

The type of microorganisms in early-onset neonatal sepsis in the infant care unit

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Abstract— Background. Based on health research in 2007 found that infections were the cause of death of newborn number 3 (12%). **Diagnosis Early-Onset neonatal sepsis (EO)** was not easy to get prove of kind of microorganism, if only based on clinical symtoms due to systemic respons. **Objective. Knowing the type of microorganisms (MO) in EO with gram staining and from ear-swab cultures.** **Methods.** We collected the data of neonates who were born in three hospitals; General Hospital of South Tangerang City, Women & Children Hospital Permata Sarana Husada, and Hospital of Syarif Hidayatullah with diagnosis EO. We do gram staining and ear-swab cultures. **Results.** Out of 20 babies, 55% were females, gestational age similar between less of 37 weeks and more than 37 weeks, majority of birth weight were ≤ 2500 grams (60%), and majority of babies deliver through sectio caesaria (60%), and babies went home alive were 85%. **Type of MO found was Staphylococcus haemophylus (gram positive).** The ears-swab cultures positive found were 16 of 20 samples, and MO found were 18 of 20 samples. **Conclusions.** EO was found majority in low birth weight babies. Culture of ears swab majority was found MO gram positive, which normal flora in the skin. Blood culture as a gold standard to take a MO to diagnosed EO rather than culture of ears swab.

Keywords— microorganisms, culture from ear swab, EO.

I. INTRODUCTION

The Millenium Development Goals (MDG's) number 4 is to decrease under-five mortality rate. Of all under-five mortality rates, infant mortality is relatively high. In 20 years infant mortality rate (IMR) decreased dramatically from 88 per 1000 live birth in 1990 to 57 per 1000 live birth in 2010. In the world IMR is still high 32 per 1000 live birth. Risesdas in 2007 mentioned the cause of neonatal death 0-6 days in Indonesia are asphyxia (37%), premature (34%), and sepsis (12%). The cause of neonatal death 7-28 days are sepsis (20.5%), congenital (19%), pneumonia (17%), respiratory distress syndrome (RDS) (14%), and premature (14%)[1]. Neonatal sepsis is still the major cause of morbidity and mortality in newborn [2]. Sepsis can also occur when the baby is hospitalized [3-5].

Neonatal sepsis is a collection of clinical symptoms due to

the systemic response of neonates to infection in the first month of life. dalah kumpulan gejala klinis akibat respons sistemik neonatus terhadap infeksi pada bulan pertama kehidupannya. Neonatal sepsis classified to 2 categories early-onset sepsis (EO) and late-onset sepsis (LO). EO occurs when sepsis manifestations appear within the first 72 hours of life. LO occurs when sepsis manifestation appear more than 72 hours of life [6,7]. The microorganism causes sepsis can be bacteria, viruses, fungi, and protozoa [8,9]. Incidence of EO in the developed countries 1-4 cases per 1000 live birth [6]. In the developing countries incidence EO 20-37 cases per 1000 live birth [10]. In 2009 division of neonatology of the department of pediatric Cipto Mangunkusumo Hospital IMF 42.7 per 1000 live births and incidence of neonatal sepsis in 2013 of 98 per 1000 live births [11].

Diagnosis neonatal sepsis are combination clinical manifestation and laboratories [12]. Neonatal sepsis are terms that have been used to describe the systemic response to infection in newborn infants, but a definite diagnosis in neonate is not easy because the symptoms and signs are not specific. Haematologic tests with complete peripheral blood does not reflect the patient's condition [13].

Gold standard diagnosis neonatal sepsis is blood culture, but rarely positive less than 12-24 hours, need more blood volume, quantitative blood cultures are expensive, and takes a long time [13].

Studies in 2006 compare surface body culture to correlation with blood culture. Ear swab, throat swab, and gastric aspirates have correlation with blood culture [7]. But throat swab and gastric aspirates have a higher difficulty to collect the samples compared with ear swab [14]. Gram staining methods was developed by Hans Christian Gram in 1884 which differentiate microorganisms Gram positive and negative [15]. The aim this study is to know the type of microorganims in our infant care unit with non-invasive procedure (ear-swab cultures and Gram staining) and can provide rational antibiotics.

The research was funded by the UIN research Institute Syarif Hidayatullah Jakarta

II. METHODS

This study used a cross-sectional design. The data of maternal and infant characteristics are summarized descriptively. We collected the data of neonates who were born in three hospitals; General Hospital of South Tangerang City, Women & Children Hospital Permata Sarana Husada, and Hospital of Syarif Hidayatullah with diagnosis EO, from June until October 2015. The patients were hospitalized in infant care unit with diagnosis EO. The sample from ear-swab and laboratory test such as Gram staining and culture. The number of subjects in this study was 20 samples through consecutive sampling.

The inclusion criteria were neonates age 0-3 days and diagnosis EO. The exclusion criteria were neonates who have been given antibiotic therapy prior to sampling, has a major congenital abnormality, and the parents refused to participate in this study. After ethical approval, data including subjects characteristic, laboratory results such as Gram staining and ear-swab cultures were collected and recorded in study data collection forms. Then, the data were analyzed through univariate and bivariate using SPSS 21 for Windows. The data were summarized in narration and tables.

The collecting sample from the suspect EO neonates, before 6 hours after birth before bathing and getting antibiotic. External ear canal the baby is taken with sterile cotton swabs, for at least 2 movements, taken at least 2 swabs, done on the right and left ear. Getting samples with aseptic procedure, sterile cotton swabs as soon as possible put into Amies transport medium to protect contamination from microorganisms in environment and bring to Microbiology Department. For Gram staining, make a smear in the object glass, then dried. Inserted the steril cotton swabs to sterile plastic, labelling with baby identity (name, age, gender, address, medical record) time (date, time of birth, time of sampling), specimen identity (ear swab, right or left ear), transportation with Amies transport medium, and write the clinical condition the neonates.

Transportation sample with Amies transport medium and refrigerate the specimen. If the sample will be sent more than 2 hours, then the specimen inserted to refrigerator, the slowest sample must have been reached in the laboratory within 24 hours, but if the sample will be sent less than 2 hours can be placed at room temperatur.

Direct smear with Gram staining to make temporary diagnosis, to select empirical antibiotics, and help determine the next step of identifying microorganisms. Make a thin film of the ear-swab on a clean glass slide using a sterile cotton swab for viscous specimen. Air dry, then heat fix the slide by passing it several times through a flame (the slide should not become too hot to touch). Flood slide with crystal (or gentian) violet about 60 seconds. Flood with Grams's iodine about 180 seconds, carefully decolorize with 95% ethanolor alcohol until thinnest parts of the smear are colorless (wash with water). Flood with safranin (pink color) 10% Fuchsine bout 60 seconds (wash with water). Air dry or blot with absorbend paper.

Organisms retain the violet-iodine complexes after washing in ethanol stain purple and are termed Gram-positive. Those that lose this complex stain red from the safranin counter stain aren termed Gram-negative. Result culture depend on Amies transport medium, incubation environment, methods, and incubation duration. Culture from outer ear (meatus externus) final reported within 5 days. Interpretation and reporting of results by considering the possibility of isolates as aetiology, the possibility of contamination. If sterile results, it is necessary to consider causes such as the use of antibiotics in the mother, less volume, endotoxine from microorganisms, or microorganisms need anaerob transport medium.

III. RESULTS

A. *Subject's characteristic.*

We collect sample from the neonatal sepsis EO. The number of samples based on the calculation should be 32, but due to time constraints and budget, then samples that can be checked only 20 samples. The 16 positive ear-swab cultures from the General Hospital South Tangerang City and 2 negative microorganisms, negative in 2 samples from the Women & Children Hospital Permata Sarana Husada, in Hospital of Syarif Hidayatullah no babies diagnosis EO. This study performed from June until October 2015. Ideally if we checked sample from the other organ, we must compare with the gold standard. The gold standard to prove microorganisms is sample from blood culture. The limitation in this study is not to do blood cultures as a gold standard examination of microorganisms.

Mostly baby females got sepsis in 11 from 20 babies, gestational age is the same preterm and aterm about 10 babies, mostly body weight less and equal 2500 grams is about 12 babies, mostly labour with C-section in 12 babies, and mostly back home healthy 17 from 20 babies.

B. *Results from ear-swab culture and Gram staining*

Ear-swab culture neonates with suspected EO positive in 16 from 20 babies, and 4 negative. Two baby found two microorganisms in ear-swab cultures. Gram staining at 4 samples not defined microorganisms and there are corelated with ear-swab cultures.

C. *The type of microorganisms*

Bacteria that grow in ear-swab cultures are 10 samples gram positive and 10 samples gram negative. Most of type microorganisms that grow in ear-swab cultures are normal skin flora such as *Staphylococcus haemolyticus*. The kind of microorganisms mostly gram negative with different names. The type of microorganisms that grows on ear-swab culture can be explain in table 1.

Table 1. Type of microorganisms

Type of microorganisms	Gram	Amount
Staphylococcus haemolyticus	Positive	4
Escherichia coli	Negative	1
Staphylococcus aureus	Positive	1
Corynebacterium amycoltum	Positive	1
Bacillus sp	Positive	2
Enterobacter cloacae	Negative	1
Acinetobacter baumannii	Negative	2
Streptococcus viridians	Positive	2
Acinetobacter sp	Negative	1
Sphingomonas paucimobilis	Negative	1
Comamonas testosteron	Negative	1
Kocuria kristinae	Negative	1

IV. DISCUSSION

The number of participants completed this study was 20 babies, of whom mostly are female. We found that 2 baby females and 1 baby male is died. It is different with study Naeye et.al who found that male infants have excessive risk for neonatal death compare with females [16]. Angela MK quotes from Chandra in 2014 found that elevated level of IL-6 and procalcitonin is significantly in male sepsis. The gender-specific effects on different immune cell functions and compartments are potentially influenced by an X-chromosome mosaicism that exists naturally in females. Therefore, heterozygous cellular mosaicism presents a unique biological circumstance in females due to the fact that either the maternal or the paternal X-chromosomes are inactivated in each individual cell whereas males carry exclusively the maternal X-chromosome. Experimental studies revealed that this female X-chromosome mosaicism diversifies leukocyte responses during endotoxemia and may contribute to the dimorphic character of the inflammatory response. Male sex hormones, i.e., androgens, have been shown to be suppressive on cell-mediated immune responses. In contrast, female sex hormones exhibit protective effects which may contribute to the natural advantages of females under septic conditions [17].

Based on gestational age the number of preterm babies equals to a term is 10 babies. The number of samples is small in this study, so it can not show gestational age less than 37 weeks more susceptible to neonatal sepsis. The study in Nigeria 2011 found that babies delivered at less than 37 weeks of gestation recorded a higher rate of mortality than those of 37 weeks and above [18]. In the other study found that the risk increased with decreasing gestational age, the OR for infection was 4.8 for any baby < 37 weeks of gestation and 21.7 for babies < 28 weeks of gestation [13]. Study in Turkey 2015 found that risk increased to infant mortality if gestational age less than 28 weeks [19]. This study found mostly body weight ≤ 2500 grams is about 12 from 20 babies. In the other study in 2011 found that neonatal sepsis in the mean birth weights of preterm babies were 1.88 ± 0.47 kg. Eleven of the babies that died were preterm low birth weight [18].

The type of labor is one of the risk factors for neonatal sepsis. The normal delivery may cause neonates to expose bacterial colonization in the genitourinary tract of the mother as the babies pass through the birth canal [7]. From another study that vaginal group Streptococcus B test positive at delivery (OR 15.4) [20]. In this study we found different way to delivery the baby caused the General Hospital of South Tangerang City was the referral hospital from puskesmas or the other Hospital. The babies mostly labour with C-section in 12 from 20 babies.

The babies mostly back home healthy 17 from 20 babies. It is the same with another study that found the neonatal early-onset sepsis found in 142 babies and 14 babies died, so the 90% babies back home healthy [20] and in Nigeria 2011 found that 12 from 278 babies died, so 95.6% babies back home healthy [18].

In this study ear-swab cultures positive in 16 from 20 babies. The samples from General Hospital South of Tangerang City found the 14 babies positive ear-swab cultures, 2 samples negative. In women and children Hospital Permata Sarana Husada 2 infants suspected EO ear-swab culture negative, neither patient in Hospital of Syarif Hidayatullah Jakarta diagnosis EO. Sampling is done by trained nurses and doctor. The examination is done in a complete laboratory. Two baby found two microorganisms in ear-swab cultures. We must be careful to say that the microorganisms caused EO in the babies, preferably confirmed by blood culture as gold standard. Limitation in this study we did not perform blood culture.

The microorganisms that grow in ear-swab cultures are 10 samples gram positive and 10 samples gram negative, with 2 babies with 2 microorganisms. One baby positive ear-swab culture *Sphingomonas paucimobilis* and *Escherichia coli*, and the other baby positive *Streptococcus viridans* and *Kocuria kristinae*. Type of microorganisms that grow in ear-swab cultures were *Staphylococcus haemolyticus* (4 samples); but the other *Bacillus sp*, *Acinetobacter baumannii*, and *Streptococcus viridans* (each 2 samples); *Escherichia coli*, *Corynebacterium amycoltum*, *Enterobacter cloacae*, *Acinetobacter sp*, *Sphingomonas paucimobilis*, *Comamonas testosteron*, and *Kocuria kristinae* (each 1 samples). Heshmati [21] found *Staphylococcus epidermidis* but Kerur [7] found mostly microorganisms caused EO is *Escherichia coli*. *Escherichia coli* was a normal flora in gastrointestinal tract, if we found in skin we must be careful it can be infected and sepsis can occur. Mostly microorganisms in vaginal mother such as *Lactobacillus*, *Prevotella*, and *Sneathia* spp. In the deliver baby with C-section, we found *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*. *Sphingomonas paucimobilis* is gram-negative bacillus are the cause of nosocomial infections and are opportunistic pathogenic bacteria [22].

The type of bacteria that causes neonatal sepsis varies widely between regions, countries, and EO or LO. Most of the microorganisms in developing countries were gram-negative enteric bacteria such as *Enterobacter sp*, *Klebsiella sp*, and

Escherichia coli [23-25]. In this study mostly negative-gram in staining gram. Study in Cipto Mangunkusumo Hospital 2005 found that mostly coloni *Acinetobacter calcoaceticus* (35.67%), *Enterobacter sp* (7.0%), and *Staphylococcus sp* (6.8%) [26].

ACKNOWLEDGMENT

Thank to nurses, midwife, and laboratory officer for their help until we finished this study.

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