacteremia or meningitis) because their immune systems are less well developed which leads in higher mortality and morbidity than in children or adults. The long-term sequelae of sepsis are also troublesome, including higher incidence rates of both Gram-negative bacilli (GNB) bacteremia [1] and drug resistance [2] which have been increasing in neonatal intensive care units (NICUs) in developing countries.

The current practice is to use empirical broad spectrum antimicrobials (BSA) in the fragile preterm or surgically-assisted neonate who is immunocompromised or has subtle presentations of sepsis or both. Adequate antimicrobial treatment in neonatal sepsis can reduce case the fatality rate (CFR). However, antimicrobial-resistant organisms may colonize from antibiotic selective pressure due to frequent and prolonged BSA use. The emergence of multidrug-resistant GNB (MDRGNB) sepsis is particularly worrisome due to growing off-label use of antibiotics such as meropenem and colistin [3]. On the other hand, there are higher rates of fatality in neonates who receive inadequate empiric antimicrobial therapy in high MDR areas [4] which creates something of a conundrum for physicians in such settings.

There is very little information on the risk factors, types of organisms, and outcomes in neonatal MDRGNB sepsis, including the increasing incidence of BSA resistance which is creating a growing therapeutic challenge in NICUs. To gain a better understanding of this problem and possible ways of dealing with it, we performed a case-case-control study to evaluate the potential risk factors and outcomes in neonatal MDRGNB sepsis.

Methodology

Setting and care practice

This study was conducted at the NICU of Songklanagarind Hospital, Songkhla, Thailand. The

NICU is a 15-bed, level III, single room in a university-affiliated teaching hospital, which is also the major tertiary care and referral center in southern Thailand. There are approximately 3000-3500 live births at the hospital yearly, with about 450-550 neonates admitted to the NICU. When early onset sepsis (EOS) develops, a neonate is started on empirical therapy with ampicillin and gentamicin. Cefotaxime or ceftazidime and amikacin are used for late-onset sepsis (LOS). Further adjustments in the antibiotic regimens are based on antimicrobial susceptibility results or if the infant's clinical status does not improve within 48–72 hours in the judgment of the attending neonatologist.

Study design

This was a retrospective case-case-control study. The case-case-control study design can be used to identify risk factors for resistant organisms more accurately and with fewer flaws than the case-control study design [5]. This design uses two separate case-control analyses within a single study: the first analysis compared case patients infected with MDRGNB (resistant cases) with control patients; and the second compared case patients infected with non-MDRGNB (susceptible cases) with control patients. Control patients were patients without infection caused by the GNB and selected from the source population [5].

Patients and data collection

The medical records of all neonates with Gram-negative sepsis, as identified from the hospital's clinical microbiological laboratory database, contracted at any time during admission to the NICU from January 1991 to December 2016, were included in the study.

The inclusion criteria were all patients admitted to the NICU during the defined study period with positive GNB cultures including all species of *Enterobacteriaceae* and some non-*Enterobacteriaceae* species (*Pseudomonas aeruginosa* and *Acinetobacter* spp.) from blood or cerebrospinal fluid (CSF) culture or both. The case-case patients were all neonates with positive blood or CSF culture or both with MDRGNB or non-MDRGNB. The control group was selected from the same source records of all neonates with clinical or suspected sepsis but without a proven culture and from whom blood or CSF culture or both had been obtained 1 day prior to, the same day, and 1 day after the date of positive cultures of all identified case patients.

After the study subjects were identified through the microbiological laboratory database, their hospital records were reviewed. The risk factors for both case and control patients were abstracted and evaluated from the time of admission until the onset of sepsis. The study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University with a waiver of informed consent.

The data were categorized by the numbers of septic patients, episodes of sepsis, and pathogenic organisms. Gestational age (GA), birth weight, sex, location of birth (inborn or outborn), mode of delivery, Apgar scores, CFR, length of stay, and hospital costs were taken from the medical records of the septic patients. Other variables were reported by the episodes of sepsis. A new episode of sepsis was defined as sepsis with additional pathogens or the same pathogens reoccurring 14 days or more following recovery from the previous episode. The percentages of susceptibility of each pathogenic organism were recorded.

Definitions

A MDR organism was defined as an isolate that was non-susceptible to at least 1 agent in at least 3 antimicrobial classes. These antimicrobial classes included aminoglycosides, carbapenems, fluoroquinolones, non-extended-spectrum cephalosporins, extended-spectrum cephalosporins, cephamycins, folate pathway inhibitors, glycylcyclines, monobactams, penicillins, penicillins/beta-lactamase inhibitors, phenicols, phosphonic acids, polymyxins and tetracyclines [6]. At least 1 mL of blood specimen was obtained under sterile conditions and processed in an automatic blood culture machine (BacT/Alert™ or BACTEC FX™). Uncentrifuged CSF specimens were inoculated onto each of one 5% sheep blood plate and one chocolate agar plate and 1.0 mL was inoculated into 5 mL of brain heart infusion broth with X and V factors. Agar plates were incubated at 35°C in 5% carbon dioxide and examined daily for 2 days. Broth cultures were incubated at 35°C and examined daily for 5 days. Susceptibility testing of each patient was performed by the disk diffusion method (zone diameter interpretive criteria) according to the last updated editions of Clinical and Laboratory Standards Institute (CLSI) guidelines [7].

EOS and LOS were defined as positive blood or CSF culture or both before or after 72 hours after birth, respectively. Cardiovascular disease was defined as congenital complicated heart disease, cyanotic heart disease or acyanotic heart disease with heart failure signs. Bronchopulmonary dysplasia or chronic lung disease was defined as a neonate who needed oxygen therapy for at least 28 days [8]. Pulmonary hypertension or cor pulmonale or both in an infant were diagnosed by either clinical signs or echocardiography

or both. Congenital gastrointestinal (GI) tract pathology was defined as esophageal atresia, pyloric stenosis, small or large bowel obstruction, omphalocele or gastroschisis or a combination of these. A low Apgar score was defined as less than 8 at 5 minutes of life.

Previous antibiotic exposure was defined as intravenous antibiotic use for at least 72 hours before obtaining the culture. The criteria for the diagnosis of ventilator-associated pneumonia (VAP) and central line (CL)-associated bloodstream infection followed the Centers for Disease Control and Prevention and National Healthcare Safety Network guidelines for infants < 1 year old [9-12]. If the diagnosis/treatment occurred in the preceding 7 days before the onset of sepsis, invasive mechanical ventilation, VAP, total parenteral nutrition, use of CL, and central line-associated bloodstream infection were considered as risk factors. CL was defined as an umbilical arterial/venous catheter or catheter inserted by the cutdown technique before or on the date of either positive blood culture or CSF culture or both. Septic shock was defined as sepsis plus tachycardia, hypotension (GA–dependent) or reduced peripheral perfusion evidence and needing vasopressor agents to maintain blood pressure within 48 hours after sepsis onset [13].

Inadequate empiric antimicrobial therapy was defined as the use of antibiotics for more than 48 hours after the day that either blood culture or CSF culture or both were performed that did not cover the microorganisms causing the sepsis or administration of antibiotics that failed to cover resistant microorganisms. Inadequate antimicrobial therapy included the absence of a prescribed antimicrobial agent directed against the specific class of either recovered microorganisms or administration of antimicrobial agents or both to which the microorganism responsible for the infection was resistant. A microbiological cure was defined as either successive negative blood cultures or CSF cultures or both within 7 days from the time the original pathogen was isolated from either a positive blood culture or CSF culture or both. Length of stay and CFR were defined as the duration of admission until discharge from Songklanagarind Hospital and the rate of crude mortality after onset of sepsis, respectively.

Statistical analysis

The R program was used to develop a database of categorical and continuous variables. Categorical variables were analyzed as frequency and percentage and compared using the χ^2 test or Fisher's exact test. Continuous (nonparametric) variables were analyzed as median (interquartile range [IQR]) and compared using the Mann-Whitney U-test. All p-values are 2-tailed and p-values less than 0.05 indicate statistical significance. Univariate and multivariate analyses were performed. Variables with $p < 0.2$ in univariate analysis or variables that had a priori clinical significance (e.g., exposure to antimicrobial regimens, and types of CL) were entered into backward stepwise logistic regression models in multivariate analysis. The model with the lowest Akaike information criteria was judged as the most parsimonious model. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were computed for significant variables independently associated with MDRGNB versus non-MDRGNB versus control group. Risk factors associated with death in MDRGNB sepsis were analyzed.

Results

Between 1991 and 2016 there were 74,944 inborn live births and 9,908 NICU admissions in our institution. Overall during the 26 years, the numbers of GNB sepsis patients, episodes, and organisms were 245, 271, and 320, respectively. The numbers of inborn neonates with MDRGNB and non-MDRGNB sepsis were 93 and 43, respectively. The incidences of GNB and MDRGNB sepsis in inborn neonates were 1.8 and 1.2 per 1000 live births, respectively. The numbers of patients (episodes) who developed sepsis in the MDRGNB, non-MDRGNB, and control groups were 157 (174), 88 (97), and 218 (218), respectively. The median (IQR) GAs and birth weights of neonates with GNB sepsis were 33 (9) weeks and 1,767.5 (1,690) grams, respectively. The CFRs in the GNB and MDRGNB sepsis groups were 33.5% (82/245) and 37.6% (59/157), respectively. From the analysis of the episodes, the rate of MDRGNB sepsis in bacteremia cases (169/256, 66.0%) was significantly higher than in meningitis cases (5/15, 33.3%) (OR 3.89; 95% CI: 1.29-11.72; $p = 0.01$). From the analysis of the organisms, the numbers (percentages) of MDRGNB and non-MDRGNB organisms were 212 (66.3%) and 108 (33.8%), respectively. The top 5 pathogenic organisms (MDRGNB/non-MDRGNB) were *Klebsiella pneumoniae* (83/15), *Acinetobacter baumannii* (57/38), *Escherichia coli* (28/17), *Enterobacter cloacae* (28/3), and *P. aeruginosa* (5/22). The rate of MDRGNB was significantly higher during 2004-2016 (153/211, 72.5%) compared with 1991-2003 (59/109, 54.1%) ($p = 0.001$). The minimum, 5^{th}, 10^{th}, and 50^{th} percentiles of onset of MDRGNB sepsis were 0.2, 1.3, 2.2, and 8.9 days after birth, respectively.

Population characteristics (patient data analysis) and risk factors (episode data analysis) compared between the MDRGNB sepsis, non-MDRGNB sepsis, and control groups are shown in Table 1. Multivariate analysis found that MDRGNB sepsis was more likely to be found in infants who had a significantly lower GA, were outborn, who had congenital or acquired neurologic sequelae, and had previous aminoglycoside exposure compared with the control group (Table 2). Multivariate analysis also indicated that non-MDRGNB sepsis was more likely in infants who were outborn, who had congenital or acquired neurologic sequelae, and previous cephalosporin (cefotaxime or ceftazidime, or cefoperazone plus sulbactam) exposure, while the infants in this group were less likely to have pulmonary hypertension compared with the control group (Table 2). In GNB sepsis, outborn neonates had significantly higher rates of congenital GI tract pathology (OR 4.98; 95% CI: 2.42-10.20; $p < 0.001$), previous surgery (OR 4.18; 95% CI: 2.10-8.33; $p < 0.001$), VAP (OR 3.48; 95% CI: 1.30-9.35; $p = 0.01$), and cutdown procedure (OR 2.58; 95% CI: 1.14-5.85; $p = 0.02$) than inborn neonates.

The results of the univariate analyses of the outcomes in the patients with MDRGNB sepsis, non-MDRGNB sepsis, and control group are shown in Table 3.

Table 1. Baseline characteristics and risk factors of multidrug-resistant (MDR), non-MDR Gram-negative bacilli (GNB) sepsis, and control groups.

Characteristic analysed by persons	**MDRGNB (n = 157), %**	**Non-MDRGNB (n = 88), %**	**Control (n = 218), %**
Gestational age (week)*	32 (8)	37 (9)	34 (9)
less than 37 weeks	75.2	43.2	62.4
less than 32 weeks	49.0	25.0	34.4
less than 28 weeks	204.	11.4	9.6
Birthweight (g)*	1570 (1500)	2316 (1605)	2010 (1690)
less than 1,500 g	47.1	28.4	33.5
less than 1,000 g	24.2	13.6	12.4
Appropriate for gestational age	79.0	80.7	79.4
Male	58.0	60.2	60.1
Outborn	40.7	51.1	30.4
Vaginal delivery	45.9	56.8	50.5
Low Apgar score at 5 minutes	29.9	23.9	22.9
Risks analysed by episodes	**MDRGNB (n = 174), %**	**Non-MDRGNB (n = 97), %**	**Control (n = 218), %**
Weight at onset of sepsis (g)*	1750 (1650)	2785 (943)	1910 (1650)
Age at onset of sepsis (d)*	8.87 (18.28)	8.86 (12.15)	4.03 (12.47)
Late-onset sepsis	86.8	89.7	45.9
Underlying chronic condition			
Neurologic sequelae, congenital or acquired	9.2	12.3	1.8
Cardiovascular disease	37.9	36.1	27.5
Bronchopulmonary dysplasia	12.1	11.3	12.8
Pulmonary hypertension	4.6	5.2	10.6
Congenital gastrointestinal tract pathology	18.4	21.6	11.5
Previous antibiotic exposure	77.6	70.1	39.5
Cefotaxime, ceftazidime	43.1	42.3	13.3
Cefoperazone plus sulbactam	12.1	17.5	5.5
Carbapenem	17.8	20.6	6.0
Aminoglycoside	78.7	63.9	35.8
Previous surgery	19.0	24.7	12.8
Invasive mechanical ventilation†	71.8	63.9	56.9
Ventilator-associated pneumonia†	10.3	7.2	2.8
Use of total parenteral nutrition†	66.7	55.7	43.6
Use of central line (CL), % (n)	52.1 (86/165)	45.7 (42/92)	33.8 (73/216)
Umbilical arterial catheter	32.3 (53/164)	22.2 (20/90)	25.0 (54/216)
Umbilical venous catheter	29.8 (48/161)	20.7 (19/92)	16.2 (35/216)
Cutdown	13.3 (20/150)	19.1 (17/89)	5.1 (11/216)
CL-associated bloodstream infection, % (n)†	7.4 (12/162)	4.6 (4/87)	0.5 (1/216)
Duration of CL use before sepsis (d)*	6 (8)	6 (9)	1 (7)

*median (IQR), †within 7 days before or on date of sepsis

Table 2. Risk factors for multidrug-resistant (MDR) and non-MDR Gram-negative bacilli (GNB) acquisition by multivariate analysis.

Risk Factor	MDRGNB vs control		Non-MDRGNB vs control	
	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Gestational age (weeks)	0.92 (0.87-0.96)	< 0.001		
Outborn	2.08 (1.30-3.45)	0.003	2.63 (1.56-4.35)	< 0.001
Underlying chronic condition				
Neurologic sequelae, congenital or acquired	11.58 (1.11-120.46)	0.04	48.25 (4.47-520.75)	0.001
Pulmonary hypertension			0.17 (0.03-0.85)	0.03
Previous antibiotic exposure				
Cefotaxime, ceftazidime	2.65 (0.90-7.77)	0.06	6.28 (2.26-17.45)	< 0.001
Cefoperazone plus sulbactam			6.48 (1.43-29.32)	0.02
Carbapenem	2.65 (0.91-7.74)	0.08		
Aminoglycoside	2.43 (1.07-5.53)	0.03		
Use of central line (CL)				
Umbilical arterial catheter	1.99 (0.88-4.50)	0.10		
Cutdown	2.73 (0.90-8.25)	0.08		

aOR (95% CI) = adjusted odds ratio (95% confidence interval).

Table 3. Outcomes of patients with multidrug-resistant (MDR), non-MDR Gram-negative bacilli (GNB) sepsis, and control group.

Outcome	MDRGNB (n = 157)	Non-MDRGNB (n = 88)	Control (n = 218)
Septic shock, %	31.8	11.4	3.7
Case fatality rate, %	37.6	26.1	14.2
Length of stay in survivors (d), median (IQR)	55 (47)	45 (49)	28 (54)
Daily hospital cost ($US), median (IQR)	169.64 (170.27)	158.05 (199.15)	135.83 (111.81)
Daily hospital cost more than 150 $US, % (n)	57.7 (64/111)	53.5 (23/43)	43.6 (61/140)

Table 4. Univariate and multivariate analyses of risk factors associated with death in multidrug-resistant Gram-negative bacilli sepsis.

Risk Factor	Univariate analysis			Multivariate analysis	
	Non-survivors (n = 59), %	Survivors (n = 98), %	p-value	aOR (95% CI)	p-value
Gestational age (week)*	30.5 (18)	32.0 (8)	0.06		
less than 37 weeks	84.7	69.4	0.01		
Birthweight (g)*	1305.0 (1539)	1677.5 (1380)	0.049		
less than 1,000 g	30.5	20.4	0.12		
Female	59.3	31.6	0.001		
Small for gestational age	72.9	82.7	0.28		
Date of onset of sepsis (days)*	4.86 (16.17)	8.26 (11.68)	0.001		
Early-onset sepsis	27.1	6.1	< 0.001	5.0 (1.23-16.67)	0.02
Low Apgar score at 5 min	47.5	19.4	< 0.001		
History of pulmonary hypertension	8.5	3.1	0.13		
Septic shock	66.7	12.2	< 0.001	5.07 (1.43-17.94)	0.01
Invasive mechanical ventilation†	83.0	65.3	0.01		
Use of central line†	61.0	37.8	0.003		
Umbilical arterial catheter†	50.8	13.4	< 0.001	3.74 (0.96-14.05)	0.06
Umbilical venous catheter†	35.6	24.5	0.13		
Cut down†	15.3	7.1	0.09	16.98 (3.36-85.90)	0.001
Inadequate empirical antibiotic therapy	40.7	31.6	0.14		
Inadequate antibiotic therapy	23.7	10.2	0.006		
Microbiological cure, % (n)	64.1 (25/39)	97.8 (90/92)	< 0.001	0.05 (0.01-0.33)	0.002

*median (IQR), †within 7 days before or on date of sepsis, aOR (95% CI) = adjusted odds ratio (95% confidence interval).

CFR (OR 3.63; 95% CI: 2.21-5.98; p < 0.001), septic shock (OR 12.81; 95% CI: 5.85-28.02; p < 0.001), length of stay in survivors (p < 0.001), daily hospital cost (p = 0.01), and daily hospital cost more than 150 $US (OR 1.76; 95% CI: 1.07-2.92; p = 0.03) in the MDRGNB sepsis group were significantly higher than in the control group. Univariate analysis also found that CFR (OR 2.13; 95% CI: 1.16-3.92; p = 0.01), septic shock (OR 3.58; 95% CI: 1.36-9.41; p = 0.006), and length of stay in survivors (p = 0.004) were significantly higher in the non-MDRGNB sepsis group than in the control group.

The univariate and multivariate analyses of the risk factors associated with death in MDRGNB sepsis are shown in Table 4. The multivariate analysis found that nonsurvivors were more likely to have had EOS, septic shock, and a cutdown procedure, and less likely to have had a microbiological cure attempted compared with the survivors. The top 3 GNB organisms that caused death were *P. aeruginosa* (13/27, 48%), *E. coli* (20/45, 44%), and *A. baumannii* (34/95, 36%). Similarly, the top 3 MDRGNB organisms that caused death were *P. aeruginosa* (5/5, 100%), *E. coli* (17/28, 61%), and *A. baumannii* (25/57, 44%).

Discussion

Some implications can be drawn from our study. First, understanding the risk factors of MDRGNB sepsis is very important because neonatologists and pediatricians must be aware of these factors when considering their choice of antimicrobials. In this study, the major risk factors for MDRGNB sepsis were outborn neonates, lower GA, history of neurological defect, and aminoglycoside use before sepsis. Second, when to start and step up to a more BSA treatment must be considered. Our current study found that EOS increased the risk of mortality in MDRGNB sepsis patients. The cut-off point between EOS and LOS is still controversial (48 hours, 72 hours, 3 days or 7 days) [14]. Most researchers use 72 hours as the cut-off point after which the risk of infection increases from environmental colonization [15]. If we start or step up to BSA after 72 hours of life, there seems to be a delay and ineffective treatment. Accordingly, we suggest a cut-off point of 48 hours (the 10th percentile onset of MDRGNB sepsis) as the appropriate time to start or step up to BSA, especially in cases of septic shock or in a moribund neonate in a high MDR area. Finally, in our study the crude excess length of stay and daily hospital costs of MDRGNB sepsis patients in the non-MDRGNB sepsis and control groups were 10 and 27 days, and 12 and 34 $US, respectively (Table 3). Several studies have found that patients with an MDRGNB infection had poorer outcomes than non-MDRGNB and control groups [3,16,17]. Regarding antibiotic stewardship programs, one study found such programs significantly reduced the incidence of infections and colonizations by MDRGNB (51% reduction; incidence ratio, 0.49; 95% CI: 0.35-0.68) [18]. Our study is further confirmation that the rate of MDRGNB sepsis is increasing in developing countries [3,12,17], and physicians and other health personnel should become ever more vigilant concerning prevention and infection control.

Our study found that MDRGNB sepsis was more likely to be found in infants who had a significantly lower GA, were outborn, who had congenital or acquired neurologic sequelae, and had previous aminoglycoside exposure compared with the control group. Unsurprisingly, neonates with a lower GA or prior antimicrobial use (third-generation cephalosporins and aminoglycosides) have been found to have a higher risk of MDRGNB sepsis or ESBL-gram-negative bacteremia [4,19-22]. Multivariate analysis in one of these studies found that outborn neonates were at higher risk for ESBL-Gram negative bacteremia compared with the control group (aOR 2.23; p = 0.01) [22]. However, another study found that outborn neonates were at a higher risk of MDRGNB sepsis only in the univariate analysis (p = 0.005), but found no difference in the multivariate analysis (p = 0.1) [4]. The current study found that outborn neonates had more congenital GI pathologies, previous surgeries, VAP events, and cutdown procedures than inborn neonates. Because our institute is a referral center for GI and neurological surgery for the large area of southern Thailand, some neonates received BSA during both pre- and post-operative care with prolonged endotracheal tube and central line use [23]. Consequently, colonization of antimicrobial-resistant organisms may flourish in GI and respiratory reservoirs in neonates [24,25]. Longer length of stay was associated with higher rates of antimicrobial use [26] and MDRGNB colonization [27]. However, our study found no difference in duration from admission until onset of sepsis between the MDRGNB and non-MDRGNB sepsis groups (8.9 days in both groups) (Table 1). Therefore, from the data in our study, we suggest that the mechanisms of antibiotic-induced resistance may not develop in a neonate but MDR organisms from the environment in an NICU may colonize and cause sepsis at higher rates in MDR areas.

A recent study reported that the prevalence (average 30%) and incidence density of MDRGNB colonization

showed an upward trend in Italy [27]. Our study showed a high percentage of MDRGNB episodes of sepsis (64.2%, 174/271). However, this incidence was lower than a study from Egypt (83.4%) but the data in that study were not clear because there was no clear definition of MDR sepsis [28]. The data from studies in Taiwan (18.6%) [4] and Hungary (including both neonatal and pediatric ICUs, 33.6%) [29] were much lower compared to our study. We found in our study that the CFR in MDRGNB sepsis was 37.6%, which again was lower than the data from Egypt (79%) [28] but higher than the reported data from Taiwan (28.6%) [4] and Hungary (24.4%) [29]. Reports from Egypt, Taiwan, Hungary, and this study found a correlation between the incidence rate of MDRGNB and the CFR. In this current study, EOS, septic shock, use of catheter from a cutdown procedure and absence of microbiological cure were the risk factors for death in MDRGNB patients. In the previous study noted above from Taiwan, the presence of infectious complications after bacteremia and underlying secondary pulmonary hypertension were identified as independent risk factors for overall CFR [4]. The most common organism in our study that caused death in both MDRGNB and non-MDRGNB sepsis was *P. aeruginosa*, which was similar to previous neonatal [28,30] and pediatric [29] sepsis reports.

This study had some limitations. First, there were changes in clinical practices, empirical antimicrobial use, drug-resistance patterns and NICU flora during the long time period of the study. Second, the rate of antibiotic use may be involved with MDR colonization and sepsis but this study did not examine antibiotic use rates due to incomplete long-term data so we were unable to comment on this. Third, our study only included two species (*P. aeruginosa* and *Acinetobacter* spp.) of non-*Enterobacteriaceae* because only four organisms (*P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*) were interpreted by the disk diffusion (zone diameter) method according to the CLSI guidelines [7] and only two organisms (*P. aeruginosa* and *Acinetobacter* spp.) have a clear definition of MDRGNB [6]. Other species need minimal inhibitory concentration (MIC) values to identify susceptibility levels. Fourth, "duration of previous antibiotic exposure" has no standard definition. Some previous studies and our study defined "duration of previous antibiotic exposure" as 72 hours [4,22]. Fifth, selection and information bias occurred because not all healthy neonates were enrolled in the control group and the database was incomplete. The risk factors were difficult to identify in healthy neonates because of no data on the date of sepsis, exposure to antimicrobials, and duration of device (CL or ventilator) use. However, we were aware of this potential problem and we were careful to select the control group from the blood or CSF cultures which were matched with the dates of the case (MDRGNB and non-MDRGNB) groups. We did not select Gram-positive (Group B *Streptococcus*, *Listeria* or coagulase-negative *Staphylococcus*) cases to be in the control group due to the low incidences of these organisms in our NICU [1,31,32]. Finally, we cannot explain the association between non-MDRGNB sepsis and the lower incidence of pulmonary hypertension compared with the control group; however, we do note a previous study which similarly found a lower rate of pulmonary hypertension in non-ESBL-producing GNB bacteremia (1.3%) than in uninfected controls (3.9%) in an NICU [22].

Conclusion

MDRGNB sepsis was more likely in infants who had a lower GA and previous aminoglycoside exposure than non-MDRGNB sepsis compared with the control group. However, GNB (MDRGNB and non-MDRGNB) sepsis was more likely in infants who were outborn with lower GA, neurologic sequelae, and previous cephalosporin or aminoglycoside exposure compared with the control group. Intervention to reduce MDRGNB sepsis in the NICU is cost-effective because of crude excess daily hospital costs of MDRGNB sepsis. Moreover, infection control and prevention of neonatal GNB sepsis decreased CFR, septic shock, and length of stay.

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Authors' contributions

AT participated in the design of the study, performed the statistical analysis, drafted, reviewed and revised the manuscript. NP participated in its design and coordination, performed the initial analyses, and helped to draft the manuscript. WJ, SD and GM participated in the design of the study and acquisition of data. All authors read and approved the final manuscript.

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