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Antimicrobial Resistance among neonates with neonatal Sepsis Morogoro Tanzania

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Abstract

Background: Neonatal sepsis increases neonatal morbidity and mortality in low- and middle-income countries. However, the prevalence of neonatal sepsis, etiological agents, and antimicrobial resistance patterns have not been documented in areas with the highest neonatal mortality rates in Tanzania.

Aim: This study aimed to investigate the prevalence of neonatal sepsis, identify the primary causative agents, and understand their resistance patterns at Morogoro Regional Hospital.

Methods: The study involved 252 admitted neonates at Morogoro Regional Hospital and was carried out between March and June 2019. Clinical and demographic information for each neonate was collected using a standardized questionnaire. Blood samples were obtained from all 252 neonates, and 50 swabs were randomly taken from neonates with umbilical pus discharge. The samples were cultured using aseptic techniques on blood, chocolate, and MacConkey agar. The identification of the causative agents relied on the characteristics of colony morphology, gram staining, and biochemical tests. Antimicrobial resistance patterns were determined using the disc diffusion method with Muller Hinton agar against Ampiclox, Erythromycin, Gentamycin, Nalidixic acid, Ciprofloxacin, Norfloxacin, Ofloxacin, Kanamycin, Co-trimoxazole, Cephalexin, Ceftriaxone, and Amikacin.

Results: The prevalence of neonatal sepsis, as determined through blood culture, was 40 % (102 /252). The predominant bacteria isolated from blood cultures were *E. coli* 31 %, *Staphylococcus aureus* 23 %, and *Citrobacter* spp 16%. Around 50% of the gram-negative bacteria resisted Ceftriaxone, a third-generation cephalosporin. Both gram-negative bacteria and *Staphylococcus aureus* displayed resistance to Ampiclox.

Conclusion: *E. coli, Staphylococcus aureus*, and Citrobacter spp. were shown to be the most frequent bacteria in neonatal sepsis in Morogoro. Many isolates were Ampicillin-resistant. Neonatal sepsis is common in Morogoro, highlighting the need for innovative neonatal care and preventative techniques.

Keywords: Neonatal sepsis, antimicrobial resistance, Tanzania

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Introduction

Children in Low- and Middle-Income countries are 18 times more likely to die before the age of five than those in developed countries (Chan and Lake, 2013, WHO 2018). Among the numerous health challenges affecting neonates in LMICs, neonatal sepsis emerges as a predominant cause of most hospital admissions (Shitaye et al., 2010). As a result, sepsis causes nearly 2 million newborn deaths in Africa (UNICEF, 2017). In Tanzania, neonatal sepsis is responsible for a neonatal mortality ranging from 29% to 38.9% (Manji, 2009; Kayange et al., 2010; Mhada et al., 2012). Neonatal sepsis is a life-threatening clinical syndrome characterized by dysregulated host immune responses due to infection, often leading to organ dysfunction (Pop-Begun et al., 2014). While the prevalence of neonatal sepsis varies from country to country (Kaistha et al., 2009), its profound impact is prominently observed in developing countries. In contrast, sepsis in adults has been extensively studied and documented worldwide (WHO, 2018), whereas neonatal sepsis remains a relatively underexplored area (*Ibid*.). The sustainable development goal (SDG) 3.2 targets newborn and child mortality by 2030 is to end preventable deaths of newborns and children under five years of age. All countries shall aim to reduce neonatal mortality to as low as 12 per 1,000 live births and under-five mortality as low as 25 per 1,000 live births(https://www.undp.org/sustainable-development-goals/good-health).

The diagnosis and treatment of neonatal sepsis pose substantial challenges in many LMICs, partly due to inadequate healthcare personnel and the absence of essential laboratory facilities (Lawn et al., 2009). The scarcity of appropriate laboratory resources hinders the identification of causative agents, and their drug sensitivity profiles, resulting in limited data regarding the incidence, prevalence, and etiological factors associated with neonatal sepsis in developing nations like Tanzania. Furthermore, research has indicated dynamic changes in the susceptibility patterns of bacteria causing sepsis (Thapa and Sapkota, 2019). This has led to the absence of a one-size-fits-all antibiotic recommendation (Kayange et al., 2010; Mhada et al., 2012; Thapa and Sapkota, 2019). Most etiological agents responsible for neonatal sepsis have developed multiple resistances to antimicrobial therapies, resulting in high mortality rates, increased healthcare costs, and clinical failures (Iqbal, 2013; Friedman et al., 2016). The variation in etiological agents and drug susceptibility patterns among bacteria causing neonatal sepsis underscores the need for regular antimicrobial resistance monitoring programs.

Studies on neonatal sepsis in Tanzania have focused on regions such as Mwanza and Dar es Salaam (Mshana et al., 2009; Jabiri et al., 2016). The Tanzania Demographic Health Surveillance estimates the infant mortality rate at Morogoro Regional Hospital to be 82 per 1000 live births (DHS, 2015 – 2016). However, updated information on the causes of neonatal deaths in the region is required. This study determined neonatal sepsis's prevalence, identified the associated etiological agents, and assessed the susceptibility status to commonly used antimicrobial agents among neonates admitted to Morogoro Regional Hospital.

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Materials and Method

Study Area and Design

The study was conducted in Morogoro Region Hospital, in the eastern part of mainland Tanzania, from January to July 2019. The region is characterized by a high infant mortality rate, with approximately 82 deaths per 1000 live births (DHS, 2015). Data were collected from neonates admitted to Morogoro Regional Hospital, a referral hospital for the region's eight districts. All laboratory work was conducted at Morogoro National Institute of Medical Research laboratories.

The study involved cross-sectional and cohort study designs involving neonates aged from 0 to 30 days after delivery who were admitted to the neonatal ward at Morogoro Regional Hospital and were suspected to have sepsis. Demographic data such as age, sex, and area of residence were obtained from consented parents or guardians. Clinical information, including the neonates' weight, gestational age, premature rupture of membranes, fever, breathing rate, delivery mode, cyanosis, convulsions, feeding ability, jaundice, and umbilical redness were sourced from hospital records.

Inclusion Criteria and Informed Consent

The study's neonates were selected based on findings from physical and clinical examinations. Enrollment included all neonates admitted to the Morogoro Regional Referral Hospital's neonatal ward, aged between 0 and 30 days postpartum, who were clinically suspected of having sepsis, with parents available. Informed consent was obtained from the parents of the neonates.

Sample Size Estimation

The sample size was calculated using Fisher's formula, based on research conducted at Muhimbili National Hospital, which showed a prevalence of neonatal sepsis of 24% (Mhada et al., 2012). Using the formula $N = Z^2P(1-P)/\delta^2$, N is the minimum sample size, Z is the standard average deviation (1.96 for the 95% confidence interval), and P is the expected prevalence. Δ is the acceptable margin of error; a minimum sample size of 280 neonates was anticipated to be used.

Ethical permit

The ethical approval was obtained from the National Health Research Committee (NatHREC) of the National Institute of Medical Research (NIMR/HQ/R.8a/Vol.IX/1896).

Neonatal Blood and Swab Samples Collection

Structured and semi-structured questionnaires were used to obtain demographic and clinical information from admitted neonates. This included physical examinations, neonate weight, and information about premature rupture of membranes, fever, breathing rate, delivery mode, cyanosis, convulsions, feeding ability, jaundice, and umbilical redness. Blood and pus samples were collected from all neonates suspected of having sepsis and admitted to Morogoro Regional Hospital with the consent of their parents. Blood collection was aseptically done, with 2 to 3 ml of intravenous blood taken from each suspected neonate. The blood samples were coded and transferred to the microbiology laboratory for storage, culturing, identifying, and determining

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antimicrobial resistance patterns. Neonates positive for sepsis were monitored to assess their health outcomes (El-Halik et al, 2018).

Microbial analysis: Sub-culturing of neonatal blood and swab samples

The collected blood samples and swabs were mixed with sterile 10mls of Brain Heart Infusion broth under aseptic environmental conditions. The mixture was then incubated at 37°C for 24 hours to allow maximum multiplication of pathogens in the given swab and blood samples. The mixture was sub-cultured using a sterile inoculating wire loop into a dry and clean surface of blood agar, chocolate agar and MacConkey agar. Then the inoculated petri dishes were incubated at 37°C overnight, followed by observation for 24, 48,72 96 hours until there was no growth of colonies. The colony morphologies were recorded based on the colour, texture, forms, shape, and reaction on blood agar whether the colonies had undergone β, α or no hemolysis. Also, the reaction of the isolates on MacConkey agar and gram staining were recorded. Microscopic observations of the slides were done using an objective lens with 100x at medium lighting using immersion oil. After identifying gram-positive and gram-negative bacteria, biochemical tests were set depending on the staining nature of the isolates. Gram-negative bacteria were put into the triple iron sugar agar reaction test (TSI), SIM test (involving motility, hydrogen sulphide test and Indole test), citrate, and urease tests. In contrast, Gram-positive bacteria were set into catalase reaction, coagulase activity, and haemolytic activity on horse blood agar plates.

Susceptibility testing

To identify the extent to which isolates were resistant or susceptible to the standard antibiotics used in the treatment of bacterial infections, a sensitivity test was carried out using the Kirby-Baeur disc diffusion susceptibility method. Muller Hinton agar was employed as a growth medium because it supports the growth of most non-fastidious pathogens satisfactorily and exhibits acceptable batch-to-batch reproducibility for susceptibility testing. It also has low concentrations of sulfonamide, trimethoprim, and tetracycline inhibitors. (Clinical and Laboratory Standard institute 2017).To ascertain the isolates' susceptibility, the following antibiotics were used: Ampiclox 30 µg (LOT 110714054), Erythromycin with 15µg (LOT 327996), Gentamycin with 30µg (LOT 2856439), Nalidixic acid 30µ (LOT 0000322969), Ciprofloxacin 30µg (LOT 320778), Norfloxacin 10µg (LOT 327289), Ofloxacin with five µg (LOT 325442), Kanamycin 30µg (LOT 321894), Clo-Trimoxazole with 25µg (LOT326434), Cephalexin 30µg, Ceftriaxone 30µg (LOT 2855442), and Amikacin with 30µg (LOT 2440768). Susceptibility testing followed Ruangpan, 2004, and Wayne, 2017.

Data analysis

Early onset neonatal sepsis was defined as the onset of symptoms 0-72 hours after birth, and late onset was more than 72 hours after birth. The prevalence of early-onset was compared to that of late-onset using the Chi-Squire test. Neonatal death due to sepsis was calculated from the number of neonates who died due to sepsis over the total number of neonates with positive blood culture sepsis. Fisher's exact test compared the

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etiological agents of early and late-onset newborn sepsis. A statistical significance was defined as a P value of less than 0.05.

Results

Sub-culturing blood and swabs on Brain Heart Infusion Broth revealed that 40.5% (102 out of 252) had positive blood culture results among the tested neonates, while 59.5% (150 out of 252) showed negative blood culture results. Of the neonate samples with positive blood culture results, 74.5% (78 out of 102) grew on MacConkey agar, while 24.5% (24 out of 102) did not. In addition, 80% (40 out of 50) of the swab samples collected showed growth on MacConkey agar, with 20% not exhibiting any growth. Gram Staining Results indicated that 23.5% (24 out of 102) of the bacterial isolates were gram-positive bacteria, while 76.5% (78 out of 102) were gram-negative bacteria. Among the gram-positive bacteria, 96% (23 out of 24) were gram-positive cocci in clusters, and 4% (1 out of 24) were gram-positive diplococci in chains. All isolated gram-negative bacteria had a rod-shaped morphology.

Biochemical characterization of gram-positive bacteria, including colony morphology on blood agar and hemolytic reaction, revealed that 96% of gram-positive bacteria from pure growth exhibited β-hemolysis colonies, which were large, convex, and wet. In comparison, 4% displayed α-hemolysis on blood agar with transparent and mucoid colonies. In the Catalase test, 96% (23 out of 24) of gram-positive bacteria tested positive for catalase, while 4% (1 out of 24) tested negative for catalase. Furthermore, the catalase-positive samples (23) were subjected to the coagulase test, and all of them exhibited a positive coagulase test by forming clumps on the glass slide after inoculation.

Biochemical characterization of gram-negative bacteria showed that about 78% (78 out of the total) of the gram-negative bacteria grown on MacConkey agar were lactose-fermenting, leading to pinkish colonies. The remaining 22% (17 out of 78) were non-lactose-fermenting and had clear colonies (see Table 1 for details).

Table 1 Summary of biochemical characterization of gram-negative bacteria

Total samples	Morphology on BA	Morphology on CA	Morphology on MCA	Gram stain test	TSI test			SIM test			Ureastest	Simon citrate test	Probable isolate
					Butt	slant	Gas	Sulphur	Indole	Motility			
14	Pure growth of non-hemolytic, circular in shape, convex, opaque colons.	Pure growth of whitish colons, circular in shape, opaque in opacity.	- Pure growth of non-lactose ferment colons, whitish in colour and circular in shape.	Gram-negative rod	Red	red	negative	Negative	Negative	Positive	Positive	Positive	Pseudomonas spp
06	Pure growth of non-hemolytic colons, medium in size, circular and opaque.	Pure growth of whitish colons, circular and opaque.	Pure growth of lactose fermenting colons, pink in colour and opaque	Gram-negative rod	Yellow	yellow	positive	Negative	Negative	Positive	Negative	Positive	Enterobacter spp
32	Pure growth of non-hemolysis colons, circular in shape, entire and opaque colons.	Pure growth of circular colons, opaque in opacity.	Pure growth of lactose fermenting colons, pinkish in colour and circular in shape.	Gram-negative rod	yellow	yellow	positive	negative	positive	Positive	Negative	Negative	*E. coli*
16	Pure growth of non-hemolysis colons, medium in size, convex, opaque and circular.	Pure growth of whitish colons, circular opaque in opacity.	Pure growth of lactose fermenting colons, pinkish in colour and convex in shape.	Gram-negative rod	Yellow	red	positive	positive	Negative	Positive	Positive	Positive	Citrobacter spp
07	Pure growth of non-hemolytic mucoid colons,	Pure growth of mucoid colons	Pure growth of lactose fermenting pinkish colons with	Gram-negative rod	Yellow	yellow	positiv	Negative	Positiv	Negative	Positive	Positive	*Klebsiella spp*

Total samples	Morphology on BA	Morphology on CA	Morphology on MCA	Gram stain test	TSI test			SIM test			Ureastest	Simon citrate test	Probable isolate
					Butt	slant	Gas	Sulphur	Indole	Motility			
	flat on the surface	whitish in colour.	mucoid and irregular shape										
02	Pure growth of entire, pale-whitish, flat swarming the blood agar		Pure growth of non-lactose fermenting colons with flat entire irregular shape	gram-negative rod	Yellow	red	positive	positive	negative	Positive	Positive	Positive	Proteus Spp
01	Pure growth of non-hemolysis colons with whitish colour, circular and raised.	Pure growth of whitish colons, convex and circular	Pure growth of non-lactose fermenting colons with a whitish colour.	Gram-negative rod	Yellow	red	negative	positive	negative	Positive	Negative	Negative	Salmonella spp

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Prevalence of Neonatal Sepsis at Morogoro Regional Hospital

This study found that 40% (102 out of 252) of the admitted neonates had positive blood cultures indicating sepsis. Notably, late-onset sepsis accounted for 58% (59 out of 102) of these cases, while early neonatal sepsis represented 42% (43 out of 102) ($\chi2$=2.46, df = 1, p = 0.005). Additionally, 38% (19 out of 50) of premature neonates included in the study tested positive for sepsis in blood cultures.

The predominant bacteria responsible for neonatal bacterial sepsis in Morogoro were Gram-negative bacteria, accounting for 76% (78 out of 102) of all isolates. Gram-positive bacteria constituted only 24% (24 out of 102) ($\chi2$ = 28.58, d f= 1, p = 0.005). Among the bacterial isolates identified in blood samples, *Escherichia coli* was the most common, making up 31% (32 out of 102) of the cases, followed by *Staphylococcus aureus* at 23% (23 out of 102), *Citrobacter* spp at 16% (16 out of 102), and *Pseudomonas* spp at 14% (14 out of 102). Other less common isolates included *Klebsiella* spp (7 out of 102, 7%), *Enterobacter* spp (6 out of 102, 6%), *Proteus* spp (2 out of 102, 2%), *Salmonella* spp (1 out of 102, 1%), and *Streptococcus* spp (1 out of 102, 1%). The distribution of etiological agents in the early and late onset of sepsis is depicted in Figure 1.

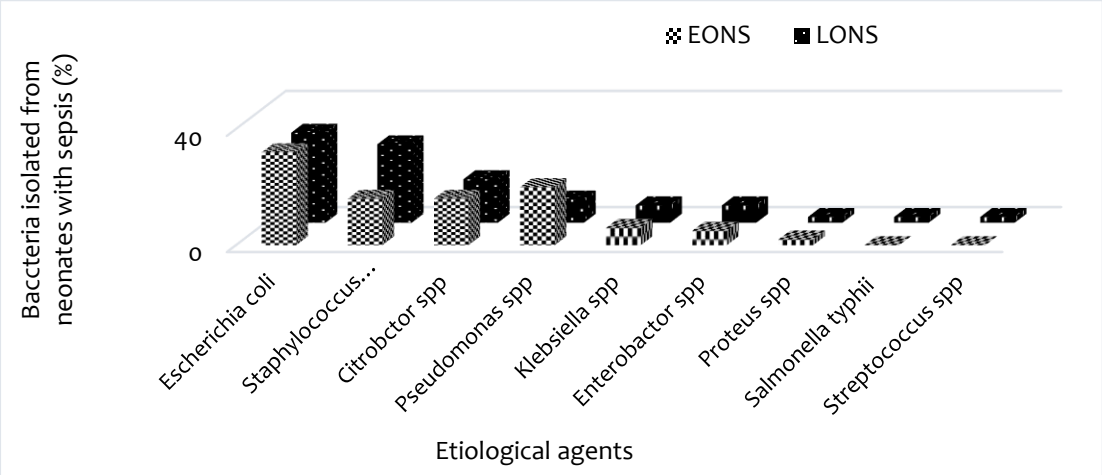

Figure 1 Etiology of neonatal sepsis among neonates admitted in Morogoro Regional Hospital

Common pathogens were isolated from swabs taken from the discharging umbilical cords of selected neonates admitted to Morogoro Hospital. The predominant bacteria found in these swabs included *E. coli* at 40% (20 out of 50 samples), *Citrobacter* spp at 28% (14 out of 50 samples), Staphylococcus aureus at 21% (10 out of 50 samples), and *Klebsiella* spp at 12% (6 out of 50 samples) (see Table 2).

Table 2 Common Pathogens Isolated from Neonatal Swabs in neonates admitted in Morogoro Hospital

Organism	EONS	LONS	Total N (%)
Escherichia coli	07	13	20(40.0)
Citrobactor spp	06	08	14(27.5)
Staphylococcus aureus	04	06	10(20.5)
Klebsiella spp	02	04	06(12.0)
Total	**19**	**31**	**50(100)**

Antibiotic Sensitivity Pattern in Neonates with Neonatal Sepsis at Morogoro Regional Hospital

The antibiotic sensitivity test followed the 2020 Clinical and Laboratory Standards Institute guidelines. The diameters of the zones of inhibition were recorded as Sensitive (S), Intermediate (I), and Resistant (R), with Intermediate (I) considered as Resistant (R). The gram-positive bacteria *S. aureus* isolates were resistant to Ampiclox and Erythromycin, while all Streptococcus isolates were susceptible to all antibiotics.

Most gram-negative bacteria showed high resistance to Ampiclox and Nalidixic acid and moderate resistance to Ceftriaxone. At the same time, most gram-negative bacteria were less resistant to Gentamicin, Cephalexin, Ciprofloxacin, Norfloxacin, and Ofloxacin. All gram-negative bacteria were sensitive to Amikacin.

Seventy-six per cent of *E. coli* were resistant to Ampiclox, 87% to Nalidixic acid, 45% to ceftriaxone and highly sensitive to Gentamicin, Ciprofloxacin, Cephalexin, Ofloxacin, Norfloxacin and Amikacin

Table 3 Sensitivity Pattern of Gram-Positive Bacteria to the Common Antibiotics used in Tanzania

	Staphylococcus aureus		**Streptococcus spp**	
	S	R	S	R
Ampiclox	0	**100**	100	0
Erythromycin	9.5	**90.5**	100	0
Gentamycin	85.7	14.3	100	0
Vancomycin	61.9	38.1	100	0
Kanamycin	57.1	42.5	100	0
Clo-Trimoxazole	57.1	42.5	100	0
Ciprofloxacin	100	0	100	0
Norfloflaxin	100	0	100	0
Amikacin	100	0	100	0

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Citrobacter spp isolates from both blood and swab were 100% resistant to Ampiclox, 33.3% to ceftriaxone, 8.3% Gentamicin, 8.3% Ciprofloxacin and sensitive to the rest of the antibiotics. *Klebsiella* isolates showed resistance to Ampiclox, Nalidixic acid, Gentamicin and Ceftriaxone. Enterobacter *spp* isolates were resistant to Ampiclox and Nalidixic acid and susceptible to Gentamicin, Ciprofloxacin, Ofloxacin, Norfloxacin, Cephalexin and Amikacin (Table 4). *Pseudomonas spp* were 100% sensitive to ceftriaxone, cephalexin, amikacin, Ofloxacin, and Ciprofloxacin and slightly resistant to Norfloxacin. *Proteus spp* and *Salmonella typhii* isolates from blood were sensitive to all antibiotics, including Gentamicin, Ceftriaxone, Ciprofloxacin, Norfloxacin and amikacin.

Table 4 Sensitivity Pattern of Gram-Negative Bacteria Isolates to the Common Antibiotics Used in Tanzania.

	Escherichia coli		*Citrobacter spp*		*Enterobacter spp*		*Klebsiella spp*		*Proteus spp*		*Salmonera typhi*		*Pseudomonas*	
Antibiotic	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
APL	23.6	**76.4**	0	**100**	0	**100**	0	**100**	NA	NA	0	**100**	NA	NA
GEN	87.5	12.5	91.7	8.3	100	0	73.3	26.7	100	0	100	0	100	0
CEFT	56.2	**43.8**	66.7	**33.3**	50	**50**	73.3	**26.7**	100	0	100	0	100	0
CIP	91.2	18.8	91.7	8.3	100	0	100	0	100	0	100	0	100	0
NX	94.1	5.9	100	0	100	0	100	0	100	0	100	0	76	**14**
OF	91.2	18.8	100	0	100	0	100	0	100	0	100	0	100	0
CL	90.5	9.5	100	0	100	0	100	0	100	0	100	0	100	0
AK	100	0	100	0	100	0	100	0	100	0	100	0	100	0
NAL	12	**56**	NA	NA	33.5	**66.5**	NA	NA	NA	NA	NA	NA	NA	NA

APL* Ampiclox, GEN* Gentamicin, CEFT* Ceftriaxone, NAL*Nalidixic acid, CIP* Ciprofloxacin, NX*Norfloxacin, OF*Ofloxacin, CL*Cephalexin, AK*Amikacin, S*Sensitive, R*Resistant, NA* Not applied.

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Clinical Outcomes among Neonates with Sepsis at Morogoro Regional Hospital

Sixteen per cent of the neonates whose sepsis was confirmed by a positive blood culture passed away from their illness. *Escherichia coli,* which was the most common isolate found in blood and swabs, was linked to 31% (5 out of 16) of the deaths that occurred in neonates, followed by *Citrobacter* spp at 25%, *S. aureus* at 19% (3 out of 16), *Pseudomonas* spp at 13%, *S. typhii* at 6%, and *Proteus* spp at 6%. Bacterial sepsis caused by gram-negative bacteria resulted in a higher mortality rate than gram-positive bacteria ($\chi 2 = 1.12$, df =1, $p < 0.05$).

Discussion

The prevalence of neonatal sepsis in Morogoro is notably high. Among the neonates admitted to Morogoro Hospital, late-onset neonatal sepsis surpassed early-onset cases. This higher incidence of late-onset neonatal sepsis suggests a greater prevalence of substandard neonatal care environments. Blood cultures from neonatal sepsis cases revealed that *E. coli* was the most common pathogen, followed by *S. aureus* and *Citrobacter* spp. Similarly, neonatal swab samples predominantly featured *E. coli*, Citrobacter spp, and *S. aureus*. An unexpected rise in *Pseudomonas* spp. cases were noted, although it is an uncommon cause of blood infection. In this study, gram-positive bacteria, particularly. *S. aureus* exhibited significant resistance to Ampiclox and erythromycin, with moderate resistance to Clo-trimoxazole. Ceftriaxone resistance was more common among gram-negative bacteria. Mortality from gram-negative bacterial infections remained higher than that from gram-positive infections, consistent with findings in other Tanzanian hospitals.

Both early and late-onset neonatal sepsis in Morogoro was primarily attributed to *E. coli*, followed by *S. aureus*, *Citrobacter* spp, and other isolates, which contrasts with studies in Mwanza that reported similar rates of early and late sepsis among neonates (Kayange et al. 2010). Furthermore, *E. coli*, *S. aureus*, and *Citrobacter* spp. were the predominant causes of neonatal sepsis in blood samples, aligning with findings in Tanzania and other low-income countries where *S. aureus*, *Klebsiella* spp, and *E. coli* were the leading culprits (Mhada et al. 2012, Fuchs et al. 2016). An increase in *E. coli* infections as the primary bacteria in both early and late-onset sepsis from both blood and swab samples may be attributed to antibiotic selective pressure. Gram-negative bacteria outnumbered gram-positive bacteria in this study, consistent with earlier studies that emphasized the predominance of gram-negative bacteria as the primary etiological agent of neonatal sepsis in various hospitals throughout Tanzania.

Surprisingly, this study identified a rise in neonatal sepsis cases caused by *Pseudomonas* spp. Although *Pseudomonas* typically causes infrequent blood infections and outbreaks in developed nations (Harnein et al. 2015), it was found to be the second most common bacteria causing early onset neonatal sepsis, following *E. coli*, which was more prevalent in early sepsis than late sepsis. These findings suggest

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maternal risk factors might have contributed to *Pseudomonas* infections among neonates. However, the study could not establish a causal link between specific environmental and maternal factors in either early or late sepsis. In addition to *E. coli*, *Klebsiella* spp, *Proteus* spp, *Salmonella* spp, and *Streptococcus* spp were also associated with neonatal sepsis. The current findings emphasize the need for further research to determine and assess the risk factors for neonatal sepsis.

The study findings revealed that most gram-positive and gram-negative bacteria isolates from blood and swab samples were susceptible to Amikacin, Norfloxacin, Ofloxacin, and Ciprofloxacin. The infrequent use of these antibiotics, considered second or third-line drugs for neonatal sepsis treatment, may explain the lack of antibiotic resistance. *Staphylococcus aureus* isolates from this study exhibited high resistance to Ampiclox and erythromycin, along with moderate resistance to Clo-Trimoxazole, consistent with similar findings at Muhimbili Hospital in Tanzania (Mhada et al. 2012). Erythromycin resistance by *S. aureus* has also been reported in Uganda, where 72.4% of all Staphylococci tested were resistant (Tumuhamye et al. 2020). The increasing antibiotic resistance among gram-positive bacteria challenges the standard protocol for neonatal sepsis treatment that relies on these drugs in the current setting.

On the other hand, gram-negative bacteria displayed high sensitivity to Amikacin, Cephalexin, Norfloxacin, Ofloxacin, and Ciprofloxacin, which might be due to their limited use, reserved for neonates with multidrug-resistant sepsis strains. Despite the observed sensitivities, most gram-negative bacteria isolates in this study resisted Ampiclox, a common antibiotic for bacterial infections in this setting. Additionally, *Klebsiella*, *Citrobacter*, *E. coli*, and *Enterobacter* spp demonstrated resistance to Ceftriaxone, consistent with previous research indicating widespread Ampicillin, Cloxacillin, Erythromycin, and Ceftriaxone resistance among bacteria (Amir et al. 2015, Tumuhamye et al. 2020, Shehab et al. 2015, Kayange et al. 2010). These observations emphasize the need for routine susceptibility tests to reassess the use of these antibiotics in neonatal sepsis treatment.

In contrast to other studies in Tanzania and various countries, the findings from this setting showed low resistance to Gentamycin, ranging from 0 to 17%. Conversely, a study in a tertiary hospital in India found up to a 70% Gentamycin resistance rate among gram-negative bacteria (Shah et al. 2016). The median percentage of antimicrobial resistance for gram-negative bacteria in neonates in low- and low-middle-income countries in Africa and Asia varied widely, indicating that resistance patterns to specific antibiotics differ from one setting to another. Therefore, the judicious use of Gentamycin in this setting remains recommended.

Mortality due to gram-negative bacterial infections exceeded those due to gram-positive infections. This is in line with findings in other Tanzanian hospitals (Kayange et al. 2010, Mhada et al. 2012) but in contrast with studies by Tumuhamye et al. (2020) where gram-positive bacteria were associated with more fatalities than gram-negative

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bacteria. Gram-negative bacteria's higher mortality rate can be attributed to their primary role in inducing a potent systemic inflammatory response, resulting in multiple organ dysfunction and a more severe form of sepsis. This necessitates early detection and reduction of risk factors associated with neonatal sepsis.

Conclusion

Late-onset newborn sepsis outnumbers early-onset cases in Morogoro hospitals, indicating poor neonatal care and the urgent need for improved standards. The most common pathogens identified in neonatal sepsis cases were *E. coli, S. aureus*, and *Citrobacter* spp, with *E. coli* being the predominant bacterium. Notably, there was an unexpected increase in cases of neonatal sepsis caused by *Pseudomonas* spp., which is typically infrequent in causing blood infections. Gram-positive bacteria, particularly *S. aureus*, were resistant to common antibiotics like Ampiclox and Erythromycin. Gram-negative bacteria were more sensitive to Amikacin, Cephalexin, Norfloxacin, Ofloxacin, and Ciprofloxacin but showed Ampiclox and Ceftriaxone resistance. Gram-negative bacteria-induced newborn sepsis with a higher fatality rate than gram-positive bacteria. This study underscores the critical need for improved neonatal care, increased awareness of antibiotic resistance, and the importance of tailored antibiotic treatment regimens to combat neonatal sepsis effectively. The findings stressed the significance of frequent susceptibility testing for antibiotic treatment guidance.

Conflict of Interest

All authors declare that they have no conflicts of interest that could potentially influence the integrity, objectivity, or credibility of the research findings presented in this paper.

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Reference

Chan M, Lake A. Towards ending preventable child deaths. The Lancet. 2012 Jun 9;379(9832):2119-20.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests for Bacteria Isolated from Animals: CLSI Supplement VET01S; Replaces VET01-S2. Clinical and Laboratory Standards Institute; 2015.

Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of neonatal sepsis and implicated pathogens: a study from Egypt. BioMed research international. 2015 Jun 4;2015.

Elhalik M, Habibullah J, El-Atawi K. Epidemiology of sepsis in NICU; A 12 years study from Dubai. J

 https://dx.doi.org/10.4314/thrb.v25i2.1

Pediatr Neonatal Care. 2018;8(2):84-8.

Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clinical Microbiology and Infection. 2016 May 1;22(5):416-22.

Fuchs A, Bielicki J, Mathur S, Sharland M, Van Den Anker J. Antibiotic use for sepsis in neonates and children: 2016 evidence update. WHO-Reviews (2016).

https://www.undp.org/sustainable-development-goals/good-health accessed November 15, 2013

Iqbal Q, Bashir C, Mushtaq S, Ahmad A, Baba AR. Thrombocytopenia and other hematological parameters in culture positive neonatal sepsis and their impact. Journal of Pediatric Infectious Diseases. 2013 Jan 1;8(1):25-9.

Jabiri A, Wella HL, Semiono A, Saria A, Protas J. Prevalence and factors associated with neonatal sepsis among neonates in Temeke and Mwananyamala Hospitals in Dar es Salaam, Tanzania. Tanzania Journal of Health Research. 2016 Oct 23;18(4).

Kaistha N, Mehta M, Singla N, Garg R, Chander J. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. The Journal of Infection in Developing Countries. 2010;4(01):055-7..

Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC pediatrics. 2010 Dec;10:1-9.

Lawn JE, Kerber K, Enweronu-Laryea C, Massee Bateman O. Newborn survival in low resource settings—are we delivering?. BJOG: An International Journal of Obstetrics & Gynaecology. 2009 Oct;116:49-59.

Mhada TV, Fredrick F, Matee MI, Massawe A. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome. BMC public health. 2012 Dec;12(1):1-6.

Manji K. Situation analysis of newborn health in Tanzania: current situation, existing plans, and strategic next steps for newborn health. Dar es Salaam: Ministry of Health and Social Welfare; 2009.

Ministry of health and Social Welfare Ministry of Health, Community development, Gender, Elderly and Children 2016 MoHCDGEC/Tanzania Mainland, Ministry of Health - MoH/Zanzibar, National Bureau of Statistics - NBS/Tanzania, Office of Chief Government Statistician - OCGS/Zanzibar, and ICF. 2016. Tanzania Demographic and Health Survey and Malaria Indicator Survey 2015-2016. Dar es Salaam, Tanzania: MoHCDGEC, MoH, NBS, OCGS, and ICF.

Mshana SE, Kamugisha E, Mirambo M, Chakraborty T, Lyamuya EF. Prevalence of multiresistant gram-negative organisms in a tertiary hospital in Mwanza,

Tanzania. BMC research notes. 2009 Dec;2(1):1-6.

Pop-Began V, Păunescu V, Grigorean V, Pop-Began D, Popescu C. Molecular mechanisms in the pathogenesis of sepsis. Journal of medicine and life. 2014;7(Spec Iss 2):38.

Shitaye D, Asrat D, Woldeamanuel Y, Worku B. Risk factors and etiology of neonatal sepsis in Tikur Anbessa University Hospital, Ethiopia. Ethiopian medical journal. 2010 Jan 1;48(1):11-21.

Ruangpan L, Tendencia E. Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment. Aquaculture Department, Southeast Asian Fisheries Development Center.; 2004.

Thapa S, Sapkota LB. Changing trend of neonatal septicemia and antibiotic susceptibility pattern of isolates in Nepal. International journal of pediatrics. 2019 Feb 6;2019.

Tumuhamye J, Sommerfelt H, Bwanga F, Ndeezi G, Mukunya D, Napyo A, Nankabirwa V, Tumwine JK. Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk. PLoS One. 2020 Aug 10;15(8):e0237085.

United Nations Children Fund Maternal and new born health disparity in Uganda. 2017 *United Nations Children Fund.*

Wayne PA. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement. CLSI document M100-S20. 2010.

Lawn JE, Kerber K, Enweronu-Laryea C, Massee Bateman O. Newborn survival in low resource setting are we delivering? BJOG: An International Journal of Obstetrics & Gynaecology. 2009 Oct; 116:49-59.

World Health Organization. WHO sepsis technical expert meeting, 16-17 January 2018. World Health Organization; 2018.