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[IJM] Editor Decision

2 messages

Prof. Mohammad Mehdi Feizabadi <ijm@tums.ac.ir> Mon, Apr 5, 2021 at 9:29 AM
To: Risa Etika <risa_etika@yahoo.com>, Khadijah Rizky Sumitro <khadijahrizkysumitro@gmail.com>, Martono Tri Utomo <mrmartono73@gmail.com>, Agung Dwi Wahyu Widodo <agungimunologi@gmail.com>

Dear Corresponding author

We have reached a decision regarding your submission to Iranian Journal of Microbiology, "CULTURE-PROVEN NEONATAL SEPSIS IN INDONESIAN TERTIARY NEONATAL INTENSIVE CARE UNIT: A HEMATOLOGICAL AND MICROBIOLOGICAL PROFILE".

It needs major revision.

Please see the comments we received from the reviewers and revise the manuscript accordingly. The revised version and your answers to the reviewer will be checked by the reviewers before making final decision. Select 5- 7 keywords from MeSH database for your manuscript. We do not accept any keywords which are not found at MeSH. Make sure that the references are searchable at PubMed and Scopus. The references should be written according to IJM format. Save the revised manuscript in word version 2003 before uploading the files.

Best Regards

Prof. Mohammad Mehdi Feizabadi

Editor

Reviewer A:

Dear Aurtors:

1. please explain kind of blood culture (BACTEC or NOT).

2.How do you rule out contamination of blood culture samples?

3.please explain if you check procalcitonin level in blood samples.

Recommendation: Revisions Required

Reviewer B:
Recommendation: Revisions Required

Comment from Author

First of all we are very grateful and appreciate the comments that have been given on our manuscript. The suggestions and corrections given are invaluable inputs. The keyword already revised and selected from MeSH database. The references already checked and revised according to IJM format.

Reviewer A:

1. Please explain kind of blood culture (BACTEC or NOT).
   Author: *In our study, liquid blood culture media used was Bactec® bottle and continue subcultured into solid culture media (blood agar plate, MacConkey agar plate and chocolate agar plate) if bacterial growth was positive in Bactec®. Methods section already revised.*
2. How do you rule out contamination of blood culture samples?
   Author: *Neonates with positive blood culture result without clinically or laboratory results supporting neonatal sepsis were excluded from our study.*
3. Please explain if you check procalcitonin level in blood samples.
   Author: *In our study, procalcitonin was not the routine examination performed for neonatal sepsis.*

Reviewer B:

1. It is necessary to review the whole article once again in terms of writing and spelling problems. (such as names of bacteria ,….)
   Author: *Thanks in advanced for the suggestion. Revisions have been made and written in red color as attached.*
2. In method section, describe the full working for example media, temperature,…and method to identification of ESBL
   Author: *Methods section already revised as attached (in red color)*
3. *B. ceruse* is a gram positive, that you categorized in the gram negative bacteria, and why do you think this bacterium should be isolated from your case?
   Author: *Sorry for the missclassification and thanks for the correction. The Table 2. Microbiological profile of proven neonatal sepsis already revised.*
   Explanantion of *B. ceruse* infection in our study has been added in discussion section
4. Is there a connection with nosocomial infections and pathogens affecting neonatal sepsis? According to your description in the discussion section
   (The causative pathogens of culture-proven neonatal sepsis vary according to geographical differences and countries. It is vary from one hospital and other different hospital and even in the same hospital at different time (31,35).
   Author: *In our study, the high incidence of Klebsiella pneumoniae infection and especially the ESBL strain was due to nosocomial infection, as previously reported in 2011 and 2015 in our hospital. Information about this has been added in the discussion section (in red color).*

CULTURE-PROVEN NEONATAL SEPSIS IN INDONESIAN TERTIARY NEONATAL INTENSIVE CARE UNIT: A HEMATOLOGICAL AND MICROBIOLOGICAL PROFILE

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ABSTRACT

Background : Neonatal sepsis is the third leading cause of neonatal death in the world. The patterns of pathogen causing neonatal sepsis varies in many countries.

Objectives : This study was aimed to identify hematological and microbiological profile of culture-proven neonatal sepsis in Indonesian tertiary neonatal intensive care unit (NICU).

Methods : Hospital based cross-sectional study was conducted in all inborn neonates with suspected sepsis neonatal over a period of six months from April to September 2019. Complete blood count, c-reactive protein (CRP) and blood culture were examined before antibiotic administration. Statistical analysis were using Chi-Square's Test and Mann-Whitney U test and $p < 0.05$ considered significant.

Results: One hundred four inborn neonates admitted to NICU and diagnosed with suspected neonatal sepsis were recruited. Culture-proven neonatal sepsis were confirmed in 52 (50%) neonates, 13 (25%) in early-onset neonatal sepsis (EONS) and 39 (75%) in late-onset neonatal sepsis (LONS). The most common abnormal hematological profile were anemia and thrombocytopenia, with amount of 61.5% and 75%, respectively. High CRP only detected in 36.4% and only 18.5% experienced leukopenia. Gram-negative bacteria responsible in 75% from total isolated pathogens. *Klebsiella pneumonia* accounted for 48.1% followed by *coagulase negative staphylococci* (CONS) for 17.3% and *Enterobacter cloacae* for 11.5%.

Conclusion: Anemia and thrombocytopenia were the top two hemaotological profile of culture-proven neonatal sepsis. Most causes of culture-proven neonatal sepsis was gramnegative bacteria and most pathogens was *Klebsiella pneumonia*.

Keywords: *neonate, neonatal sepsis, gram-negative bacteria, Klebsiella, Indonesia*

Introduction

Neonatal sepsis is a clinical syndrome that occurs in infants in the first month of life characterized by systemic infection and bacteremia. It is classified into two types based on the age of onset of the findings, early-onset neonatal sepsis (EONS) occurring within ≤ 72 hours and late-onset neonatal sepsis (LONS) occurring after 72 hours (1–4). Neonatal sepsis is the third leading cause of neonatal death in the world (5). There has been a decrease in neonatal mortality between 1990 and 2017, from 36.6 (35.5 - 37.8) to 18 (17 - 19.9) deaths per 1000 live births. Nonetheless, the SDGs stipulate that all countries should aim to reduce the neonatal mortality rate (NMR) to 12 deaths per 1000 live births or less by 2030 (6).

Blood culture is the gold standard examination for neonatal sepsis diagnosis (2). The presence of pathogen isolation on blood culture examination or positive PCR examination in neonates with clinically neonatal sepsis is categorized as a culture-proven neonatal sepsis (7).

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The patterns of pathogen causing neonatal sepsis differ in many countries according to the local microbial pattern and to the onset of neonatal sepsis (2,8). Little is known about the incidence and distribution of neonatal sepsis pathogens and profile in Indonesia, especially in Surabaya, so this study aims to provide an overview of the hematological and microbiological profile of culture-proven neonatal in tertiary neonatal intensive care unit in Indonesia.

Methods

Study design and ethical clearance

This hospital based observational analytic cross-sectional study conducted for 6 months, between April and September 2019. An ethical clearance certificate was approved by Ethical Committee in Health Research of Dr. Soetomo General Academic Surabaya (ref. no. 1047/KEPK/III/2019).

Study population

All inborn neonates admitted to the NICU and with suspected sepsis neonatal were eligible in this study. For this study, diagnostic criteria for suspected neonatal sepsis was according to definitions of blood stream infection in the newborn by Haque (2005) (7). Suspected neonatal sepsis was the presence of clinically neonatal sepsis accompanied by increasing CRP (>10 mg/dL or >2 SD above normal value) or at least 2 abnormal inflammatory laboratory results. Local Clinical Practice Guidelines are carried out by examining complete blood count, creactive protein (CRP) and blood culture before administered antibiotics. Neonatal sepsis was categorized into early-onset neonatal sepsis (EONS; ≤ 72 hours) and late-onset neonatal sepsis (LONS; >72 hours) based on the age of onset sepsis and also into culture-proven and suspected neonatal sepsis based on the positivity blood culture result. Neonatal characteristic consisting of gender, mode of delivery, birth weight, gestational age, and maternal risk factors (premature rupture of membranes, preeclampsia/ eclampsia, and prenatal history of steroids) were reported in this study.

Hematological and microbiological profile

Complete blood count performed by automated hematology analyzer and include white blood count (WBC) differential as evaluation of the WBC based on light scattering characteristics. Anemia defined hemoglobin level <14 g/dL. Leukopenia defined total leukocyte count (TLC) <4000/mm^3 while leukocytosis defined TLC >34000/mm^3. Thrombocytopenia defined platelet count <150000/mm^3 and classified into mild thrombocytopenia (100000 - 150000/mm^3), moderate thrombocytopenia (50000 – 99000/mm^3) and severe thrombocytopenia (<50000/mm^3). C-reactive protein (CRP) performed with particle enhanced turbidimetric immunoassay (PETIA) principal. High CRP defined > 10 mg/dL. Blood culture was obtained from venous blood with a minimum volume of 1 ml. Blood culture sample directly inoculated into bottle containing blood culture media and incubated for five days by automatic biochemistry methods.

Data and statistic analysis

Data was listed by number (percentage) and by median (interquartile range) methods. SPSS was used for data dan statistical analyzing. Categorical data were analyzed using Chi-square's test or Fisher's exact test. Numerical data were analyzed using Mann-Whitney U test with significance defined as p-value <0.05.

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Results

Table 1. Proven neonatal sepsis basic characteristics and hematological profiles

Characteristics	Total n (%)	EONS n (%)	LONS n (%)	p
Neonatal Risk Factors				
Gender				0.331*
Male	30 (57.7)	9 (69.2)	21 (53.8)	
Female	22 (42.3)	4 (30.8)	18 (46.2)	
Mode of delivery				0.017**
Vaginal delivery	16 (30.7)	8 (61.5)	9 (23.1)	
Sectio Caesaria	35 (67.3)	5 (38.5)	30 (61.5)	
Birth Weight (grams)	1500 (1250 – 1800)$	1500 (1000 – 1900)$	1500 (1250 – 1750)$	0.589#
< 1000				
1000 – <1500				
1500 – <2500	27 (51.9)	7 (53.8)	20 (51.3)	
> 2500	3 (5.8)	0 (0)	3 (7.7)	
	2 (3.8)	2 (15.4)	0 (0)	
	20 (38.5)	4 (30.8)	16 (41.0)	0.564**
Gestational Age (weeks)	33 (31.5 – 34)$	33 (31 – 34)$	33 (32 – 34)$	0.414#
< 28	5 (9.6)	2 (15.4)	3 (7.7)	
28 – <32	8 (15.4)		5 (12.8)	1.000**
32 – <37	33 (63.5)		26 (66.7)	
≥ 37	6 (11.5)	1 (7.7)	5 (12.8)	
			3 (23.1)	
			7 (53.8)	
Maternal Risk Factors				
Premature rupture of membrane	15 (28.8)	4 (30.8)	11 (28.2)	1.000*
Preeclampsia/ Eclampsia	21 (40.4)	4 (30.8)	17 (43.6)	0.415*
Prenatal Steroid		3 (23.1)	9 (23.1)	1.000*
Hematological Profiles	12 (23.1)			
Hemoglobin level (g/dL)	13. 15 (12.45 – 15.45)$	15.5 (13.0 – 17.7)$	12.9 (11.85 – 14.3)$	0.012#
< 14	32 (61.5)	4 (30.8)	28 (71.8)	0.008*
Total leucocyte count (TLC, /mm^3)	11700 (6405 – 17590)$	11690 (3980 – 14530)$	11710 (6955 – 18785)$	0.315#
<4000	8 (15.4)	4 (30.8)	4 (10.3)	0.096**
Absolute neutrophil count (ANC, /mm^3)	7310 (4920 – 11580)$	5620 (2620 – 11070)$	7400 (4555 – 12130)$	0.492#
Absolute lymphocyte count (/mm^3)	1840 (1120 – 3145)$	1690 (1050 – 2090)$	2080 (1190 – 3245)$	0.286#

Platelet count (/mm^3)	35500 (9000 – 152500)$^{\$}$	117000 (11000 – 231000)$^{\$}$	29000 (9000 – 128000)$^{\$}$	0.375$^{\#}$
100000 – 150000	5 (9.6)	2 (15.4)	3 (7.7)	
50000 – 99000	6 (11.5)	1 (7.7)	5 (12.8)	0.512*
<50000	28 (53.8)	5 (38.5)	23 (59)	
CRP (mg/dL)	6. 4 (3.0 – 13.05)$^{\$}$	6.49 (2.8 – 10.9)$^{\$}$	6.4 (3.2 – 13.2)$^{\$}$	0.575$^{\#}$
High CRP (>10)	20 (38.5)	4 (30.8)	16 (41)	0.541*

$^{\$}$median (inter-quartile range) **Chi-Square's* test **Fisher's exact test $^{\#}$Mann Whitney U-test

Culture-proven neonatal sepsis basic characteristic

During the study period, a total of 492 inborn neonates were admitted to the NICU and 104 neonates fulfill the diagnostic criteria for suspected neonatal sepsis. Identified pathogen from blood culture were confirmed in 52/ 104 (50%) suspected neonatal sepsis. Incidence rate of culture-proven neonatal sepsis in this study was 10.6% from the total NICU admissions. Culture-proven LONS were reported 39/ 52 (75%) from all positive blood culture. The median birth weight and gestational age were 1500 (1250 – 1800) grams and 33 (31.5 – 34) weeks, respectively. Table 1 listed the basic characteristics of culture-proven neonatal sepsis.

Haematological profiles of culture-proven neonatal sepsis

Anemia and thrombocytopenia were the two most common abnormal hematological profile in this study. Thirty two (61.5%) neonates had anemia. Anemia more experienced in LONS than EONS. Neonates with culture-proven LONS significantly had lower hemoglobin level than culture-proven EONS. Only 15.4% (8/52) culture-proven neonatal sepsis had an abnormal leukocyte count. All of these neonates had leukopenia and none had leukocytosis. Thrombocytopenia and high CRP were observed in 75% (39/52) and 38.5% (20/52) cultureproven neonatal sepsis, respectively. The hematological profiles of culture-proven neonatal sepsis are listed in table 1.

Microbiological profile of culture-proven neonatal sepsis

Fifty two neonates identified pathogen isolated from the blood. According to the gram staining, gram negative bacterias were responsible for 75% of culture-proven neonatal sepsis (76.9% EONS and 74.4% LONS). *Coagulase negative Staphylococci (CoNS)* dominated gram positive bacteria, whilst *Klebsiella spp* dominated gram negative bacteria as a cause of culture-proven neonatal sepsis in this study. More than half of the pathogen isolated and identified in this study were *Klebsiella spp* and nearly 90% of them were *Klebsiella pneumoniae.* There was onnly one *Klebsiella pneumoniae* bacteria which did not produce extented sprectrum beta lactamase (ESBL). The three common etiology of culture-proven neonatal sepsis in this study were *Klebsiella pneumonia, Coagulase negative Staphylococci (CoNS)* and *Enterobacter cloacae*. Three neonates isolated more than one identified bacteria from the blood culture (mix pathogen), with *Aeromonas hydrophila – Enterococcus cloacae* in EONS and *Klebsiella pneumonia – Enterococcus faecalis* and *Klebsiella pneumonia ESBL (+) – E. Coli ESBL (+)*

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Commented [PR14]: bacteria

Commented [PR15]: gram negative pathogen

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in LONS. The other of microbiological profiles in culture-proven neonatal sepsis are listed in table 2.

Table 2. Microbiological profile of proven neonatal sepsis

Blood Culture Results	Total n (%)	EONS n (%)	LONS n (%)
Gram positive	10 (19.2)	2 (15.4) 0	8 (20.5) 1
Staphylococcus aureus	1 (1.9)	(0)	(2.6)
Coagulase negative staphylococci (CONS)	9 (17.3)	2 (15.4)	7 (17.9)
Gram negative	39 (75.0)	10 (76.9)	29 (74.4)
Achromobacter spp	1 (1.9)	1 (7.7)	0 (0)
Acinetobacter baumannii	1 (1.9)	0 (0)	1 (2.6)
Bacillus cereus	1 (1.9)	0 (0)	1 (2.6)
Enterobacter cloacae	6 (11.5)	2 (15.4)	4 (10.3)
Klebsiella spp			
Klebsiella oxytoca	2 (3.8)	1 (7.7)	1 (2.6)
Klebsiella ozaenae	1 (1.9)	0 (0)	1 (2.6)
Klebsiella penumonia	25 (48.1)	6 (46.2)	19 (48.7)
Proteus mirabilis	1 (1.9)	0 (0)	1 (2.6)
Serratia marcescens	1 (1.9)	0 (0)	1 (2.6)
Mix pathogens (>1 isolated bacteria)	3 (5.8)	1 (7.7)	2 (5.1)

Commented [PR20]: B. Cereus is gram positive

Discussions

Fifty two neonates (10.6%) were identified as culture-proven neonatal sepsis from 492 inborn neonates during this study. This incidence is comparable to incidences reported in others countries as Ethiopia (9.8%) (9), South Africa (10.3%) (10), Nigeria (10.6%) (11) and Iran (12.17%) (12). Among all of the culture-proven neonatal sepsis, 57.7% were male, 94.2% were weighing less than 2500 grams and 88.5% were born <37 weeks of gestational age. Higher rate of culture-proven neonatal sepsis in male may be related with X-linked immunoregulatory genes and haploid of X chromosome in males (13). Animal studies also have shown a predominance of the Th-1 type immune response, higher production of proinflammatory cytokines (IL-2 and TNF-α) in males rats after LPS administration, and vice versa in female rats (14). The risk of neonatal sepsis increases in proportion to the decrease in birth weight and gestational age (2). Meta-analysis study in Ethiopia reported low birth weight and preterm were 1.42 and 3.36 times more likely to develop neonatal sepsis compared to normal birth weight and term neonates (15). Culture-proven neonatal sepsis dominated in male, preterm and low birth weight also reported by several previous study in India, Saudi Arabia, South Africa, and China (9,16–19).

More neonates born by *sectio caesaria* in this study and significantly higher in the culture proven LONS compared to culture-proven EONS (table 1). However, study from Saudi Arabia, China and Canada found that birth route was have no impact on culture-proven LONS (16,17,20). *Sectio caesaria* may altered colonization with normal human commensals compared to term infants born vaginally, who will have a variety of colonization beneficial microbiota from the maternal vagina, intestinal and skin microbiota immediately after birth, and responsible for the subsequent alteration of the immune system (20,21). Intestinal microbiota dysbiosis may causing bacterial translocation across the intestinal into the bloodstream and increases the risk of LONS (22).

Systemic bacterial infection in an immature neonatal hemopoietic system may lead to malfunctions of the hemopoietic system (19). Anemia, leukopenia and thrombocytopenia were

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observed in 32/52 (61.5%), 8/52 (15.4%), and 39/52 (75%), respectively, in our study. Anemia was observed 12.5% in EONS and 87.5% in LONS. Significantly lower hemogoblin level was detected in LONS than EONS. A study in Iran agreed this study, anemia was detected in 32.7% and was higher in culture-proven LONS (60%) than in EONS (27%). Mean hemoglobin level also reported significantly lower in LONS than EONS (13.5 □ 1.8 and 15 □ 2, p=0.002) (23). Different definition of anemia was used in a six years retrospective study in China with hemoglobin level < 9 d/dL. Anemia experienced 25% of the neonatal sepsis (19). The development of anemia in septic patient related with alteration in red blood cells (RBCs). Binding of RBCs membrane and the endotoxin (lipopolysaccarhide, LPS) altered the RBCs morphology and rheology and increased the clearance of affected RBCs from circulation (24,25).

Commented [PR23]: Hemoglobin

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Leukopenia was the only abnormal leukocyte count in this study and detected in only 15.4%. Leukopenia only observed 30.8% in EONS and even less 10.3% in LONS. Retrospective study in Saudi Arabia in line with this study, only 9.4% leukopenia observed and developed 25.3% and 9.4% in EONS and LONS (16). About 35% of neonatal sepsis in China also experienced leukopenia, but with the higher limit of leukopenia (<7500/ mm^3) (19). In contrast, leukocytosis only reported by 17.7% in Saudi Arabia, even less by 4% in China and none of neonates experienced leukocytosis in this study. The definition of leukocytosis differs from that used in this study. The total leukocyte count (TLC) is a very unreliable indicator of neonatal infection. A normal TLC does not rule out sepsis because as many as 50% of culture-proven neonatal sepsis within normal limit of TLC (26). The TLC was associated with a higher likelihood ratio of neonatal sepsis only in leukopenia (27). Leukopenia had a higher spesificity compared to leukocytosis (87.5% vs. 25%) as predictor of culture-proven neonatal sepsis, however the sensitivity was low (20%) (28).

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Three-quarter of culture-proven neonatal sepsis in this study experienced thrombocytopenia. As many as 72% of them classified as severe thrombocytopenia. In a study conducted by Guo et al (19), thrombocytopenia detected in 29% and 43% of them had a severe thrombocytopenia. Thrombocytopenia also found in 26.5% neonatal sepsis in Saudi Arabia, even with a different limit (16). The pathogenesis of thrombocytopenia in neonatal sepsis is a combination of increased destruction and inadequate production of platelets, even the mechanism is not fully understood. A direct pathophysiological mechanism of endotoxins produced by gram negative bacteria may also contribute (29,30). Neonatal sepsis caused by *K. pneumoniae* and *Candida spp* encountered more anemia, more leukopenia and more thrombocytopenia than caused by other pathogens (19). *Klebsiella pneumoniae* was the predominance pathogen in this study and justified for the many cases of anemia, leukopenia and thrombocytopenia, although we did not evaluate fungi as the cause of the culture-proven neonatal sepsis.

High CRP (>10 mg/dL) only took place in 38.5% culture-proven neonatal sepsis in this study. High CRP was positive in only 30.8% and 41% of the EONS and LONS, respectively. Lesser positive CRP, even with lesser cut-off (>5 g/dL), was reported only 17.3% from total positive blood culture sample in Iran (14.4% EONS and 30% LONS) (23). Not concordance with this study, 58% neonates in Saudi Arabia had a high CRP and significantly more common in LONS than EONS (76.4% and 33.3%, p<0.001). Altough the study did not explained the meaning of high CRP used (16). C-reactive protein (CRP) is an acute phase reactant which most frequently used laboratory tests in the diagnosis of neonatal sepsis (31). However, neonates with fetal hypoxia, respiratory distress syndrome (RDS), meconium aspiration, after trauma/ surgery, and

after immunizations also had an elevated CRP. A false-positive rate of 8% has been found in healthy neonates (2). A meta-analysis from 31 study reported the sensitivity and spesificity of CRP in diagnosting neonatal sepsis were 69% (95% CI 66 – 71%) and 77% (95% CI 76 – 78%), respectively, with area under curve (AUC) 0.8458 (32).

In all culture-proven neonatal sepsis and by the age of neonatal sepsis (EONS and LONS), gram negative bacteria dominate the isolated pathogens in this study over gram positive bacteria. The most common identified pathogens were *Klebsiella pneumonia*, and followed by *Coagulase negative Staphylococci (CoNS)* and *Enterobacter cloacae*, respectively. *Klebsiella pneumoniae* as the main pathogen in culture-proven neonatal sepsis also reported by Hasibuan et al in Medan (33) and Wilar et al in Manado (34) in other Indonesian tertiary NICU. These mostly isolated gram negative bacteria were also reported in previous study in developing countries by Arowosegbea et al in 2017 (11), Hematyar et al in 2017 (23), El-Mashad et al in 2019 (35), Gao et al in 2019 (36) and also in developed country by Al-Matary et al in 2019 (16).

Arowosegbea et al provided gram negative bacteria for 78.9% of all isolated bacteria in 85 culture-proven neonatal sepsis with 26% case-fatality rate. *Klebsiella* spp (31.6%), *Enterobacter* spp (21.1%) and CoNS (15.8%) were the predominantly isolated pathogen reported in Nigeria, identically with isolated pathogen in this study. Gram negative bacteria was the only recognized pathogen in EONS and dominated with *Klebsiella* spp. However, gram positive bacteria mainly CoNS dominated the isolated pathogens in LONS (11). Study in Iran reported 72.7% organism isolated from blood culture were gram negative bacteria (*Escherichia coli* and *Klebiella spp*) (23). A 12-month-prospective-study in Egypt also revealed *Klebsiella* (31.03%), gram negative bacteria, as the predominat microorganism followed by *Staphylococcus aures* (20%) (35). Gram negative bacteria (59.8%) dominate over gram positive bacteria also reported in total isolates of neonatal sepsis in China. The four most predominant bacteria were *Klebsiella pneumoniae* (21.9%), *Escherichia coli* (21.9%), *group B Streptococcus* (GBS, 13.2%), and *Staphylococcus aureus* (6.8%). In EONS, GBS (30.0%) and E. coli (20.0%) were dominant, whereas in LONS, *K. pneumoniae* (25.6%) and *E. coli* (22.4%) were dominant (36). Culture-proven neonatal sepsis in Saudi Arabia also confirmed 61.1% of gram negative bacteria in blood culture and was the leading cause of mortality in EONS and LONS. However, the most frequently isolated pathogen based on onset of neonatal sepsis was gram positive bacteria, group B *Streptococcus* in EONS and *Klebsiella spp* in LONS (16).

In contrast, two years retrospective cohort study in India reported the majority of total isolates pathogens were gram positive bacteria. However, the most common isolated microorganism were *Klebsiella spp* (31.1%), *Staphylococcus aures* (24.5%) and CoNS (22.9%). Gram positive bacteria had a higher isolation rate in EONS and LONS, whereas the most frequent microorganism was *Klebsiella* spp in EONS and *Staphylococcus aures* in LONS (18). Different result also reported from culture-proven neonatal sepsis in South Africa with gram positive bacteria constituted 53.4% of all positive blood cultures, mainly CoNS (25%), *E. coli* (20.3%) and *S. aureus* (18.2%). In EONS, gram positive bacteria (mostly CoNS) was dominated eventhough gram negative bacteria (mostly *E. coli* and *Klebsiella*) was domintaed in LONS (9). The causative pathogens of culture-proven neonatal sepsis vary according to geographical differences and countries. It is vary from one hospital and other different hospital and even in the same hospital at different time (31,35).

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Given the conditions of the your hospital, what is your explanation for the existence of these pathogens such as klebsiella ?

Conclusion

Culture-proven neonatal sepsis mostly occurred in preterm and low birth weight neonates. Our study showed that late-onset neonatal sepsis (LONS) were significantly more in infant born by *sectio caesaria* and significantly more anemia experienced than EONS. Anemia and thrombocytopenia were the most common hematological abnormalities. Severe thrombocytopenia was observed in 72% thrombocytopenia neonates. Isolated pathogens were dominated by gram-negative bacteria over gram-positive bacteria, in all culture-proven neonatal sepsis, in EONS and in LONS. *Klebsiella pneumoniae* was the most pathogen identified as cause of neonatal sepsis in this study, followed by *coagulase negative staphylococci (CONS)* and *Enterobacter cloacae*. The causative pathogens causing neonatal sepsis was very much dependent on the local microbial pattern.

Acknowledgements

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Author Contributions

RE and MTU was developed the research design. KRS was responsible for data collection. KRS, RE and MTU was responsible for data analysis and revised the manuscript.

Funding

No funding was received for this study.

Conflicts of Interest

None

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CULTURE-PROVEN NEONATAL SEPSIS IN INDONESIAN TERTIARY NEONATAL INTENSIVE CARE UNIT: A HEMATOLOGICAL AND MICROBIOLOGICAL PROFILE

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ABSTRACT

Background : Neonatal sepsis is the third leading cause of neonatal death in the world. The patterns of pathogen causing neonatal sepsis varies in many countries.

Objectives : This study was aimed to identify hematological and microbiological profile of culture-proven neonatal sepsis in Indonesian tertiary neonatal intensive care unit (NICU).

Methods : Hospital based cross-sectional study was conducted in all inborn neonates that were suspected sepsis neonatal over a period of six months from April to September 2019. Complete blood count, c-reactive protein (CRP) and blood culture were examined before antibiotic administration. Statistical analysis were were calculated based on Chi-Square's Test and Mann-Whitney U test and $p < 0.05$ considered significant.

Results: One hundred four inborn neonates admitted to NICU and diagnosed with suspected neonatal sepsis were recruited. Culture-proven neonatal sepsis were confirmed in 52 (50%) neonates, 13 (25%) in early-onset neonatal sepsis (EONS) and 39 (75%) in late-onset neonatal sepsis (LONS). The most common abnormal hematological profile were anemia and thrombocytopenia, with amount of 61.5% and 75%, respectively. High CRP only detected in 36.4% and only 18.5% experienced leukopenia. Gram negative bacteria responsible in 75% from total isolated pathogens. *Klebsiella pneumonia* accounted for 48.1% followed by *coagulase negative staphylococci* (CONS) for 17.3% and *Enterobacter cloacae* for 11.5%.

Conclusion: Anemia and thrombocytopenia were the top two hematological profile of culture-proven neonatal sepsis. Most causes of culture-proven neonatal sepsis was gram negative bacteria and most pathogens was *Klebsiella pneumonia*.

Keywords: *neonatal sepsis, bacteremia, blood culture, gram negative bacteria, Klebsiella*

Introduction

Neonatal sepsis is a clinical syndrome that occurs in infants in the first month that characterized by systemic infection and bacteremia. It is classified into two types based on the age of onset of the findings, early-onset neonatal sepsis (EONS) occurring within ≤ 72 hours and late-onset neonatal sepsis (LONS) occurring after 72 hours (1–4). Neonatal sepsis is the third leading cause of neonatal death in the world (5). There has been a decrease in neonatal mortality between 1990 and 2017, from 36.6 (35.5 - 37.8) to 18 (17 - 19.9) deaths per 1000 live births. Nonetheless, the Sustainable Development Goals (SDGs) stipulate that all countries should aim to reduce the neonatal mortality rate (NMR) to 12 deaths per 1000 live births or less by 2030 (6).

Blood culture is the gold standard examination for neonatal sepsis diagnosis (2). The presence of pathogen isolation on blood culture examination or positive PCR examination in neonates with clinically neonatal sepsis is categorized as a culture-proven neonatal sepsis (7). The patterns of pathogen causing neonatal sepsis differ in many countries according to the local microbial pattern and to the onset of neonatal sepsis (2,8). Little information is known about the incidence and distribution of neonatal sepsis pathogens and profile in Indonesia, especially in Surabaya, so this study aims to provide an overview of the hematological and

microbiological profile of culture-proven neonatal in tertiary neonatal intensive care unit in Indonesia.

Methods

Study design and ethical clearance

This hospital based observational analytic cross-sectional study conducted for 6 months, between April and September 2019. An ethical clearance certificate was approved by Ethical Committee in Health Research of Dr. Soetomo General Academic Hospital Surabaya (ref. no. 1047/KEPK/III/2019).

Study population

All inborn neonates admitted to the NICU and with suspected sepsis neonatal were eligible in this study. For this study, diagnostic criteria for suspected neonatal sepsis was according to definitions of blood stream infection in the newborn by Haque (2005) (7). Suspected neonatal sepsis was the presence of clinically neonatal sepsis accompanied by increasing c-reactive protein/ CRP (>10 mg/dL or >2 SD above normal value) or at least 2 abnormal inflammatory laboratory results. Local Clinical Practice Guidelines are carried out by examining complete blood count, c-reactive protein (CRP) and blood culture before administered antibiotics. Neonatal sepsis was categorized into early-onset neonatal sepsis (EONS; ≤ 72 hours) and late-onset neonatal sepsis (LONS; >72 hours) based on the age of onset sepsis and also into culture-proven and suspected neonatal sepsis based on the positivity blood culture result. Neonatal characteristic consisting of gender, mode of delivery, birth weight, gestational age, and maternal risk factors (premature rupture of membranes, preeclampsia/ eclampsia, and prenatal history of steroids) were reported in this study.

Hematological and microbiological profile

Complete blood count performed by automated hematology analyzer and include white blood count (WBC) differential as evaluation of the WBC based on light scattering characteristics. Anemia defined hemoglobin level <14 g/dL. Leukopenia defined total leukocyte count (TLC) <4000/mm^3 while leukocytosis defined TLC >34000/mm^3. Thrombocytopenia defined platelet count <150000/mm^3 and classified into mild thrombocytopenia (100000 - 150000/mm^3), moderate thrombocytopenia (50000 – 99000/mm^3) and severe thrombocytopenia (<50000/mm^3). C-reactive protein (CRP) performed with particle enhanced turbidimetric immunoassay (PETIA) principal. High CRP defined > 10 mg/dL. Blood culture was obtained from venous blood with a minimum volume of 1 ml and directly inoculated into Bactec® bottle containing liquid blood culture media. The specimens were sent to the Clinical Microbiology Laboratory of Dr. Soetomo General Academic Hospital and incubated at 35 – 37^oC for five days or until indicator of bacterial growth to be positive. Medium that showed bacterial growth was subcultured into solid culture media (blood agar plate, MacConkey agar plate and chocolate agar plate) and incubated for 18-24 hours in aerobic condition. Identification and susceptibility test were carried out using the BD Phoenix semi-automated system, and interpreted as extended spectrum beta lactamase (ESBL) based on Clinical and Laboratory Standards Institute (CLSI) 2015 (9).

Data and statistical analysis

Data was listed by number (percentage) and by median (interquartile range) methods. SPSS was used for data and statistical analyzing. Categorical data were analyzed using Chi-square's test or Fisher's exact test. Numerical data were analyzed using Mann-Whitney U test with significance defined as p-value <0.05.

Results

Table 1. Proven neonatal sepsis basic characteristics and hematological profiles

Characteristics	Total n (%)	EONS n (%)	LONS n (%)	p
Neonatal Risk Factors				
Gender				0.331*
Male	30 (57.7)	9 (69.2)	21 (53.8)	
Female	22 (42.3)	4 (30.8)	18 (46.2)	
Mode of delivery				0.017**
Vaginal delivery	16 (30.7)	8 (61.5)	9 (23.1)	
Sectio Caesaria	35 (67.3)	5 (38.5)	30 (61.5)	
Birth Weight (grams)	1500 (1250 – 1800)$	1500 (1000 – 1900)$	1500 (1250 – 1750)$	0.589#
< 1000	2 (3.8)	2 (15.4)	0 (0)	
1000 – <1500	20 (38.5)	4 (30.8)	16 (41.0)	0.564**
1500 – <2500	27 (51.9)	7 (53.8)	20 (51.3)	
≥ 2500	3 (5.8)	0 (0)	3 (7.7)	
Gestational Age (weeks)	33 (31.5 – 34)$	33 (31 – 34)$	33 (32 – 34)$	0.414#
< 28	5 (9.6)	2 (15.4)	3 (7.7)	
28 – <32	8 (15.4)	3 (23.1)	5 (12.8)	1.000**
32 – <37	33 (63.5)	7 (53.8)	26 (66.7)	
≥ 37	6 (11.5)	1 (7.7)	5 (12.8)	
Maternal Risk Factors				
Premature rupture of membrane	15 (28.8)	4 (30.8)	11 (28.2)	1.000*
Preeclampsia/ Eclampsia	21 (40.4)	4 (30.8)	17 (43.6)	0.415*
Prenatal Steroid	12 (23.1)	3 (23.1)	9 (23.1)	1.000*
Hematological Profiles				
Hemoglobin level (g/dL)	13. 15 (12.45 – 15.45)$	15.5 (13.0 – 17.7)$	12.9 (11.85 – 14.3)$	0.012#
< 14	32 (61.5)	4 (30.8)	28 (71.8)	0.008*
Total leucocyte count (TLC, /mm^3)	11700 (6405 – 17590)$	11690 (3980 – 14530)$	11710 (6955 – 18785)$	0.315#
<4000	8 (15.4)	4 (30.8)	4 (10.3)	0.096**
Absolute neutrophil count (ANC, /mm^3)	7310 (4920 – 11580)$	5620 (2620 – 11070)$	7400 (4555 – 12130)$	0.492#
Absolute lymphocyte count (/mm^3)	1840 (1120 – 3145)$	1690 (1050 – 2090)$	2080 (1190 – 3245)$	0.286#
Platelet count (/mm^3)	35500 (9000 – 152500)$	117000 (11000 – 231000)$	29000 (9000 – 128000)$	0.375#
100000 – 150000	5 (9.6)	2 (15.4)	3 (7.7)	
50000 – 99000	6 (11.5)	1 (7.7)	5 (12.8)	0.512*
<50000	28 (53.8)	5 (38.5)	23 (59)	
CRP (mg/dL)	6. 4 (3.0 – 13.05)$	6.49 (2.8 – 10.9)$	6.4 (3.2 – 13.2)$	0.575#
High CRP (>10)	20 (38.5)	4 (30.8)	16 (41)	0.541*

$median (inter-quartile range) *_Chi-Square's_ test **Fisher's exact test #Mann Whitney U-test

Culture-proven neonatal sepsis basic characteristic

During the study period, a total of 492 inborn neonates were admitted to the NICU and 104 neonates fulfill the diagnostic criteria for suspected neonatal sepsis. Identified pathogen from blood culture were confirmed in 52/ 104 (50%) suspected neonatal sepsis. Incidence rate of culture-proven neonatal sepsis in this study was 10.6% from the total NICU admissions. Culture-proven LONS were reported 39/ 52 (75%) from all positive blood culture. The median birth weight and gestational age were 1500 (1250 – 1800) grams and 33 (31.5 – 34) weeks, respectively. Table 1 listed the basic characteristics of culture-proven neonatal sepsis.

Hematological profiles of culture-proven neonatal sepsis

Anemia and thrombocytopenia were the two most common abnormal hematological profile in this study. Thirty two (61.5%) neonates had anemia. Anemia more experienced in LONS than EONS. Neonates with culture-proven LONS significantly had lower hemoglobin level than culture-proven EONS. Only 15.4% (8/52) culture-proven neonatal sepsis had an abnormal leukocyte count. All of these neonates had leukopenia and none had leukocytosis. Thrombocytopenia and high CRP were observed in 75% (39/52) and 38.5% (20/52) culture-proven neonatal sepsis, respectively. The hematological profiles of culture-proven neonatal sepsis are listed in table 1.

Microbiological profile of culture-proven neonatal sepsis

Fifty two neonates identified pathogen isolated from the blood. According to the gram staining, gram negative bacteria were responsible for 75% of culture-proven neonatal sepsis (76.9% EONS and 74.4% LONS). *Coagulase negative Staphylococci (CoNS)* dominated gram positive bacteria, whilst *Klebsiella spp* dominated gram negative bacteria as a cause of culture-proven neonatal sepsis in this study. More than half of the gram negative pathogen isolated and identified in this study were *Klebsiella spp* and nearly 90% of them were *Klebsiella pneumoniae.* There was only one *Klebsiella pneumoniae* bacteria which did not produce extended spectrum beta lactamase (ESBL). The three common etiology of culture-proven neonatal sepsis in this study were *Klebsiella pneumonia, Coagulase negative Staphylococci (CoNS)* and *Enterobacter cloacae*. Three neonates isolated more than one identified bacteria from the blood culture (mix pathogen), with *Aeromonas hydrophila – Enterococcus cloacae* in EONS and *Klebsiella pneumonia – Enterococcus faecalis* and *Klebsiella pneumonia ESBL(+) – E. Coli ESBL(+)* in LONS. The other of microbiological profiles in culture-proven neonatal sepsis are listed in table 2.

Table 2. Microbiological profile of proven neonatal sepsis

Blood Culture Results	Total n (%)	EONS n (%)	LONS n (%)
Gram positive	11 (21.2)	2 (15.4)	9 (22.5)
Staphylococcus aureus	1 (1.9)	0 (0)	1 (2.6)
Coagulase negative staphylococci (CONS)	9 (17.3)	2 (15.4)	7 (17.9)
Bacillus cereus	1 (1.9)	0 (0)	1 (2.6)
Gram negative	38 (73.0)	10 (76.9)	29 (74.4)
Achromobacter spp	1 (1.9)	1 (7.7)	0 (0)
Acinetobacter baumannii	1 (1.9)	0 (0)	1 (2.6)
Enterobacter cloacae	6 (11.5)	2 (15.4)	4 (10.3)
Klebsiella spp			
Klebsiella oxytoca	2 (3.8)	1 (7.7)	1 (2.6)
Klebsiella ozaenae	1 (1.9)	0 (0)	1 (2.6)
Klebsiella penumonia	25 (48.1)	6 (46.2)	19 (48.7)
Proteus mirabilis	1 (1.9)	0 (0)	1 (2.6)
Serratia marcescens	1 (1.9)	0 (0)	1 (2.6)
Mix pathogens (>1 isolated bacteria)	3 (5.8)	1 (7.7)	2 (5.1)

Discussions

Fifty two neonates (10.6%) were identified as culture-proven neonatal sepsis from 492 inborn neonates during this study. This incidence is comparable to incidences reported in others countries as Ethiopia (9.8%) (10), South Africa (10.3%) (11), Nigeria (10.6%) (12) and Iran (12.17%) (13). Among all of the culture-proven neonatal sepsis, 57.7% were male, 94.2% were weighing less than 2500 grams and 88.5% were born <37 weeks of gestational age. Higher rate of culture-proven neonatal sepsis in male may be related with X-linked immunoregulatory genes and haploid of X chromosome in males (14). Animal studies also

have shown a predominance of the Th-1 type immune response, higher production of pro-inflammatory cytokines (IL-2 and TNF-α) in males rats after LPS administration, and vice versa in female rats (15). The risk of neonatal sepsis increases in proportion to the decrease in birth weight and gestational age (2). Meta-analysis study in Ethiopia reported low birth weight and preterm were 1.42 and 3.36 times more likely to develop neonatal sepsis compared to normal birth weight and term neonates (16). Culture-proven neonatal sepsis dominated in male, preterm and low birth weight also reported by several previous study in India, Saudi Arabia, South Africa, and China (10,17–20).

More neonates born by *sectio caesaria* in this study and significantly higher in the culture-proven LONS compared to culture-proven EONS (table 1). However, study from Saudi Arabia, China and Canada found that birth route was have no impact on culture-proven LONS (17,18,21). *Sectio caesaria* may altered colonization with normal human commensals compared to term infants born vaginally, who will have a variety of colonization beneficial microbiota from the maternal vagina, intestinal and skin microbiota immediately after birth, and responsible for the subsequent alteration of the immune system (21,22). Intestinal microbiota dysbiosis may causing bacterial translocation across the intestinal into the bloodstream and increases the risk of LONS (23).

Systemic bacterial infection in an immature neonatal hematopoietic system may lead to malfunctions of the hematopoietic system (20). Anemia, leukopenia and thrombocytopenia were observed in 32/52 (61.5%), 8/52 (15.4%), and 39/52 (75%), respectively, in our study. Anemia was observed 12.5% in EONS and 87.5% in LONS. Significantly lower Hemogoblin (Hb) level was detected in LONS than EONS. A study in Iran agreed this study, anemia was detected in 32.7% and was higher in culture-proven LONS (60%) than in EONS (27%). Mean hemoglobin level also reported significantly lower in LONS than EONS (13.5 ± 1.8 and 15 ± 2, p=0.002) (24). Different definition of anemia was used in a six years retrospective study in China with hemoglobin level < 9 d/dL. Anemia experienced 25% of the neonatal sepsis (20). The development of anemia in septic patient related with alteration in red blood cells (RBCs). Binding of RBCs membrane and the endotoxin (lipopolysaccaride, LPS) altered the RBCs morphology and rheology and increased the clearance of affected RBCs from circulation (25,26).

Leukopenia was the only abnormal leukocyte count in this study and detected in only 15.4%. Leukopenia only observed 30.8% in EONS and even less 10.3% in LONS. Retrospective study in Saudi Arabia in line with this study, only 9.4% leukopenia observed and developed 25.3% and 9.4% in EONS and LONS (17). About 35% of neonatal sepsis in China also experienced leukopenia, but with the higher limit of leukopenia (<7500/ mm^3) (20). In contrast, leukocytosis only reported by 17.7% in Saudi Arabia, even less by 4% in China and none of neonates experienced leukocytosis in this study. The definition of leukocytosis differs from that used in this study. The total leukocyte count (TLC) is a very unreliable indicator of neonatal infection. A normal TLC does not rule out sepsis because as many as 50% of culture-proven neonatal sepsis within normal limit of TLC (27). The TLC was associated with a higher likelihood ratio of neonatal sepsis only in leukopenia (28). Leukopenia had a higher specificity compared to leukocytosis (87.5% vs. 25%) as predictor of culture-proven neonatal sepsis, however the sensitivity was low (20%) (29).

Three-quarter of culture-proven neonatal sepsis in this study experienced thrombocytopenia. As many as 72% of them classified as severe thrombocytopenia. In a study conducted by Guo et al (20), thrombocytopenia detected in 29% and 43% of them had a severe thrombocytopenia.

Thrombocytopenia also found in 26.5% neonatal sepsis in Saudi Arabia, even with a different limit (17). The pathogenesis of thrombocytopenia in neonatal sepsis is a combination of increased destruction and inadequate production of platelets, even the mechanism is not fully understood. A direct pathophysiological mechanism of endotoxins produced by gram negative bacteria may also contribute (30,31). Neonatal sepsis caused by *K. pneumoniae* and *Candida spp* encountered more anemia, more leukopenia and more thrombocytopenia than caused by other pathogens (20). *Klebsiella pneumoniae* was the predominance pathogen in this study and justified for the many cases of anemia, leukopenia and thrombocytopenia, although we did not evaluate fungi as the cause of the culture-proven neonatal sepsis.

High CRP (>10 mg/dL) only took place in 38.5% culture-proven neonatal sepsis in this study. High CRP was positive in only 30.8% and 41% of the EONS and LONS, respectively. Lesser positive CRP, even with lesser cut-off (>5 g/dL), was reported only 17.3% from total positive blood culture sample in Iran (14.4% EONS and 30% LONS) (24). Not concordance with this study, 58% neonates in Saudi Arabia had a high CRP and significantly more common in LONS than EONS (76.4% and 33.3%, $p<0.001$). Altough the study did not explained the meaning of high CRP used (17). C-reactive protein (CRP) is an acute phase reactant which most frequently used laboratory tests in the diagnosis of neonatal sepsis (32). However, neonates with fetal hypoxia, respiratory distress syndrome (RDS), meconium aspiration, after trauma/ surgery, and after immunizations also had an elevated CRP. A false-positive rate of 8% has been found in healthy neonates (2). A meta-analysis from 31 study reported the sensitivity and spesificity of CRP in diagnosing neonatal sepsis were 69% (95% CI 66 – 71%) and 77% (95% CI 76 – 78%), respectively, with area under curve (AUC) 0.8458 (33).

In all culture-proven neonatal sepsis and by the age of neonatal sepsis (EONS and LONS), gram negative bacteria dominate the isolated pathogens in this study over gram positive bacteria. The most common identified pathogens were *Klebsiella pneumonia*, and followed by *Coagulase negative Staphylococci (CoNS)* and *Enterobacter cloacae*, respectively. *Klebsiella pneumoniae* as the main pathogen in culture-proven neonatal sepsis also reported by Hasibuan et al in Medan (34) and Wilar et al in Manado (35) in other Indonesian tertiary NICU. These mostly isolated gram negative bacteria were also reported in previous study in developing countries by Arowosegbea et al in 2017 (12), Hematyar et al in 2017 (24), El-Mashad et al in 2019 (36), Gao et al in 2019 (37) and also in developed country by Al-Matary et al in 2019 (17).

Arowosegbea et al provided gram negative bacteria for 78.9% of all isolated bacteria in 85 culture-proven neonatal sepsis with 26% case-fatality rate. *Klebsiella* spp (31.6%), *Enterobacter* spp (21.1%) and CoNS (15.8%) were the predominantly isolated pathogen reported in Nigeria, identically with isolated pathogen in this study. Gram negative bacteria was the only recognized pathogen in EONS and dominated with *Klebsiella* spp. However, gram positive bacteria mainly CoNS dominated the isolated pathogens in LONS (12). Study in Iran reported 72.7% organism isolated from blood culture were gram negative bacteria (*Escherichia coli* and *Klebiella spp*) (24). A 12-month-prospective-study in Egypt also revealed *Klebsiella* (31.03%), gram negative bacteria, as the predominat microorganism followed by *Staphylococcus aures* (20%) (36). Gram negative bacteria (59.8%) dominate over gram positive bacteria also reported in total isolates of neonatal sepsis in China. The four most predominant bacteria were *Klebsiella pneumoniae* (21.9%), *Escherichia coli* (21.9%), *group B Streptococcus* (GBS, 13.2%), and *Staphylococcus aureus* (6.8%). In EONS, GBS (30.0%) and E. coli (20.0%) were dominant, whereas in LONS, *K. pneumoniae* (25.6%) and *E. coli* (22.4%) were dominant (37). Culture-proven neonatal sepsis in Saudi Arabia also confirmed 61.1% of

gram negative bacteria in blood culture and was the leading cause of mortality in EONS and LONS. However, the most frequently isolated pathogen based on onset of neonatal sepsis was gram positive bacteria, group B *Streptococcus* in EONS and *Klebsiella spp* in LONS (17).

In contrast, two years retrospective cohort study in India reported the majority of total isolates pathogens were gram positive bacteria. However, the most common isolated microorganism were *Klebsiella spp* (31.1%), *Staphylococcus aures* (24.5%) and CoNS (22.9%). Gram positive bacteria had a higher isolation rate in EONS and LONS, whereas the most frequent microorganism was *Klebsiella* spp in EONS and *Staphylococcus aures* in LONS (19). Different result also reported from culture-proven neonatal sepsis in South Africa with gram positive bacteria constituted 53.4% of all positive blood cultures, mainly CoNS (25%), *E. coli* (20.3%) and *S. aureus* (18.2%). In EONS, gram positive bacteria (mostly CoNS) was dominated eventhough gram negative bacteria (mostly *E. coli* and *Klebsiella*) was domintaed in LONS (10). The causative pathogens of culture-proven neonatal sepsis vary according to geographical differences and countries. It is vary from one hospital and other different hospital and even in the same hospital at different time (32,36). In 2015, *Klebsiella pneumoniae* was reported as the predominant cause of bacteremia in preterm neonates admitted to NICU in Dr. Soetomo General Academic Hospital (38). High incidence of ESBL producer strain among *Klebsiella pneumoniae* in Dr. Soetomo General Academic Hospital was also reported as 50.28% in 2011 (39). The high incidence of nosocomial infections is the reason for the high infection of *Klebsiella pneumoniae* and especially the ESBL strains in this study.

Bacillus cereus is a rare pathogenic bacterium, but in premature neonatesit can be potentially serious infection of the bloodstream, lungs, and central nervous system (40). In our study, *Bacillus cereus* was isolated in one pretem neonates (34 weeks of gestational age and 1900 grams) as a cause of neonatal sepsis. A previous study in 2013 reported the first report of severe *Bacillus cereus* infections in premature neonates possibly originating from pooled breast milk (PBM). However, the origin of the B cereus strains in the breastmilk remain unknown (41). In our study, we did not perform environmental sample evaluation and bacteriological analysis from PBM or from breast milk itself. Meanwhile, breast milk is the main choice for nutrition in premature neonates used in our NICU.

Conclusion

Culture-proven neonatal sepsis mostly occurred in preterm and low birth weight neonates. Our study showed that late-onset neonatal sepsis (LONS) were significantly more in infant born by *sectio caesaria* and significantly more anemia experienced than EONS. Anemia and thrombocytopenia were the most common hematological abnormalities. Severe thrombocytopenia was observed in 72% thrombocytopenia neonates. Isolated pathogens were dominated by gram negative bacteria over gram positive bacteria, in all culture-proven neonatal sepsis, in EONS and in LONS. *Klebsiella pneumoniae* was the most pathogen identified as cause of neonatal sepsis in this study, followed by *coagulase negative staphylococci (CONS)* and *Enterobacter cloacae*. The causative pathogens causing neonatal sepsis was very much dependent on the local microbial pattern.

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Author Contributions
MTU and ADWD were developed the research design. KRS was responsible for data collection. KRS and MTU was responsible for data analysis and revised the manuscript.

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Conflicts of Interest
None

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CULTURE-PROVEN NEONATAL SEPSIS IN INDONESIAN TERTIARY NEONATAL INTENSIVE CARE UNIT: A HEMATOLOGICAL AND MICROBIOLOGICAL PROFILE

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ABSTRACT

Background : Neonatal sepsis is the third leading cause of neonatal death in the world. The patterns of pathogen causing neonatal sepsis varies in many countries.

Objectives : This study was aimed to identify hematological and microbiological profile of culture-proven neonatal sepsis in Indonesian tertiary neonatal intensive care unit (NICU).

Methods : Hospital based cross-sectional study was conducted in all inborn neonates with suspected sepsis neonatal over a period of six months from April to September 2019. Complete blood count, c-reactive protein (CRP) and blood culture were examined before antibiotic administration. Statistical analysis were using Chi-Square's Test and Mann-Whitney U test and $p < 0.05$ considered significant.

Results: One hundred four inborn neonates admitted to NICU and diagnosed with suspected neonatal sepsis were recruited. Culture-proven neonatal sepsis were confirmed in 52 (50%) neonates, 13 (25%) in early-onset neonatal sepsis (EONS) and 39 (75%) in late-onset neonatal sepsis (LONS). The most common abnormal hematological profile were anemia and thrombocytopenia, with amount of 61.5% and 75%, respectively. High CRP only detected in 36.4% and only 18.5% experienced leukopenia. Gram-negative bacteria responsible in 75% from total isolated pathogens. *Klebsiella pneumonia* accounted for 48.1% followed by *coagulase negative staphylococci* (CONS) for 17.3% and *Enterobacter cloacae* for 11.5%.

Conclusion: Anemia and thrombocytopenia were the top two hemaotological profile of culture-proven neonatal sepsis. Most causes of culture-proven neonatal sepsis was gramnegative bacteria and most pathogens was *Klebsiella pneumonia*.

Keywords: *neonate, neonatal sepsis, gram-negative bacteria, Klebsiella, Indonesia*

Introduction

Neonatal sepsis is a clinical syndrome that occurs in infants in the first month of life characterized by systemic infection and bacteremia. It is classified into two types based on the age of onset of the findings, early-onset neonatal sepsis (EONS) occurring within ≤ 72 hours and late-onset neonatal sepsis (LONS) occurring after 72 hours (1–4). Neonatal sepsis is the third leading cause of neonatal death in the world (5). There has been a decrease in neonatal mortality between 1990 and 2017, from 36.6 (35.5 - 37.8) to 18 (17 - 19.9) deaths per 1000 live births. Nonetheless, the SDGs stipulate that all countries should aim to reduce the neonatal mortality rate (NMR) to 12 deaths per 1000 live births or less by 2030 (6).

Blood culture is the gold standard examination for neonatal sepsis diagnosis (2). The presence of pathogen isolation on blood culture examination or positive PCR examination in neonates with clinically neonatal sepsis is categorized as a culture-proven neonatal sepsis (7).

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The patterns of pathogen causing neonatal sepsis differ in many countries according to the local microbial pattern and to the onset of neonatal sepsis (2,8). Little is known about the incidence and distribution of neonatal sepsis pathogens and profile in Indonesia, especially in Surabaya, so this study aims to provide an overview of the hematological and microbiological profile of culture-proven neonatal in tertiary neonatal intensive care unit in Indonesia.

Methods

Study design and ethical clearance

This hospital based observational analytic cross-sectional study conducted for 6 months, between April and September 2019. An ethical clearance certificate was approved by Ethical Committee in Health Research of Dr. Soetomo General Academic Surabaya (ref. no. 1047/KEPK/III/2019).

Study population

All inborn neonates admitted to the NICU and with suspected sepsis neonatal were eligible in this study. For this study, diagnostic criteria for suspected neonatal sepsis was according to definitions of blood stream infection in the newborn by Haque (2005) (7). Suspected neonatal sepsis was the presence of clinically neonatal sepsis accompanied by increasing CRP (>10 mg/dL or >2 SD above normal value) or at least 2 abnormal inflammatory laboratory results. Local Clinical Practice Guidelines are carried out by examining complete blood count, creactive protein (CRP) and blood culture before administered antibiotics. Neonatal sepsis was categorized into early-onset neonatal sepsis (EONS; ≤ 72 hours) and late-onset neonatal sepsis (LONS; >72 hours) based on the age of onset sepsis and also into culture-proven and suspected neonatal sepsis based on the positivity blood culture result. Neonatal characteristic consisting of gender, mode of delivery, birth weight, gestational age, and maternal risk factors (premature rupture of membranes, preeclampsia/ eclampsia, and prenatal history of steroids) were reported in this study.

Hematological and microbiological profile

Complete blood count performed by automated hematology analyzer and include white blood count (WBC) differential as evaluation of the WBC based on light scattering characteristics. Anemia defined hemoglobin level <14 g/dL. Leukopenia defined total leukocyte count (TLC) <4000/mm^3 while leukocytosis defined TLC >34000/mm^3. Thrombocytopenia defined platelet count <150000/mm^3 and classified into mild thrombocytopenia (100000 - 150000/mm^3), moderate thrombocytopenia (50000 – 99000/mm^3) and severe thrombocytopenia (<50000/mm^3). C-reactive protein (CRP) performed with particle enhanced turbidimetric immunoassay (PETIA) principal. High CRP defined > 10 mg/dL. Blood culture was obtained from venous blood with a minimum volume of 1 ml. Blood culture sample directly inoculated into bottle containing blood culture media and incubated for five days by automatic biochemistry methods.

Data and statistic analysis

Data was listed by number (percentage) and by median (interquartile range) methods. SPSS was used for data dan statistical analyzing. Categorical data were analyzed using Chi-square's test or Fisher's exact test. Numerical data were analyzed using Mann-Whitney U test with significance defined as p-value <0.05.

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Results

Table 1. Proven neonatal sepsis basic characteristics and hematological profiles

Characteristics	Total n (%)	EONS n (%)	LONS n (%)	p
Neonatal Risk Factors				
Gender				0.331*
Male	30 (57.7)	9 (69.2)	21 (53.8)	
Female	22 (42.3)	4 (30.8)	18 (46.2)	
Mode of delivery				0.017**
Vaginal delivery	16 (30.7)	8 (61.5)	9 (23.1)	
Sectio Caesaria	35 (67.3)	5 (38.5)	30 (61.5)	
Birth Weight (grams)	1500 (1250 – 1800)$	1500 (1000 – 1900)$	1500 (1250 – 1750)$	0.589#
< 1000				
1000 – <1500				
1500 – <2500	27 (51.9)	7 (53.8)	20 (51.3)	
> 2500	3 (5.8)	0 (0)	3 (7.7)	
	2 (3.8)	2 (15.4)	0 (0)	
	20 (38.5)	4 (30.8)	16 (41.0)	0.564**
Gestational Age (weeks)	33 (31.5 – 34)$	33 (31 – 34)$	33 (32 – 34)$	0.414#
< 28	5 (9.6)	2 (15.4)	3 (7.7)	
28 – <32	8 (15.4)		5 (12.8)	1.000**
32 – <37	33 (63.5)		26 (66.7)	
≥ 37	6 (11.5)	1 (7.7)	5 (12.8)	
			3 (23.1)	
			7 (53.8)	
Maternal Risk Factors				
Premature rupture of membrane	15 (28.8)	4 (30.8)	11 (28.2)	1.000*
Preeclampsia/ Eclampsia	21 (40.4)	4 (30.8)	17 (43.6)	0.415*
Prenatal Steroid		3 (23.1)	9 (23.1)	1.000*
Hematological Profiles	12 (23.1)			
Hemoglobin level (g/dL)	13. 15 (12.45 – 15.45)$	15.5 (13.0 – 17.7)$	12.9 (11.85 – 14.3)$	0.012#
< 14	32 (61.5)	4 (30.8)	28 (71.8)	0.008*
Total leucocyte count (TLC, /mm^3)	11700 (6405 – 17590)$	11690 (3980 – 14530)$	11710 (6955 – 18785)$	0.315#
<4000	8 (15.4)	4 (30.8)	4 (10.3)	0.096**
Absolute neutrophil count (ANC, /mm^3)	7310 (4920 – 11580)$	5620 (2620 – 11070)$	7400 (4555 – 12130)$	0.492#
Absolute lymphocyte count (/mm^3)	1840 (1120 – 3145)$	1690 (1050 – 2090)$	2080 (1190 – 3245)$	0.286#

Platelet count (/mm^3)	35500 (9000 – 152500)$^{\$}$	117000 (11000 – 231000)$^{\$}$	29000 (9000 – 128000)$^{\$}$	0.375$^{\#}$
100000 – 150000	5 (9.6)	2 (15.4)	3 (7.7)	
50000 – 99000	6 (11.5)	1 (7.7)	5 (12.8)	0.512*
<50000	28 (53.8)	5 (38.5)	23 (59)	
CRP (mg/dL)	6. 4 (3.0 – 13.05)$^{\$}$	6.49 (2.8 – 10.9)$^{\$}$	6.4 (3.2 – 13.2)$^{\$}$	0.575$^{\#}$
High CRP (>10)	20 (38.5)	4 (30.8)	16 (41)	0.541*

$^{\$}$median (inter-quartile range) **Chi-Square's* test **Fisher's exact test $^{\#}$Mann Whitney U-test

Culture-proven neonatal sepsis basic characteristic

During the study period, a total of 492 inborn neonates were admitted to the NICU and 104 neonates fulfill the diagnostic criteria for suspected neonatal sepsis. Identified pathogen from blood culture were confirmed in 52/ 104 (50%) suspected neonatal sepsis. Incidence rate of culture-proven neonatal sepsis in this study was 10.6% from the total NICU admissions. Culture-proven LONS were reported 39/ 52 (75%) from all positive blood culture. The median birth weight and gestational age were 1500 (1250 – 1800) grams and 33 (31.5 – 34) weeks, respectively. Table 1 listed the basic characteristics of culture-proven neonatal sepsis.

Haematological profiles of culture-proven neonatal sepsis

Anemia and thrombocytopenia were the two most common abnormal hematological profile in this study. Thirty two (61.5%) neonates had anemia. Anemia more experienced in LONS than EONS. Neonates with culture-proven LONS significantly had lower hemoglobin level than culture-proven EONS. Only 15.4% (8/52) culture-proven neonatal sepsis had an abnormal leukocyte count. All of these neonates had leukopenia and none had leukocytosis. Thrombocytopenia and high CRP were observed in 75% (39/52) and 38.5% (20/52) cultureproven neonatal sepsis, respectively. The hematological profiles of culture-proven neonatal sepsis are listed in table 1.

Microbiological profile of culture-proven neonatal sepsis

Fifty two neonates identified pathogen isolated from the blood. According to the gram staining, gram negative bacterias were responsible for 75% of culture-proven neonatal sepsis (76.9% EONS and 74.4% LONS). *Coagulase negative Staphylococci (CoNS)* dominated gram positive bacteria, whilst *Klebsiella spp* dominated gram negative bacteria as a cause of culture-proven neonatal sepsis in this study. More than half of the pathogen isolated and identified in this study were *Klebsiella spp* and nearly 90% of them were *Klebsiella pneumoniae.* There was onnly one *Klebsiella pneumoniae* bacteria which did not produce extented sprectrum beta lactamase (ESBL). The three common etiology of culture-proven neonatal sepsis in this study were *Klebsiella pneumonia, Coagulase negative Staphylococci (CoNS)* and *Enterobacter cloacae*. Three neonates isolated more than one identified bacteria from the blood culture (mix pathogen), with *Aeromonas hydrophila – Enterococcus cloacae* in EONS and *Klebsiella pneumonia – Enterococcus faecalis* and *Klebsiella pneumonia ESBL (+) – E. Coli ESBL (+)*

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in LONS. The other of microbiological profiles in culture-proven neonatal sepsis are listed in table 2.

Table 2. Microbiological profile of proven neonatal sepsis

Blood Culture Results	Total n (%)	EONS n (%)	LONS n (%)
Gram positive	10 (19.2)	2 (15.4) 0	8 (20.5) 1
Staphylococcus aureus	1 (1.9)	(0)	(2.6)
Coagulase negative staphylococci (CONS)	9 (17.3)	2 (15.4)	7 (17.9)
Gram negative	39 (75.0)	10 (76.9)	29 (74.4)
Achromobacter spp	1 (1.9)	1 (7.7)	0 (0)
Acinetobacter baumannii	1 (1.9)	0 (0)	1 (2.6)
Bacillus cereus	1 (1.9)	0 (0)	1 (2.6)
Enterobacter cloacae	6 (11.5)	2 (15.4)	4 (10.3)
Klebsiella spp			
Klebsiella oxytoca	2 (3.8)	1 (7.7)	1 (2.6)
Klebsiella ozaenae	1 (1.9)	0 (0)	1 (2.6)
Klebsiella penumonia	25 (48.1)	6 (46.2)	19 (48.7)
Proteus mirabilis	1 (1.9)	0 (0)	1 (2.6)
Serratia marcescens	1 (1.9)	0 (0)	1 (2.6)
Mix pathogens (>1 isolated bacteria)	3 (5.8)	1 (7.7)	2 (5.1)

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Discussions

Fifty two neonates (10.6%) were identified as culture-proven neonatal sepsis from 492 inborn neonates during this study. This incidence is comparable to incidences reported in others countries as Ethiopia (9.8%) (9), South Africa (10.3%) (10), Nigeria (10.6%) (11) and Iran (12.17%) (12). Among all of the culture-proven neonatal sepsis, 57.7% were male, 94.2% were weighing less than 2500 grams and 88.5% were born <37 weeks of gestational age. Higher rate of culture-proven neonatal sepsis in male may be related with X-linked immunoregulatory genes and haploid of X chromosome in males (13). Animal studies also have shown a predominance of the Th-1 type immune response, higher production of proinflammatory cytokines (IL-2 and TNF-α) in males rats after LPS administration, and vice versa in female rats (14). The risk of neonatal sepsis increases in proportion to the decrease in birth weight and gestational age (2). Meta-analysis study in Ethiopia reported low birth weight and preterm were 1.42 and 3.36 times more likely to develop neonatal sepsis compared to normal birth weight and term neonates (15). Culture-proven neonatal sepsis dominated in male, preterm and low birth weight also reported by several previous study in India, Saudi Arabia, South Africa, and China (9,16–19).

More neonates born by *sectio caesaria* in this study and significantly higher in the culture proven LONS compared to culture-proven EONS (table 1). However, study from Saudi Arabia, China and Canada found that birth route was have no impact on culture-proven LONS (16,17,20). *Sectio caesaria* may altered colonization with normal human commensals compared to term infants born vaginally, who will have a variety of colonization beneficial microbiota from the maternal vagina, intestinal and skin microbiota immediately after birth, and responsible for the subsequent alteration of the immune system (20,21). Intestinal microbiota dysbiosis may causing bacterial translocation across the intestinal into the bloodstream and increases the risk of LONS (22).

Systemic bacterial infection in an immature neonatal hemopoietic system may lead to malfunctions of the hemopoietic system (19). Anemia, leukopenia and thrombocytopenia were

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observed in 32/52 (61.5%), 8/52 (15.4%), and 39/52 (75%), respectively, in our study. Anemia was observed 12.5% in EONS and 87.5% in LONS. Significantly lower hemogoblin level was detected in LONS than EONS. A study in Iran agreed this study, anemia was detected in 32.7% and was higher in culture-proven LONS (60%) than in EONS (27%). Mean hemoglobin level also reported significantly lower in LONS than EONS (13.5 □ 1.8 and 15 □ 2, p=0.002) (23). Different definition of anemia was used in a six years retrospective study in China with hemoglobin level < 9 d/dL. Anemia experienced 25% of the neonatal sepsis (19). The development of anemia in septic patient related with alteration in red blood cells (RBCs). Binding of RBCs membrane and the endotoxin (lipopolysaccarhide, LPS) altered the RBCs morphology and rheology and increased the clearance of affected RBCs from circulation (24,25).

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Leukopenia was the only abnormal leukocyte count in this study and detected in only 15.4%. Leukopenia only observed 30.8% in EONS and even less 10.3% in LONS. Retrospective study in Saudi Arabia in line with this study, only 9.4% leukopenia observed and developed 25.3% and 9.4% in EONS and LONS (16). About 35% of neonatal sepsis in China also experienced leukopenia, but with the higher limit of leukopenia (<7500/ mm^3) (19). In contrast, leukocytosis only reported by 17.7% in Saudi Arabia, even less by 4% in China and none of neonates experienced leukocytosis in this study. The definition of leukocytosis differs from that used in this study. The total leukocyte count (TLC) is a very unreliable indicator of neonatal infection. A normal TLC does not rule out sepsis because as many as 50% of culture-proven neonatal sepsis within normal limit of TLC (26). The TLC was associated with a higher likelihood ratio of neonatal sepsis only in leukopenia (27). Leukopenia had a higher spesificity compared to leukocytosis (87.5% vs. 25%) as predictor of culture-proven neonatal sepsis, however the sensitivity was low (20%) (28).

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Three-quarter of culture-proven neonatal sepsis in this study experienced thrombocytopenia. As many as 72% of them classified as severe thrombocytopenia. In a study conducted by Guo et al (19), thrombocytopenia detected in 29% and 43% of them had a severe thrombocytopenia. Thrombocytopenia also found in 26.5% neonatal sepsis in Saudi Arabia, even with a different limit (16). The pathogenesis of thrombocytopenia in neonatal sepsis is a combination of increased destruction and inadequate production of platelets, even the mechanism is not fully understood. A direct pathophysiological mechanism of endotoxins produced by gram negative bacteria may also contribute (29,30). Neonatal sepsis caused by *K. pneumoniae* and *Candida spp* encountered more anemia, more leukopenia and more thrombocytopenia than caused by other pathogens (19). *Klebsiella pneumoniae* was the predominance pathogen in this study and justified for the many cases of anemia, leukopenia and thrombocytopenia, although we did not evaluate fungi as the cause of the culture-proven neonatal sepsis.

High CRP (>10 mg/dL) only took place in 38.5% culture-proven neonatal sepsis in this study. High CRP was positive in only 30.8% and 41% of the EONS and LONS, respectively. Lesser positive CRP, even with lesser cut-off (>5 g/dL), was reported only 17.3% from total positive blood culture sample in Iran (14.4% EONS and 30% LONS) (23). Not concordance with this study, 58% neonates in Saudi Arabia had a high CRP and significantly more common in LONS than EONS (76.4% and 33.3%, p<0.001). Altough the study did not explained the meaning of high CRP used (16). C-reactive protein (CRP) is an acute phase reactant which most frequently used laboratory tests in the diagnosis of neonatal sepsis (31). However, neonates with fetal hypoxia, respiratory distress syndrome (RDS), meconium aspiration, after trauma/ surgery, and

after immunizations also had an elevated CRP. A false-positive rate of 8% has been found in healthy neonates (2). A meta-analysis from 31 study reported the sensitivity and spesificity of CRP in diagnosting neonatal sepsis were 69% (95% CI 66 – 71%) and 77% (95% CI 76 – 78%), respectively, with area under curve (AUC) 0.8458 (32).

In all culture-proven neonatal sepsis and by the age of neonatal sepsis (EONS and LONS), gram negative bacteria dominate the isolated pathogens in this study over gram positive bacteria. The most common identified pathogens were *Klebsiella pneumonia*, and followed by *Coagulase negative Staphylococci (CoNS)* and *Enterobacter cloacae*, respectively. *Klebsiella pneumoniae* as the main pathogen in culture-proven neonatal sepsis also reported by Hasibuan et al in Medan (33) and Wilar et al in Manado (34) in other Indonesian tertiary NICU. These mostly isolated gram negative bacteria were also reported in previous study in developing countries by Arowosegbea et al in 2017 (11), Hematyar et al in 2017 (23), El-Mashad et al in 2019 (35), Gao et al in 2019 (36) and also in developed country by Al-Matary et al in 2019 (16).

Arowosegbea et al provided gram negative bacteria for 78.9% of all isolated bacteria in 85 culture-proven neonatal sepsis with 26% case-fatality rate. *Klebsiella* spp (31.6%), *Enterobacter* spp (21.1%) and CoNS (15.8%) were the predominantly isolated pathogen reported in Nigeria, identically with isolated pathogen in this study. Gram negative bacteria was the only recognized pathogen in EONS and dominated with *Klebsiella* spp. However, gram positive bacteria mainly CoNS dominated the isolated pathogens in LONS (11). Study in Iran reported 72.7% organism isolated from blood culture were gram negative bacteria (*Escherichia coli* and *Klebiella spp*) (23). A 12-month-prospective-study in Egypt also revealed *Klebsiella* (31.03%), gram negative bacteria, as the predominat microorganism followed by *Staphylococcus aures* (20%) (35). Gram negative bacteria (59.8%) dominate over gram positive bacteria also reported in total isolates of neonatal sepsis in China. The four most predominant bacteria were *Klebsiella pneumoniae* (21.9%), *Escherichia coli* (21.9%), *group B Streptococcus* (GBS, 13.2%), and *Staphylococcus aureus* (6.8%). In EONS, GBS (30.0%) and E. coli (20.0%) were dominant, whereas in LONS, *K. pneumoniae* (25.6%) and *E. coli* (22.4%) were dominant (36). Culture-proven neonatal sepsis in Saudi Arabia also confirmed 61.1% of gram negative bacteria in blood culture and was the leading cause of mortality in EONS and LONS. However, the most frequently isolated pathogen based on onset of neonatal sepsis was gram positive bacteria, group B *Streptococcus* in EONS and *Klebsiella spp* in LONS (16).

In contrast, two years retrospective cohort study in India reported the majority of total isolates pathogens were gram positive bacteria. However, the most common isolated microorganism were *Klebsiella spp* (31.1%), *Staphylococcus aures* (24.5%) and CoNS (22.9%). Gram positive bacteria had a higher isolation rate in EONS and LONS, whereas the most frequent microorganism was *Klebsiella* spp in EONS and *Staphylococcus aures* in LONS (18). Different result also reported from culture-proven neonatal sepsis in South Africa with gram positive bacteria constituted 53.4% of all positive blood cultures, mainly CoNS (25%), *E. coli* (20.3%) and *S. aureus* (18.2%). In EONS, gram positive bacteria (mostly CoNS) was dominated eventhough gram negative bacteria (mostly *E. coli* and *Klebsiella*) was domintaed in LONS (9). The causative pathogens of culture-proven neonatal sepsis vary according to geographical differences and countries. It is vary from one hospital and other different hospital and even in the same hospital at different time (31,35).

Commented [PR26]: diagnosing

Commented [PR27]: In your opinion
Given the conditions of the your hospital, what is your explanation for the existence of these pathogens such as klebsiella ?

Conclusion

Culture-proven neonatal sepsis mostly occurred in preterm and low birth weight neonates. Our study showed that late-onset neonatal sepsis (LONS) were significantly more in infant born by *sectio caesaria* and significantly more anemia experienced than EONS. Anemia and thrombocytopenia were the most common hematological abnormalities. Severe thrombocytopenia was observed in 72% thrombocytopenia neonates. Isolated pathogens were dominated by gram-negative bacteria over gram-positive bacteria, in all culture-proven neonatal sepsis, in EONS and in LONS. *Klebsiella pneumoniae* was the most pathogen identified as cause of neonatal sepsis in this study, followed by *coagulase negative staphylococci (CONS)* and *Enterobacter cloacae*. The causative pathogens causing neonatal sepsis was very much dependent on the local microbial pattern.

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Author Contributions

RE and MTU was developed the research design. KRS was responsible for data collection. KRS, RE and MTU was responsible for data analysis and revised the manuscript.

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Conflicts of Interest

None

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