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Original Research Article

Obstetric Characteristics Associated with Laboratory-Confirmed Neonatal Sepsis at the Referral Hospital, Dar Es Salaam, Tanzania. A Cross-Sectional Study

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Abstract: ***Background:*** Neonatal sepsis is among the leading causes of neonatal mortality. Due to limited laboratory services, the diagnosis is mainly based on clinical presentation. Signs and symptoms of neonatal sepsis are multiple and nonspecific. Blood culture is the gold standard for the diagnosis of neonatal sepsis. The demographic and obstetric factors associated with neonatal sepsis vary in facilities and locations. Therefore, we conceptualized the study to determine the factors that can independently predict the occurrence of neonatal sepsis. ***Methodology*****:** A prospective cross-sectional study was conducted by recruiting neonates admitted to the neonatal ward with signs and symptoms of sepsis. The blood sample was collected for culture, and isolates were identified using conventional methods. Descriptive analysis was presented as frequencies and proportions. Univariate logistic regression analysis was used to analyze the factors associated with laboratory-confirmed neonatal sepsis. Multivariable logistic regression was performed to determine the independent predictors of neonatal sepsis. A p-value of less than 0.05 was considered statistically significant. ***Results.*** Two hundred seventy-nine neonates were recruited; 161(57.7%) were aged less than four days. Fever was the most common complaint in 72.4% of neonates among the 13 clinical features used to determine the clinical diagnosis of neonatal sepsis. Of 279 blood cultures performed, 198(71.0%) were culture-positive. In bivariate analysis, birth asphyxia, vaginal delivery, and foul-smelling liquor had increased odds of neonatal sepsis. However, neonatal sepsis was independently associated vaginal delivery. Neonates born through vaginal delivery had 2.57 times the adjusted odds of getting neonatal sepsis than those born by cesarean section (aOR = 2.57, 95% CI = 1.3–28.2, p= 0.021) ***Conclusion*****:** Vaginal delivery was the only factor independently associated with laboratory-confirmed neonatal sepsis. There is a need for evaluation of pregnant women for the risk of neonatal infection to inform empirical management decisions at birth to prevent neonatal infection.

Keywords: Neonatal sepsis, Obstetric characteristics**,** spontaneous vaginal delivery.

INTRODUCTION

Neonatal sepsis is a clinical syndrome in neonates manifested by systemic signs and symptoms and isolation of a causative pathogen [1]. Two categories of neonatal sepsis have been described based on the onset time. Early-onset neonatal sepsis develops within the first 72 hours of life after birth, and late-onset develops after 72 hours [2]. Early onset infection is usually due to vertical transmission by ascending contaminated amniotic fluid or during vaginal delivery from bacteria in the mother's genital tract [3]. Late-onset sepsis is caused by bacteria acquired from the healthcare environment or community. In addition, late-onset can be caused by vertical transmission, resulting in initial neonatal colonization that evolves into a later infection [4-6]. Neonatal sepsis is one of the three leading causes of

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neonatal mortality, together with prematurity and intrapartum-related complications. [7]

Physicians caring for infected neonates face multiple challenges, including getting an accurate diagnosis. Multiple conditions resemble neonatal sepsis [8]. Signs and symptoms of neonatal sepsis are multiple and nonspecific. Blood culture is the gold standard for the diagnosis of neonatal sepsis. However, several factors, including laboratory capabilities, affect the positivity rate [7]. In most low-middle-income countries, diagnosis of neonatal sepsis is mainly clinical, and blood culture can be confirmed in up to 40% of the cases due to a low yield [9]. The clinical presentation of neonatal sepsis varies, and there are no pathognomonic features[10]. Studies report variations in features associated with neonatal sepsis; a study conducted at a tertiary hospital in Tanzania reported an inability to breastfeed, lethargy, convulsion, chest wall in-drawing, jaundice, and umbilical redness to be strongly associated with neonatal sepsis[11].

The treatment option for neonatal sepsis depends on the severity of the disease. It may include administering antibiotics, providing supportive care, correcting metabolic derangements, transfusion of blood products, oxygen therapy, or other forms of respiratory support [12]. The challenges for clinicians are promptly identifying neonates with a high likelihood of sepsis and initiating antimicrobial therapy. The World Health Organization (WHO) guideline recommends penicillin and gentamycin as the first-line antibiotics [13]. Some resource-limited settings have adopted the Integrated Management of Childhood Illnesses guidelines for managing neonatal sepsis[14]. However, the etiology of neonatal sepsis and the response to antimicrobial agents vary significantly, which may affect the success of empirical management.[10, 15]

Several infection-preventive interventions have been advocated to decrease the possibility of infection in neonates. These measures and practices are required during prenatal, delivery, and afterward[16]. However, some infrastructural challenges remain in obtaining all the set targets for alleviating neonatal sepsis. Following the dynamic nature of the etiological agents and the influencing factors, local data are needed to determine the factors independently associated with neonatal sepsis. The findings highlight the need to improve obstetric practices and strengthen healthcare promotion guidelines.

MATERIALS AND METHODS

Study design and setting

We conducted a cross-sectional study at the neonatal ward of Mwananyamala Reginal Referral Hospital (MRRH). The facility serves a population of around 2,226,692 in Kinondoni District, Dar es Salaam region, Tanzania. The hospital has a 254-bed capacity and 87% bed occupancy. The medical services offered at the facility include reproductive and child health, including antenatal, postnatal, and newborn care. The average neonatal admission per month during the time of study was around 250, of whom approximately 20% were due to neonatal sepsis. Newborns with at least two clinical features suggestive of neonatal sepsis per WHO guidelines are admitted for further management. All neonates admitted to the ward undergo thorough clinical evaluation guided by the neonatal infection reporting tool prepared by the facility. The tool helps detect early warning signs, symptoms, and other risk factors for neonatal sepsis. Laboratory investigation follows the completion of clinical evaluations.

Study Population

The study participants were neonates born at MRRH and admitted to the neonatal ward. We defined clinically suspected neonatal sepsis as when a neonate presents with at least two signs and symptoms suggestive of sepsis. These include hypothermia (less than 36.5°C), fever (more than37.5°C), jaundice, a respiratory rate of less than 30 or more than 60 breaths per minute, grunting, central cyanosis, hypoxia (oxygen saturation less than 90%), poor feeding, pulse rate of less than100 or more than 160 beats per minute, irritability, seizures, lethargy, and altered consciousness. We excluded neonates whose mothers failed to consent to enrollment.

Sample size and sampling procedure

We estimated the minimum sample size based on the known proportion of neonatal sepsis of 20% at MRRH with the assumption of a 5% margin error and a 95 % confidence interval. We estimated the minimum sample size using the Kish and Leslie formula ($n = Z^2P(100-P)/\mathcal{E}^2$); n = sample size, z= the value for the desired confidence level at 95% confidence limits (1.96), p = estimated prevalence (20%), $\mathcal{E}$= margin of error of 5%. The estimated minimum sample was 272.

Data and sample collection

We used a standardized data collection tool to collect mothers' and neonates' demographic characteristics, obstetric characteristics, clinical presentation, and laboratory results. The mother of each participating neonate was interviewed after informed consent. In addition, physical measurements were performed for each neonate during neonatal clinical assessment. A trained research assistant collected blood samples for bacterial culture from the peripheral vein for each participating neonate, adhering to aseptic techniques. The participants' skin and the septum covering the blood culture vial were disinfected with 70% alcohol (or 1-2% iodine tincture). Blood samples were collected into commercially prepared blood culture vials for pediatrics. The blood sample volume to blood broth ratio was 1:5 [9].

Laboratory procedures

The inoculated blood culture vials were incubated at 36 – 37°C in the automated blood culture

machine designed to detect microorganisms rapidly for over five days unless flagged positive. Positive blood cultures were sub-cultured onto blood agar and MacConkey agar plates. Isolates were identified using conventional bacteriological identification methods. Microbiological techniques, including colony morphology, Gram-staining reactions, and biochemical tests, were performed using pure isolates obtained from subculture plates. The culture media were prepared according to the onsite standard operation procedures. A sterility check was performed every time a new batch of media was prepared by incubating a sample of 5% of prepared media at 37^{o}C for 48 hrs. The ability of media to support the growth of the suspected organisms was determined by inoculating the medium with a typical stock culture. We used reference strains of *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, and *Staphylococcus aureus* ATCC 25923 for quality control.

Data analysis

The data was analyzed using the Statistical Package for Social Science version 23. The frequencies and proportions were used to analyze categorical variables. Bivariate logistic regression analysis was used to determine the factors associated with laboratory-confirmed neonatal sepsis. Factors with a p-value less than 0.2 in bivariate analysis were further subjected to multivariable logistic regression to determine the independent predictors of neonatal sepsis. A p-value of less than 0.05 was considered statistically significant.

Ethical considerations

The ethical clearance was obtained from the National Institute for Medical Research (NIMR) (NIMR) ethically cleared the study with a certificate numbered NIMR/HQ/R.8a/Vol.IX/3538. The Kinondoni Municipal Council and MRRH authorities permitted data collection. Before recruitment, mothers of respective neonates provided informed written consent. Mothers of neonates were informed about voluntary participation and possible withdrawal at any time. Laboratory test results were shared with the attending clinician for the participants' management. The confidentiality was adhered to by using unique study identification numbers.

RESULTS

Participants characteristics

The study recruited 279 neonates with clinical features of neonatal sepsis from January to December 2022. Most neonates, 161(57.7%), were aged less than four days, and the same were males. Neonates with low birth weight (below 2500gm) were 44(15.8%), and 52(18.4%) were born premature (less than 37 gestation weeks). Most neonates, 262(93.9%), had Apgar scores of 7 or above at one minute and 21(7.5%) had birth asphyxia. Over a quarter of mothers, 73(26.2%), had urinary tract infections during pregnancy, 224(80.3%) had spontaneous vaginal delivery, and 16(5.7%) had premature rupture of membrane, and 132(47.3%) had given birth once. Only 13/224(5.8%) mothers had prolonged membrane rupture, while 12/224(5.4%) had prolonged labor. Foul-smelling liquor was reported in 7/253(2.8%) mothers, while 19/248(7.7%) had meconium-stained liquor, and 8/243(3.3%) mothers received antibiotic prophylaxis in pregnancy. Most neonates, 201/207(97.27%), were on exclusive breastfeeding. Most mothers, 101/196 (51.5%), reported washing their hands before breastfeeding (Table 1)

Table 1: Demographic and obstetric characteristics related to participants (N=279)

Variable	Option	Frequency	Percent (%)
Age (days)	1-3 days	161	57.7
	≥4 days	118	42.3
Sex	Male	161	57.7
	female	118	42.3
Birthweight (grams)	< 2500	44	15.8
	≥ 2500	235	84.2
Gestation age at birth (weeks)	< 37	52	18.6
	≥ 37	227	81.4
Mode of delivery	Cesarian section	55	19.7
	Normal vaginal delivery	224	80.3
Apgar score at 1 min	0-6	17	6.1
	7-10	262	93.9
Birth asphyxia		21	7.5
Urinary tract infection in Pregnancy		73	26.2
Premature rupture of membrane		16	5.7
Parity	1	132	47.3
	2+	147	52.7
Prolonged rupture of membranes (n=224)		13	5.8
Prolonged labor (n=224)		12	5.4
Foul-smelling liquor (n=253)		7	2.8
Meconium-stained liquor (n=248)		19	7.7

Variable	Option	Frequency	Percent (%)
Prophylactic antibiotics in pregnancy (n=243)		8	3.3
Use of antibiotic before admission (n=256)		41	16.0
Types of feeding (n=207)	Exclusive breastfeeding	201	97.1
	Others (mixed, formula) feeding	6	2.9
Handwashing before breast feeding (n=196)		101	51.5

Clinical features for neonatal sepsis

Thirteen clinical features were used to determine the clinical diagnosis of neonatal sepsis. Fever was the most common complaint found in 202(72.4%). The other three common complaints were poor feeding, 118(42.3%), jaundice 97(34.8%), and tachypnea, 76(27.2%). (Figure1)

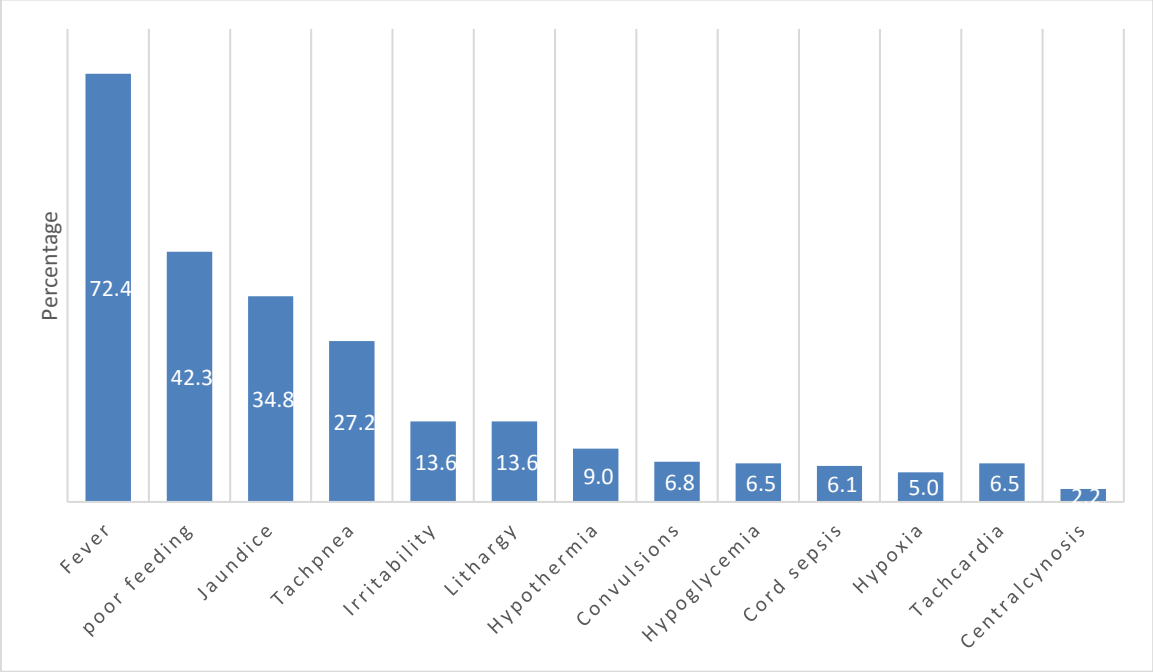

Figure 1: Frequency distribution of clinical features of neonates

These were signs and symptoms found at the initial clinical evaluation of 279 neonates before admission.

Factors associated with laboratory-confirmed neonatal sepsis

Of 279 blood cultures for neonatal sepsis, 198 (71.0%) had bacterial growth. In bivariate analysis, birth asphyxia, mode of delivery, and foul-smelling liquor were associated with laboratory-confirmed neonatal sepsis. Neonates with birth asphyxia had 3 times the odds of neonatal sepsis than those without birth asphyxia (cOR = 2.95, 95%CI 1.20-7.26, p=0.014). Neonates delivered by spontaneous vaginal delivery had about 2 times more odds of neonatal sepsis than in caesarian section delivery (cOR = 1.86, 95%CI 1.01-3.45, p = 0.046). The odd of neonatal sepsis was 6 times in more in neonates born with foul-smelling liquor than the counterpart (cOR = 5.93, 95%CI 1.12-31.24, p=0.036). (Table 2).

Table 2. Bivariate analysis for the factors associated with neonatal sepsis

Variable		Neonatal sepsis	cOR	95% CI	P-value
Gender	Male	115(71.4)	0.9	0.56-1.60	0.843
	Female	83(70.3)			
Apgar score	< 7	10(58.8)	1.78	0.65 – 4.85	0.255
	7 +	188(71.8)			
Birthweight (g)	<2500	33(75.0)	0.79	0.38-1.64	0.521
	2500+	165(70.2)			
Gestation age (wks)	<37	39(75.0)	0.78	0.39-1.55	0478
	37+	159(70.0)			
Parity	1	98(74.2)	0.74	0.44-1.24	0.253
	2+	100(68.0)			
UTI in pregnancy	Yes	54(74.0)	1.22	0.67-2.23	0.510

Variable		Neonatal sepsis	cOR	95% CI	P-value
	No	144(69.9)			
Premature rupture of membrane	Yes	11(68.8)	0.89	0.30-2.66	0.840
	No	187(71.1)			
Birth asphyxia	Yes	10(47.6)	2.95	1.20-7.26	0.014
	No	188(72.9)			
Mode of Delivery	Cesarian section	33(60.0)	1.86	1.01-3.45	0.046
	Normal vaginal delivery	167(73.7)			
Prolonged rupture of membranes	Yes	8(61.5)	0.55	0.17-1.75	0.307
	No	157(74.4)			
Prolonged labor	Yes	8(66.7)	0.70	0.20-2.42	0.572
	No	157(74.1)			
Foul smelling liquor	Yes	2(28.6)	5.93	1.12-31.24	0.036
	No	173(70.3)			
Meconium-stained liquor	Yes	11(57.9)	0.59	0.23-1.54	0.278
	No	160(69.9)			
Type of feeding	Exclusive breast feedings	147(73.1)	0.18	0.03-1.03	0.054
	Mixed/formula	2(33.3)			
Wash hands before breastfeeding	Yes	69(68.3)	0.65	0.34-1.23	0.182
	No	73(76.8)			

Five factors (birth asphyxia, mode of delivery, foul-smelling liquor, types of feedings, washing hands before breastfeeding) were included in the multivariable logistic regression analysis using the backward stepwise method. Only three variables, including mode of delivery, types of feedings, and washing hands before breastfeeding, were included in step three of the regression equation. The foul-smelling liquor and birth asphyxia were removed in step 2 and step 3 of the logistic equation, respectively. Only the delivery model was independently associated with laboratory-confirmed neonatal sepsis for the three factors that showed association with neonatal sepsis in the bivariate analysis. Neonates born through vaginal delivery had 2.57 times the adjusted odds of getting neonatal sepsis than those born by cesarean section (aOR = 2.57, 95% CI = 1.3–28.2, p= 0.021). (Table 3)

Table 3: Multivariable logistic regression analysis for the factors of neonatal sepsis

	Variable	Neonatal sepsis	a OR	95% CI	P-value
Mode of Delivery	Cesarian section	33(60.0)	1	1.15-5.76	0.021
	Normal vaginal delivery	167(73.7)	2.57		
Type of feeding	Exclusive breast feedings	147(73.1)	1	0.01–1.03	0.053
	Mixed/formula	2(33.3)	0.99		
Wash hands before breastfeeding	Yes	69(68.3)	1	0.26-1.02	0.057
	No	73(76.8)	0.52		

DISCUSSION

The study conducted a laboratory investigation to confirm the clinically suspected neonatal sepsis and determined associated factors among neonates admitted at a referral hospital. The study found that 71% of clinically suspected neonatal sepsis had positive blood cultures. A model of delivery was the only factor independently associated with laboratory-confirmed neonatal sepsis. Neonates born by vaginal delivery route were three times increased chance of acquiring neonatal sepsis compared to those delivered by caesarian section. Our finding on the yield of laboratory-confirmed neonatal sepsis is higher than the average of 40% [9]. The finding may reflect the increased accuracy of a standardized neonatal clinical evaluation tool used at Mwananyamala Regional Referral Hospital for diagnosing neonatal sepsis and the advantage of utilizing blood culture. In addition, our findings imply that neonates delivered through the vaginal model of delivery interact with potential pathogens for neonatal sepsis in the process of delivery during the intrapartum period. The findings call for evaluating the virginal delivery process in our setting to control the risky infection.

The current study reveals that normal vaginal delivery was independently associated with laboratory-confirmed neonatal sepsis. Neonates delivered through the vaginal canal had about three times more risk of neonatal sepsis than cesarian-section delivery. Our finding is comparable to other studies [17, 18]. Studies in Ethiopia[19] and Uganda [20] found the same that spontaneous vaginal delivery to be a significant predictor of neonatal sepsis. The babies born through the vaginal canal have a high chance of being exposed to potential pathogens in the vaginal and fecal bacteria [10]. Spontaneous vaginal delivery pose a risks of neonatal sepsis in a high-risk characteristics delivery like

prolonged labor and premature rupture of membranes [21]. To prevent neonatal sepsis, some countries implement universal screening of pregnant women for streptococcal colonization and provide intrapartum chemoprophylaxis to those colonized [22]. The lack of universal screening and intrapartum prophylaxis in our setting could explain the increased risk of neonatal sepsis we found related to vaginal delivery.

The factors associated with neonatal sepsis vary with different studies from different geographical areas and types of health facilities. Neonatal sepsis is generally a result of maternal, neonatal, and health-related factors, depending on the time of onset. In the current study, neonatal sepsis was more common in delivery associated with foul-smelling liquor. However, the association was not independent when related to other factors. Studies in Ethiopia [23], Uganda [20], and Sri Lanka [24] reported an association between neonatal sepsis and foul-smelling liquor. The lack of independent association of foul-smelling liquor with neonatal sepsis could be due to a low number of neonates born with foul-smelling liquor.

In the current study, meconium-stained liquor was found not to be associated with developing neonatal sepsis. A South African study found a low rate of neonatal sepsis in newborns exposed to meconium-stained liquor [25]. However, a study done in sub-Saharan Africa in 10 countries showed a strong association between meconium-stained liquor and the occurrence of neonatal sepsis [26]. In our study, the reason for not being associated with neonatal sepsis could be attributed to the severity of the meconium-stained fluid, grade 1, and the small sample size of neonates born with meconium-stained fluid.

The most common cause of early-onset sepsis is related to maternal factors, and the cause of late-onset sepsis is related to the result of neonatal care practices after delivery. Different studies report various factors contributing to neonatal sepsis, which we did not find in our study, like preterm neonates and Birth weight [19, 27-29]. Preterm neonates and those with low birthweight mostly acquire infections related to healthcare practices during hospitalization due to the low level of innate and cell-mediated immunity [30]. Therefore, the lack of association between prematurity and low birth weight with neonatal sepsis in our study is an indication that there was a low risk of neonatal infection due to healthcare practice. Furthermore, unlike a previously published study from the same setting [31], health services-related factors were not associated with neonatal sepsis in the current study, implying improved newborn healthcare services.

Our study has some limitations because we did not include in the analysis information on healthcare practices during delivery and sterilization or disinfection practices as they influence neonatal sepsis [32, 33]. We also missed information like health status and practices during pregnancy. Factors of maternal colonization with potential pathogens during pregnancy, frequency of per-vaginal examination during labour and delivery, and competence of health workers, which are missed in our study, have also been associated with neonatal sepsis [34].

CONCLUSION

Neonates with sepsis present with various clinical presentations and strict clinical evaluation increase the yield of laboratory laboratory-confirmed neonatal sepsis. Vaginal delivery was the factor independently associated with laboratory-confirmed neonatal sepsis. Evaluation of pregnant women for the risk of neonatal infection, including early screening for colonization with potential pathogens, and timely management should be applied to alleviate the burden of neonatal sepsis.

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Declaration of Interest statement: The authors report no conflict of interest.

Data Availability Statement: We confirm that relevant data generated for this study are included in the manuscript and can be obtained from the corresponding author at a reasonable request.

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Author Contributions: MM, JM, SK, and AJ conceptualized and designed the study. ZM, MK, ZB, and AK were involved in recruitment and data collection, MM, ZM, JM, and AJ performed data analysis. ZM, JM participated in drafting the manuscript led by MM. All authors approve the final version of the manuscript to be published and agree to be accountable for all aspects of the work.

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