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Original Article

Clinical and bacteriological profile of culture-negative and culture-proven neonatal sepsis in Palembang, Indonesia

Ariesti Karmila[1,2], Indrayady Barchia[1], Afifa Ramandati[1], Lixin Zhang[2]

[1] Department of Child Health, Faculty of Medicine, University of Sriwijaya, RSUP Dr. Mohmmad Hoesin, Palembang, Indonesia
[2] Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, United States

Abstract

Introduction: Culture-negative and multidrug-resistant neonatal sepsis frequently occur in developing countries and complicate neonatal sepsis management. These conditions contribute to a high neonatal mortality rate and accelerate the misuse of antibiotics. However, the extent of culture-negative and multidrug-resistant neonatal sepsis in developing countries remains poorly characterized. This study aims to describe culture-negative and culture-proven neonatal sepsis epidemiology and the antimicrobial resistance patterns in Palembang, Indonesia.

Methodology: A retrospective review of the medical records of all neonatal admissions between January 2016 and December 2018 was conducted at a tertiary-level referral hospital in Indonesia. The maternal and neonatal characteristics and microbiological results of the identified sepsis cases were obtained and analyzed.

Results: Three hundred and fifty-six neonatal sepsis cases were admitted from 2016 to 2018, accounting for 14.1% of neonatal hospital admissions. The percentages of early-onset and late-onset sepsis were comparable (49.7% vs. 50.3%), with an 18.1% case fatality rate. The proportion of culture-negative sepsis was 44%. The mortality rates between culture-proven and culture-negative sepsis cases did not differ statistically (p = 0.11). Coagulase-negative staphylococci (30.9%), *Klebsiella pneumoniae* (18.1%), and *Acinetobacter* spp. (10.7%) were the most frequently isolated pathogens. Overall, 62.6% of all isolated organisms were multidrug-resistant bacteria, with a high prevalence of extended-spectrum cephalosporin-resistant and carbapenem-resistant strains.

Conclusions: Culture-negative sepsis accounts for a significant proportion of neonatal sepsis cases. Early- and late-onset and culture-negative and culture-proven neonatal sepsis contribute to a comparable proportion of neonatal sepsis morbidity and mortality. There is an alarmingly high prevalence of resistance to extended-spectrum cephalosporin and carbapenem in neonatal sepsis cases.

Key words: Neonatal sepsis; antibiotic resistance; culture-negative.

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Introduction

Neonatal sepsis is a significant cause of morbidity and mortality among newborns. Worldwide, the estimated neonatal sepsis incidence is 22 per 1,000 live births [1]. In 2018, the World Health Organization (WHO) estimated that neonatal sepsis was responsible for over 1,000 deaths per day globally, accounting for 15% of all neonatal deaths [2]. The highest burden of neonatal sepsis occurs in developing countries, where the incidence is twofold to fourfold higher than that in developed countries. The risk of death in a newborn resulting from neonatal sepsis is 34 times higher in developing countries than in developed countries [3]. However, these numbers may not reflect the actual burden of neonatal sepsis because of data scarcity from developing countries. High-quality surveillance data are available for only one-third of WHO member countries and most of these are developed countries [4].

Culture-negative sepsis and multidrug-resistant (MDR) neonatal sepsis are conditions that often challenge the management of sepsis in newborns. Blood culture remains the most important microbiological tool in sepsis diagnosis and management; however, the inability to isolate a microbial pathogen does not exclude a sepsis diagnosis. Many neonates, especially those in developing countries, have been diagnosed with sepsis solely based on clinical suspicion, referred to as culture-negative sepsis [5]. A study showed that blood culture positivity among neonatal sepsis cases could be as low as 0.5% [6]. Recent reports suggest that antibiotic use is up to 16 times greater for culture-negative sepsis therapy than for culture-proven sepsis therapy [7]. In addition, culture-negative sepsis cases treated with antibiotics are largely ignored in epidemiological studies. Therefore, more studies are needed to obtain a better understanding of culture-

negative sepsis, as it also contributes to significant morbidity and mortality.

The high consumption of antibiotics in culture-negative sepsis cases potentially relates to an increased risk of colonization by antibiotic-resistant bacteria in neonates. Over the past decade, neonatal sepsis episodes caused by MDR organisms have become associated with significant increases in mortality rates, particularly in developing countries [8,9]. However, the extent of MDR neonatal sepsis in developing countries remains poorly characterized. Consequently, there is a critical need for more data on the population-level epidemiology of MDR neonatal sepsis in these countries to optimize neonatal sepsis management and prevent antibiotic resistance.

This study aimed to review neonatal sepsis epidemiology, including cases of culture-negative and culture-proven neonatal sepsis, and antimicrobial resistance patterns at a tertiary level referral hospital in Indonesia.

Methodology

Study design and variables of interest

This study used data from the medical records of Mohammad Hoesin Hospital from January 2016 to December 2018. The hospital is located in Palembang, Indonesia. It is a government-run teaching hospital that acts as a tertiary-level referral hospital serving patients from five neighboring provinces. The hospital's Neonatal Intensive Care Unit capacity and neonatal ward capacity are 15 and 40 beds, respectively. We identified neonatal sepsis cases based on the International Classification of Disease, 10th Revision, Clinical Modification coded P36 (i.e., bacterial sepsis of newborns) and searched the paper-based medical records of each patient. Sepsis was classified as early-onset sepsis (EOS) when symptoms occurred within the first 72 hours of life and as late-onset sepsis (LOS) when the onset occurred after 72 hours of life [10].

The medical records of the cases were screened for the onset of sepsis, gender, birth weight, gestational age, mode of delivery, place of delivery (in-born or out-born), birth attendant, and mortality. The risk factors for neonatal sepsis included a history of premature rupture of the membrane > 18 hours, maternal antepartum fever, antepartum hemorrhage, and foul-smelling amniotic fluid.

The bacteriological profile was obtained by reviewing the blood culture results database. At the study site, blood cultures were performed following the protocol of the BD BACTEC^{TM} automated blood culture systems. All cultures were incubated aerobically at 37 °C for 18-24 hours, and negative cultures were incubated for up to five days for bacteria and nine days for fungi before being reported as negative.

Identification and antimicrobial susceptibility testing of all isolates were performed using an automated method from VITEX-2 Compact (Biomérieux, Crappone, France), in accordance with the Clinical and Laboratory Standard Institute guideline [11].

The sensitivity of antimicrobial testing results was categorized as susceptible, intermediate, resistant, or not tested. Pathogens were recorded based on their resistance to various antibiotics classes, which included methicillin, vancomycin, extended-spectrum cephalosporin (any one of ceftriaxone, ceftazidime, or cefotaxime), extended-spectrum penicillin (piperacillin), carbapenem (meropenem or imipenem), fluroquinolone (ciprofloxacin or levofloxacin), and aminoglycoside (gentamycin or amikacin). Antimicrobial multidrug resistance was defined as an isolated pathogen classified as intermediate or showing resistance to at least one agent in three or more antimicrobial classes [12].

Statistical analysis

Descriptive statistics and the frequency distribution of all variables of interest were reported as a proportion for categorical variables and as a mean or median for continuous variables. The chi-square or Fisher's exact test was used to analyze categorical variables. Logistic regression was used to determine the significant risk factors for the onset of sepsis, mortality, and antimicrobial resistance. Initially, each factor was tested individually in a univariable regression model. The variables with a p value < 0.25 were then included in the multivariable regression logistic model to estimate the adjusted odds ratio (aOR). The data processing and analyses were conducted using SAS®, version 9.4.

Ethics approval

This study was approved by the Michigan State University's Biomedical and Health Institutional Review Board and by the Department of Education and Research Mohammad Hoesin Hospital.

Results

Between 2016 and 2018, the perinatology ward admitted 2,517 patients. Among all admissions, 356 (14.1%) newborns were diagnosed with neonatal sepsis from January 2016 until December 2018. We were able to retrieve and review the medical records of 306 (86%)

patients: 95 of 118 (80.5%) from 2016, 128 of 141 (90.1%) from 2017, and 83 of 97 (85.6%) from 2018. Across the calendar years, the referral cases, gender, gestational age group, mode of delivery, birth attendant, and case outcomes showed a similar proportion. Although not statistically significant, in 2018, the proportion of sepsis cases with low birth weight was smaller than that in previous years. The basic characteristics of the neonatal cases are summarized in Table 1.

Of all the cases, 152 (49.7%) were classified as EOS and 154 (50.3%) as LOS. Cases born outside the hospital (out-born) accounted for 48% of all EOS and 63% of all LOS. Out-born cases were likelier to have LOS than cases born in the hospital (in-born) (odds ratio [OR] 1.84, 95% confidence interval [CI] 1.17–2.91). Most of the newborns delivered with EOS were assisted by Obstetrics and Gynecology (OB/GYN) residents (37.5%), whereas most LOS cases were assisted by midwives (40.9%). The mean (standard deviation [SD]) birth weight was 2,833.1 ± 588.2 grams for neonates with EOS, whereas for neonates with LOS, the mean (SD) of the birth weight was 2,687.8 ± 652.4 grams. Aside from the place of delivery, we did not find any other association between other neonatal and maternal risk factors for sepsis onset (Table 2).

However, a stratified analysis based on the delivery site revealed a significant association between sepsis onset and the person who assisted the delivery in the in-born neonatal sepsis cases. LOS was likelier to occur in births delivered by OB/GYN residents than by specialist (OR 5.4, 95% CI 1.76–16.78).

Mortality of neonatal sepsis

During the study period, 55 deaths resulting from neonatal sepsis were recorded. Of these, 31 (53.4%) were EOS, and 24 (43.6%) were LOS.

Table 1. Basic characteristics of neonatal sepsis cases by year (N = 306).

Variable (%)	2016 (n = 95)		2017 (n = 128)		2018 (n = 83)	
Place of delivery						
In-born	42	(44.2)	58	(45.3)	36	(43.4)
Out-born	53	(55.8)	70	(54.7)	47	(56.6)
Gender						
Male	53	(55.8)	77	(60.2)	59	(71.1)
Female	42	(44.2)	51	(39.9)	24	(28.9)
Birthweight (grams)						
Mean (SD)	2684.2	(664.5)	2784.1	(643.2)	2809.3	(542.2)
Range	1100–4100		1200–4500		1300–4000	
Birthweight classification						
Very low birth weight (1000–1499 grams)	2	(2.1)	4	(4.7)	2	(2.4)
Low birth weight (1500–2499 grams)	30	(31.6)	27	(21.1)	11	(13.3)
Normal birthweight (≥ 2500 grams)	63	(66.3)	102	(79.7)	70	(84.3)
Gestational age (weeks)						
Mean (SD)	37.0	(3.1)	37.2	(2.9)	37.1	(2.5)
Range	26–42		28–41		28–42	
Gestational age group (%)						
Extreme preterm (< 28 wk)	2	(2.1)	0	0	0	(0)
Very preterm (28 – < 32wk)	2	(2.1)	6	(4.7)	4	(4.8)
Moderate preterm (32 – < 34 wk)	9	(9.5)	4	(3.1)	1	(1.2)
Late preterm (34 – < 37wk)	16	(16.8)	16	(12.5)	19	(22.9)
Term (32 – 42 wk)	66	(69.5)	102	(79.7)	59	(71.1)
Mode of delivery (%)						
Spontaneous	56	(59.0)	71	(55.5)	46	(55.4)
Vacuum extraction	3	(3.2)	1	(0.8)	4	(4.8)
Forceps extraction	0	0	2	(1.6)	0	(0)
C-Section	36	(37.9)	54	(42.2)	33	(39.8)
Birth assistant (%)						
Midwife	37	(39.0)	47	(36.7)	29	(35.0)
General practitioner	0	0	2	(1.6)	1	(1.2)
Ob-Gyn resident	28	(29.5)	47	(36.7)	34	(41.0)
Ob-Gyn specialist	30	(31.6)	32	(25.0)	19	(22.9)
Outcome						
Lived	83	(79.0)	102	(79.7)	70	(84.3)
Died	19	(20.0)	23	(18.0)	13	(15.7)
Discharged against medical advice	1	(1.1)	3	(2.3)	0	(0)

Table 2. Neonatal and maternal risk factors of sepsis onset.

Variable	EOS	LOS	*p**
	n = 152	n = 154	
Out-born	73 (48.0)	97 (63.0)	**0.009**
Male	93 (61.2)	96 (62.3)	0.84
Birth assistant			0.47
Midwife	50 (32.9)	63 (40.9)	
General practitioner	1 (0.7)	2 (1.3)	
Ob-Gyn resident	57 (37.5)	52 (33.8)	
Ob-Gyn specialist	44 (29.0)	37 (24.0)	
Prematurity	40 (26.3)	39 (25.3)	0.84
Birthweight < 1500 grams	33 (21.7)	43 (27.9)	0.21
C-Section	66 (43.4)	57 (37.0)	0.25
Premature rupture of membrane (PROM)	53(34.9)	41 (26.6)	0.12
Maternal fever†	4 (2.6)	2 (1.3)	0.45**
Antepartum hemorrhage	5 (3.3)	4 (2.6)	0.72
Foul-smelling amniotic fluid	9 (5.9)	10 (6.5)	0.84

*chi square; **Fisher-exact; †1 missing data.

Table 3. Predictors of mortality by univariate and multivariate logistic regression (N = 306).

Variables	*p*-value*	OR (95% CI)		aOR (95% CI)	
Early-onset sepsis	0.23	0.70	(0.39–1.26)	0.56	(0.30–1.05)
Birth assistant	0.41	--		--	
C-Section	0.54	--		--	
Premature rupture of membrane	0.98	--		--	
Maternal fever	0.99	--		--	
Out-born	0.05	1.85	(1.00–3.42)	**2.07**	(1.09–3.92)
Female	0.14	1.54	(0.86–2.79)	1.46	(0.78–2.67)
Very low birth weight (< 1500 grams)	**0.01**	**2.24**	**(1.20–4.17)**	2.01	(0.97–4.15)
Prematurity	0.12	1.67	(0.89–3.12)	1.21	(0.59–2.50)
Foul-smelling amniotic fluid	0.34	--		--	
Antepartum hemorrhage	0.37	--		--	
Culture proven sepsis	0.11	1.67	(0.32–1.13)	1.80	(0.93–3.47)

*Chi-square; Fisher exact.

Table 4. Predictors of mortality for culture-proven sepsis only (n=147).

Variables (%)	Death (+) (n = 27)	Death (-) (N=119)	*p-value**	OR (95% CI)	aOR (95% CI)
Late-onset sepsis	11 (40.7)	61 (50.8)	0.36	--	--
Birth assistant			0.96	--	--
Midwife	9 (33.3)	40 (33.3)	--	--	--
General Practitioner	0 (0)	2 (1.7)	--	--	--
OB/GYN resident	10 (37.0)	48 (40.0)	--	--	--
OB/GYN specialist (ref)	8 (29.6)	30 (25.0)	--	--	--
C-Section	9 (33.3)	51 (42.5)	0.41	--	--
Premature rupture of membrane	8 (29.6)	35 (29.2)	0.91	--	--
Maternal fever†	1 (3.7)	4 (3.4)	0.93	--	--
Out-born	16 (59.3)	50 (50)	0.37	--	--
Female	11 (40.7)	79 (65.8)	0.54	--	--
Very low birth weight (<1,500gr)	11 (40.7)	29 (24.2)	0.07	2.23 (0.93-5.37)	1.84 (0.64-5.25)
Prematurity	10 (37.0)	30 (25)	0.18	1.83 (0.75-4.42)	1.36 (0.47-3.95)
Foul-smelling amniotic fluid	2 (7.4)	7 (5.8)	0.77	--	--
Antepartum hemorrhage	0 (0)	7 (5.8)	0.97	--	--
Isolates	--	--	0.07	--	--
Gram-positive (ref)	7 (25.9)	60 (50.0)	--	--	--
Gram-negative	19 (70.4)	59 (49.2)	--	2.71 (1.06-6.94)	**2.70 (1.05-6.97)**
Fungal	1 (3.7)	1 (0.8)	--	8.43 (0.47-150.21)	7.05 (0.37-135.35)

*Chi-square; Fisher exact; †1 missing data.

Among all death cases, 20 cases (36.4%) were culture-negative sepsis, 27 (49.1%) were culture-proven sepsis, and 8 had missing culture results (14.5%). Among the 27 culture-proven sepsis cases, Gram-negative bacteria were the leading cause of death in both EOS and LOS. In culture-proven EOS, 10 deaths were due to Gram-negative bacteria, 3 were due to Gram-positive bacteria, and 1 was due to fungal infection. In culture-proven LOS, Gram-negative bacteria caused 9 deaths, and Gram-positive bacteria caused 4 deaths.

Klebsiella pneumoniae was the most common pathogen isolated from all culture-proven EOS-related cases that died, whereas *Acinetobacter* spp. and *Enterobacter* spp. were the two most common pathogens isolated from all culture-proven LOS-related cases that died. Among all pathogens isolated from all cases of neonatal sepsis-related death, 20 (36.4%) were MDR bacteria; 17 of these were Gram-negative bacteria, and 3 were Gram-positive bacteria.

In the adjusted model, our study found a significant association between out-born cases and mortality (aOR 2.07, 95% CI 1.09–3.92). Although not statistically significant, we also found that newborns whose birthweight was less than 1,500 grams were twice as likelier to die as those whose birthweight > 1,500 grams. (aOR 2.01, 95% CI 0.97–4.15) (Table 3).

An unadjusted logistic regression model that only includes culture-proven sepsis (n = 147) revealed a likelier association between Gram-negative pathogens and death than between Gram-positive pathogens and death (OR 2.71, 95% CI 1.06–6.94). This significant association persisted after adjusting for birth weight category and prematurity (aOR 2.70, 95%CI 1.05–6.97). A univariable logistic model did not reveal any specific pathogen that significantly increased the risk of mortality (Table 4).

Our study site does not require two simultaneous blood cultures to differentiate true coagulase-negative staphylococcal (CoNS) infection from contamination. Therefore, we conducted an additional analysis excluding sepsis cases resulting from CoNS infection, assuming that such cases were not a true infection. After excluding CoNS sepsis cases, although the association between Gram-negative pathogens and mortality lost its statistical significance, the aOR was similar to the aOR before CoNS exclusion (aOR 2.80, 95% CI 0.59-13.36).

Table 6. Isolated pathogens in all culture-proven neonatal sepsis (N = 149).

Isolated pathogen	N (%)
Coagulase-negative staphylococci (CoNS)	46 (30.9)
Klebsiella pneumoniae	27 (18.1)
Acinetobacter spp.	16 (10.7)
Pseudomonas aeruginosa	11 (7.4)
Enterobacter spp.	10 (6.7)
Non-beta hemolytic *streptococcus*	10 (6.7)
Staphylococcus aureus	6 (4.0)
Enterococcus spp.	5 (3.4)
Escherichia coli	5 (3.4)
Pantoea spp.	5 (3.4)
Serratia spp.	4 (2.7)
*Candida s*p.	2 (1.3)
Bacillus sp.	1 (0.7)
Kocuria sp.	1 (0.7)

Microbial profile

Overall, 266 (86.9%) cases had blood culture results. Of the 266 blood culture results, 149 (56.0%) were culture proven and 117 (44.0%) were culture negative. Gender, the onset of sepsis, prematurity, and mode of delivery were not associated with the culture results (Table 5). However, culture-proven sepsis was likelier in neonates with birth weights less than 1,500 grams than in those with birthweights equal to or greater than 1,500 grams (OR 1.8, 95% CO 1.01–3.36).

Among all culture-proven sepsis cases (n = 149), 78 (52.4%) were caused by Gram-negative bacteria, 69 (46.3%) by Gram-positive bacteria, and 2 (1.4%) by fungal infection (both *Candida albicans)*. Overall, the most frequently isolated pathogens were CoNS (46,

Table 5. Characteristics of culture-proven versus culture-negative sepsis (N = 266).

Variable (%)	Culture proven n = 149		Culture negative n = 117		*p*
Out-born delivery	77	(51.7)	72	(61.5)	0.11
Male	96	(64.4)	70	(59.9)	0.44
Late-onset sepsis	72	(48.3)	59	(50.4)	0.73
Birthweight < 1500 grams	41	(27.5)	20	(17.1)	**0.04**
Prematurity	40	(26.9)	25	(21.4)	0.30
C-Section	61	(40.9)	49	(41.9)	0.88
Premature rupture of membrane	44	(29.5)	36	(30.8)	0.83
Foul-smelling amniotic fluid	9	(6.0)	6	(5.1)	0.75
Maternal fever*	5	(3.4)	1	(0.9)	0.23
Antepartum hemorrhage	7	(4.7)	2	(1.7)	0.31

*missing 1.

30.9%), *Klebsiella pneumoniae* (27, 18.1%), and *Acinetobacter* spp. (16, 10.7%). The distribution of the isolated pathogens is shown in Table 6.

Among the Gram-negative bacteria, the predominant bacteria were *Klebsiella pneumoniae* (27, 34.6%), *Acinetobacter baumanii* (11, 14.1%), and *Pseudomonas aeruginosa* (11, 14.1%). The predominant Gram-positive bacteria causing neonatal sepsis were CoNS (46, 30.9%) and non-beta hemolytic streptococcus (10, 6.7%).

Among the neonates with culture-proven EOS, *CoNS* (26, 33.7%) was the most frequently detected pathogen, followed by *Klebsiella pneumoniae* (15, 19.5%) and non-beta hemolytic streptococcus (7, 9.1%). In the LOS cases, the most common causative organisms were also CoNS (20, 27.8%), followed by *Klebsiella pneumoniae* (12, 16.7%), and *Acinetobacter* spp. (10, 13.9%) (Figure 1).

Although further analysis did not reveal an association between Gram-negative neonatal sepsis and maternal and neonatal risk factors, the presence of Gram-negative bacteria was proportionately more frequent in LOS (55.1% vs. 40.9%, OR 1.8, 95% CI 0.93–3.41). No significant association was noted between neonatal and maternal sepsis risk factors and sepsis caused by a specific Gram-negative pathogen.

Antimicrobial susceptibility

Among the 149 culture-proven sepsis cases, 10 specimens from these cases did not have antimicrobial susceptibility test (AST) results; two had fungal infections, seven were infected with Gram-positive bacteria, and one was infected with Gram-negative bacteria. Of the 139 cases with AST results, 87 (62.6%) were MDR bacterial infections. Among the 62 Gram-positive bacteria, 44 (71.0%) were MDR, and among the 77 Gram-negative bacteria, 43 (55.9%) were MDR. CoNS were the most prevalent MDR bacteria among all Gram-positive bacteria, whereas *Klebsiella pneumoniae* were the most prevalent MDR bacteria among the Gram-negative pathogens (Figure 2).

This study revealed that 58.3% of the tested *Acinetobacter* spp., 63.6% of the *Pseudomonas aeruginosa*, and 37.2% of the *Enterobacteriaceae* isolates were carbapenem-resistant strains. In addition, 64.6% of the *Enterobacteriaceae* isolates were cephalosporin-resistant strains. At the study hospital, cephalosporin is the empiric antibiotic therapy for neonatal sepsis; this study revealed that 78.7% of the tested isolates were resistant to cephalosporins. Supplementary Table 1 shows the antibiotic resistance patterns of the major isolated organisms.

Univariable analysis did not reveal significant associations between MDR pathogens and various maternal and neonatal sepsis risk factors. This study did not show an association between MDR and mortality (OR 1.08, 95% CI 0.44–2.65). Similar results were found in the analysis that excluded cases of CoNS neonatal sepsis.

Discussion

Throughout the study, we found that the characteristics of the admitted neonatal sepsis cases were similar. However, over the years, there was a decrease in the proportion of neonatal sepsis cases with low birth weight, from 31.6% in 2016 to 13.3% in 2018. The possible reason for this is that over the years, there has been an improvement in healthcare facilities in Indonesia; an increasing number of smaller hospitals now have the capability to manage babies with low birth weights. Therefore, fewer cases of low birth weight needed to be referred to our study site. The incidence of neonatal admissions for sepsis during the study period was 14.1%. Currently, Indonesia does not have national data on neonatal sepsis incidence. However, several studies have reported that the neonatal admission

Figure 1. Causative pathogen distribution in EOS and LOS cases.

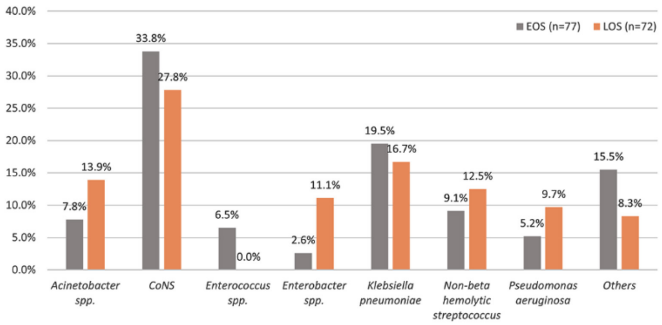
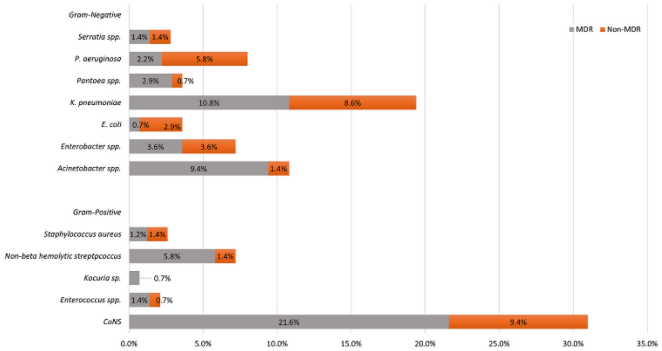


Figure 2. Distribution of isolated pathogens in culture-proven neonatal sepsis as determined by antimicrobial susceptibility test (AST) results.



incidence rates in some tertiary hospitals in Indonesia vary between 5% and 25% [13,14]. Studies from other developing countries in South Asia have shown admission incidence rates ranging from 20.5 to 45.9% [15,16]. The variation in incidence rates between referral hospitals in Indonesia may be due to the differences in the healthcare referral systems implemented during each study period and hospital capacity. In 2014, Indonesia's government developed a new national health insurance scheme that changed the referral policy and restricted those cases referred to tertiary-level hospitals. The admission incidence difference with other developing countries may also be explained by differences in patient socio-demographic status, accessibility to healthcare facilities, and the definition of neonatal sepsis used in the different studies.

The proportions of EOS and LOS in this study were similar (49.7% vs. 50.3%). Previous studies have shown a higher EOS burden in developing countries than in developed countries [17,18]. Advances in obstetric care, including prophylactic antibiotics, have significantly reduced the incidence of EOS in developed countries; however, in developing countries, the incidence of EOS remains high. The occurrence of EOS is frequently associated with colonization of the newborn by vertical transmission from the maternal genital tract, unhygienic birth practices during labor, and ultra-early horizontal transmission from the delivery room or neonatal care units; these problems are more common in developing countries [19,20].

By contrast, LOS reflects community or nosocomial infection more strongly and is highly associated with infant prematurity. Improvements in premature infant survival as a result of advances in neonatal intensive care in developed countries have led to increases in LOS incidence [19–21]. The comparable proportion of LOS and EOS observed in this study may indicate that basic obstetric practices aimed at preventing vertical infection from mother to newborns are still inadequate, despite improvements in overall neonatal care. In this study, LOS was likelier to occur in out-born cases, reflecting community-acquired infection. Poor hygiene, poor cord care, unhygienic bottle feeding, and the use of prelacteal feeds are common practices in developing countries, especially among people with a low socioeconomic status [22].

We also found that in-born cases assisted by OB/GYN residents were likelier to develop LOS than those cases assisted by OB/GYN specialists. This may be related to the two-tier healthcare system in hospitals in Indonesia, which frequently leads to discrimination in the provision and quality of care for patients [23]. Residents serve more patients with a lower socioeconomic status who occupy crowded wards. A low socioeconomic status predisposes newborns to neonatal infection, and overcrowded wards compromise the healthcare quality given to both the mother and the newborn, thereby increasing newborns' susceptibility to late infection [23,24].

The fatality rate of neonatal sepsis in the present study was 18%, whereas other studies from Indonesia have reported higher fatality rates, from 20% to 67% [13,14]. In other developing countries, the fatality rates range from 16% to 46% [15,25]. Our study had a smaller proportion of newborns who have a low-birth-weight and are premature, which could explain the lower fatality rate, as neonate mortality is frequently inversely proportional to birth weight and gestational age [25]. Our study also revealed that a low birth weight is a higher risk of mortality. The out-born cases were also associated with a higher risk of mortality, most likely because these were the more severe case and were referred from a lower healthcare facility.

Consistent with previous studies, we found increased mortality in neonates with Gram-negative sepsis compared with Gram-positive sepsis [17,26]. The most common pathogens isolated from cases with both EOS- and LOS-related deaths were the Gram-negative bacteria. Sepsis resulting from Gram-negative infection carried a higher risk of severe sepsis and mortality in all age ranges. Gram-negative bacteria are known to be more virulent because of their capability to evade hosts' immune responses, produce endotoxins that increase the severity of inflammation, and adapt to changing host and environmental conditions through multiple genetic mechanisms [27].

The proportion of culture-negative sepsis in this study was 44%, which is comparable with the proportions reported by studies in other developing countries [16,28]. In developed countries, the ratio of culture-proven versus culture-negative sepsis ranges from 1:6 to 1:12 [7]. Some concerns have been raised regarding the over-diagnosis of sepsis. This leads to higher consumption of unnecessary antibiotics; however, some of these culture-negative sepsis cases may not be truly negative. Our study supported other previous findings regarding a lack of significant mortality differences between culture-negative and culture-positive cases [29,30]. This result reflects the fact that newborns with culture-negative sepsis were also severely ill.

The reasons for the large proportion of culture-negative sepsis remain unclear. The low blood volume

obtained from newborns is likely a strong reason. In addition, anaerobic blood cultures are not routinely performed. Cultures obtained after antibiotic initiation and maternal antibiotic treatment before and during delivery are also possible explanations. Conventional microbiological methods may frequently fail to identify pathogens because of technical issues or traits intrinsic to microorganisms that limit sepsis detection. Although new diagnostic approaches have been developed to replace conventional methods, implementation in developing countries will be challenging because of a lack of resources [31,32].

Our study found the same pattern of the predominant pathogen for both EOS and LOS, which was CoNS, followed by *K. pneumoniae,* and *Acinetobacter* spp. Studies from Turkey and Brazil have also reported that CoNS is the leading cause of both EOS and LOS. The 14-years study from Turkey reported that 64.4% of neonatal sepsis cases were caused by CoNS, whereas in Brazil, the proportion of CoNS was 36.5% [33,34]. Other smaller studies from Peru, Egypt, and India also reported similar findings [16,26,35]. On the other hand, a large cohort study in India found the same three predominant pathogens - CoNS, *Acinetobacter* spp., and *Klebsiella* spp. - as the most isolated pathogens in their EOS and LOS cases but in a different order. Instead of CoNS, *Acinetobacter* spp. were ranked first as the predominant pathogen [9]. Although the determination of CoNS as a true pathogen or contamination in neonatal sepsis is still debatable, the consistent findings from multiple studies of CoNS as the most reported causative pathogen in neonatal sepsis cases should be a strong indication that CoNS have an important role in neonatal sepsis. A careful evaluation is needed before determining that CoNS isolation from blood culture is a contamination, especially when the case is supported with sepsis clinical signs and symptoms and abnormal laboratory findings and when the specimen collection was performed with an appropriate antisepsis protocol. Previous studies have suggested that CoNS bacteremia is associated with low birth weight and prematurity [16,36]; however, we found that CoNS infection was also frequently found in full-term neonates with normal birth weight. This may suggest that even in full-term neonates, the immaturity of the immune system and the ineffectiveness of neonate skin and mucous membranes to act as physical barriers may still be associated with these neonate's vulnerability to low-virulence pathogens. However, the search for approaches to increase the ability to distinguish between true bacteremia and contamination should continue.

Consistent with previous studies from developing countries reporting Group B Streptococcus (GBS) infection were rarely found, our studies also did not detect any GBS neonatal sepsis cases [9,20]. In developed countries, the most common cause of neonatal sepsis for EOS and LOS is a Gram-positive organism or GBS for EOS and CoNS for LOS [17,37]. Whether differences in the etiological agents of neonatal sepsis between developed and developing countries reflect an actual difference in the causative agents across the globe or can be attributed to differences in the case definition of sepsis, the capability to perform blood culture published reports that come from short periods of surveillance, and the numbers of neonatal sepsis cases diagnosed without blood culture or that never reached healthcare facilities and not reported remain uncertain. Therefore, further epidemiological studies that describe the various pathogens causing neonatal sepsis and their changing antibiotic susceptibility profile remain important.

Similar to previous studies, our work revealed a large number of MDR pathogens [9,15]. This confirms that antibiotic resistance is a major global health problem and needs urgent attention, particularly in developing countries. In developed countries, MDR neonatal sepsis accounts for less than 20% of cases, whereas this proportion can reach 40%–80% in developing countries [9,38–40]. We observed a high prevalence of resistance to extended-spectrum cephalosporin and carbapenem, which significantly complicates sepsis management, especially considering that the first- and second-line empirical antibiotics used at our study site are third-generation cephalosporin and carbapenem, respectively. These first and second-line antibiotics are used until blood culture, and AST results are available, or they are continued as a complete course of treatment of culture-negative sepsis cases.

Previous exposure to third-generation cephalosporin and carbapenem has been identified as an independent risk factor for acquiring resistance to Gram-negative bacteria [40]. Other factors responsible for the surge in MDR in developing countries include the overuse of empirical antibiotics and intrapartum antibiotic prophylaxis, the non-existence of antibiotic prescription guidelines, the over-the-counter sale of antibiotics, poor sanitary conditions, a lack of basic facilities and practices, and the lack of surveillance regarding organisms that cause infections [9,41,42].

Our study provides an update on neonatal sepsis burden, the bacteriological profile, and antibiotic resistance patterns in Indonesia. This study also emphasize the prevalence of culture-negative sepsis

cases, which are generally underreported. This research has some limitations. The data were obtained by reviewing medical records. The documentation may have been incomplete, which would have limited further analysis to find associations with other potential risk factors. Our work is a single-center study conducted at a tertiary-level referral hospital, so selection bias may have occurred against less severe neonatal sepsis cases. Our findings may not be representative of other neonatal units in the country. Another limitation is that the blood culture specimen collection protocol at our study site does not require two simultaneous blood cultures, so we may have overestimated the incidence of CoNS infection. However, at the study site, sepsis diagnosis is made by fulfilling clinical and laboratory criteria, and it was not solely based on blood culture results.

Conclusions

Our findings showed that EOS and LOS and culture-negative and culture-proven neonatal sepsis cases shared a comparable proportion of neonatal sepsis morbidity and mortality. Our findings emphasized the surge in multidrug antibiotic resistance occurring in developing countries and the need for significant actions that will improve efforts to prevent infection in neonates while controlling the use of antibiotics. Neonatal sepsis remains a global public health issue, so we recommend more comprehensive, extensive, and large-scale studies to better understand the magnitude of the disease. We also advocate the development of alternative, affordable pathogen identification approaches that can serve as add-ons to traditional microbiological techniques to improve the management of neonatal sepsis and the prevention of antimicrobial resistance.

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Corresponding author

Ariesti Karmila, MD
Bagian Ilmu Kesehatan Anak lt. 4
RSUP dr. Mohammad Hoesin
Jl. Jend. Sudirman, KM 3,5, Palembang, 30126
Tel. +62-711-376445 Fax. +62-711-376445
Email: a.karmila@unsri.ac.id; karmilaa@msu.edu

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Annex – Supplementary Items

Supplementary Table 1. Antimicrobial resistance patterns among isolated pathogens in neonatal sepsis cases with antimicrobial susceptibility test (AST) results (n = 139).

	Resistance/Tested	Not tested
Gram-Positive		
Coagulase-negative staphylococci (n = 43)		
Methicillin	37/41	2
Vancomycin	1/1	7
Extended-spectrum cephalosporin	22/27	16
Extended-spectrum penicillin	21/23	20
Enterococcus sp. (n = 3)		
Methicillin	2/3	0
Vancomycin	0/3	0
Extended-spectrum cephalosporin	1/1	2
Extended-spectrum penicillin	1/1	2
Kocuria sp. (n = 1)		
Vancomycin	0/1	0
Extended spectrum cephalosporin	1/1	0
Extended-spectrum penicillin	0/1	1
Non-beta hemolytic streptococcus (n = 10)		
Extended spectrum cephalosporin	7/7	3
Extended-spectrum penicillin	7/7	3
Staphylococcus aureus (n = 5)		
Methicillin	3/5	0
Vancomycin	1/5	0
Gram-Negative		
Acinetobacter sp. (n = 15)		
Extended spectrum cephalosporin	14/14	1
Extended spectrum penicillin	1/12	2
Carbapenem	7/12	3
Fluoroquinolones	9/11	4
Aminoglycosides	1/6	0
Enterobacter sp. (n = 10)		
Extended spectrum cephalosporin	8/10	0
Extended spectrum penicillin	7/8	2
Carbapenem	8/10	0
Aminoglycosides	0/10	0
Fluoroquinolones	3/10	0
Escherichia coli (n = 5)		
Extended-spectrum cephalosporin	1/3	1
Extended-spectrum penicillin	2/2	1
Carbapenem	0/1	3
Aminoglycosides	1/3	2
Fluoroquinolones	2/3	2
Klebsiella pneumoniae (n = 27)		
Extended spectrum cephalosporin	16/26	1
Extended spectrum penicillin	18/21	6
Carbapenem	1/3	1
Aminoglycosides	1/7	1
Fluoroquinolones	1/6	1
Pantoea sp. (n = 5)		
Extended-spectrum cephalosporin	5/5	0
Extended-spectrum penicillin	4/4	1
Carbapenem	4/5	0
Aminoglycosides	0/5	0
Fluoroquinolones	1/5	0
Pseudomonas aeruginosa (n = 11)		
Extended-spectrum cephalosporin	9/10	1
Extended-spectrum penicillin	0/11	0
Carbapenem	7/11	0
Aminoglycosides	6/11	0
Fluoroquinolones	3/7	1
Serratia sp. (n = 4)		
Extended-spectrum cephalosporin	1/4	0
Extended-spectrum penicillin	4/4	0
Carbapenem	1/4	0
Aminoglycosides	1/4	0
Fluoroquinolones	0/4	0

Extended-spectrum cephalosporin (any one of ceftriaxone, ceftazidime, or cefotaxime): extended-spectrum penicillin (piperacillin); carbapenems (meropenem or imipenem); fluroquinolone (ciprofloxacin or levofloxacin); and aminoglycoside (gentamycin or amikacin).