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Distribution, Isolation and Identification of Bacterial Infection among Neonates in some Hospitals in Kano Metropolis

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ABSTRACT

Neonatal sepsis continues to be one of the major causes of morbidity and mortality in newborns especially in developing nations due to the difficulties in early diagnosis and management and microbial resistance. This research was carried out to determine the pattern of distribution, isolation, and identification of bacteria causing neonatal sepsis in some selected hospitals in Kano metropolis, Kano state. Cross-sectional and descriptive study design was used for the study which involved 231 neonates from 0-28 days of age admitted in the neonatal wards of Murtala Muhammad Specialist Hospital, Muhammad Abdullahi Wase Teaching Hospital and Khalifa Isyaku Rabi'u Pediatric Hospital. Identification of bacteria isolates was done through Gram staining, biochemical testing, and use of VITEK systems. Out of the 231 neonates enrolled in the study, 88 neonates (38.1%) had positive bacterial growth while 143 neonates (61.9%) had negative cultures. Murtala Muhammad Specialist Hospital had the highest percentage of positive isolates 40 (42.6%), and Khalifa Isyaku Rabi'u Pediatric Hospital had the least number 20 (33.3%). The most common bacterial isolate was Staphylococcus aureus, which comprised of 80 (90.9%) of isolates, followed by Escherichia coli 4 (4.5%), Pseudomonas aeruginosa 2 (2.3%), Streptococcus spp. 1 (1.1%), and Klebsiella spp. 1 (1.1%). Most of the culture positive septicemia was that of late-onset neonatal sepsis, with the highest occurrence among neonates aged between 8 and 15 days. From the above results, it is clear that there is a significant level of bacterial neonatal sepsis, with S. aureus being the most common pathogen. High rate of late-onset infections suggests the possibility of environmental and contact transmission post-delivery. Hence, measures such as proper hygiene, sterilization of neonate equipment, environmental sanitation, maternal education, microbiological diagnosis, and proper antibiotic use are highly recommended.

Keywords: Neonatal sepsis; bacterial pathogens; neonatal sepsis; antimicrobial resistance;

Introduction

Neonatal sepsis is defined as a clinical syndrome characterized by systemic signs of infectionand bacteremia in the first month of life. It is commonly categorized into early-onset sepsis (EOS), typically occurring within 72 hours of birth and usually resulting from organisms transmitted vertically from the mother, and late-onset sepsis (LOS), which often arises from nosocomial or community-acquired infections (WHO., 2021). Both forms are associated with significant morbidity and mortality, especially in low-resource settings.Globally, an estimated 3 million cases of neonatal sepsis are reported annually, with over400,000 associated deaths, the majority of which occur in sub-Saharan Africa and South Asia (Fleischmann-Struzek *et al*., 2018). In Nigeria, neonatal mortality remains alarmingly high at 36 deaths per 1,0.,00 live births, and sepsis is identified as one of the leading contributors,responsible for nearly 30% of neonatal deaths (NPC and ICF, 2019). These figures underscore the urgent need for improved prevention, diagnosis, and management strategies.A neonate is a newborn baby within the first 28 days of life a period marked by significant physiological adaptations and heightened vulnerability to infections due to an underdeveloped immune system (WHO, 2021). Infections during this early life stage can progress rapidly and may result in severe complications or death if not promptly and effectively managed. Among these infections, neonatal sepsis remains one of the most serious and life-threatening conditions affecting neonates globally (Rahman*et al*., 2022). Given the increasing prevalence of neonatal sepsis and the alarming rise in antimicrobial resistance in Nigeria, especially in the northern states, there is an urgent need to generate up-to-date data on the prevalence, causative organisms, and antimicrobial susceptibility patterns. This study aims to address this gap by investigating the bacterial etiologies of neonatal sepsis in selected hospitals in Kano State, analyzing their resistance patterns, and identifying any associated virulence genes. The outcomes will guide clinicians and policymakers in formulating evidence-based strategies for managing and preventing neonatal sepsis in the region.

Materials and Method

StudyArea

The study was conducted in selected hospitals within Kano State, including Murtala Muhammad Specialist Hospital (11.9946°N, 8.5502°E), Muhammad Abdullahi Wase Teaching Hospital (11.9910°N, 8.5115°E), and Khalifa Isyaku Rabiu Pediatrics Hospital (11.9982°N, 8.5508°E). These hospitals are major healthcare providers in the region, offering specialized neonatal care and serving as referral centers for both urban and rural populations. They are well- equipped for diagnosing and managing neonatal sepsis, making them ideal settings for this research. It was estimated that about 19,899 neonates are delivered in these hospitals annually (Ogundare *et al*., 2021). The study focus on neonates admitted to the neonatal wards which is Special Care Baby Unit (SCBU) of these hospitals, ensuring a representative sample of the population affected by neonatal sepsis (Google Maps, 2025).

Research Design

The study employs a cross-sectional, descriptive design to investigate neonatal sepsis in selected hospitals within Kano State. The clinical data was collected from neonates aged 0-28 days who are diagnosed with or suspected of having sepsis. Blood samples were obtained from these neonates for microbiological analysis to identify bacterial pathogens and determinetheirantibiotic resistanceprofiles. Data on demographics, clinical signs, and treatment outcomes was recorded. The study will be conducted over a specified period to provide an accurate representation of the prevalence and resistance patterns in the region (Adewumi *et al*., 2020).

Ethical Considerations

Ethical clearance was obtained from the Kano State Ministry of Health Ethical Review Board. Written informed consent was obtained from mothers or guardians of neonates. All data were kept confidential and used solely for academic purposes.

Sample Size Determination

Usingthe formula: Adewumi *et al.,* (2020)

$$n = \frac{Z^2 x\ pq}{d^2}$$

Where:

n=required sample size

Z=standard normal deviate (1.96^2 for 95% confidence level) =3.8416

p=estimated prevalence of neonatal sepsis$=209.7=(210)$

d=precision (5% $=0.05^2=0.0025$)

p=16.3 =0.163

q=1-p

q=1-0.163=0.837

$$n = \frac{(1.96)^2 x\ 0.163x0.837}{(0.05)^2}$$

n=209.64 samples which is equals to 210 and n_2 is 10% standard error rounded up to 231 to improve precision making a total of 231 samples.

Thus, 231 neonates were recruited (Chen *et al.,* 2020).

Inclusion and Exclusion criteria

The works cover only neonates admitted with sepsis from three selected hospitals of Kano metropolis. Any other neonates without sepsis or are in the hospitals besides the scope is among the exclusion.

Sampling collection

Purposive sampling technique was used in selecting the hospitals. Proportionate sampling was used to calculate the required sample number collected from each hospital (Musicha, 2021). Thus, for each hospital the sample number was generated by dividing the numberofbedspacein the Hospitalby thetotal number of bedspacesin all the hospitals multiplied by 210 as shown below:

i. Number of beds space of neonate in Murtala Muhammad Specialist Hospital= 17
ii. Number of bed space for neonate in Muhammad Abdullahi wase Teaching Hospital = 17
iii. Number of bed space Available for neonate in Khalifa Isyaku Rabi'u Pediatric Hospital=13

Therefore, total number of bed spaces = 17+17+13=47

Sample number from Murtala Muhammadspecialist Hospital = 17/47×210= 75.95
Sample number from Muhammad Abdullahi Wase Teaching Hospital = 17/47×210=75.95
Sample number from Khalifa Isyaku Rabi'u Pediatric Hospital = 13/47×210=27.8

So 92 neonate's samples were collected from Murtala Muhammad Specialist Hospital, 78 from Muhammad Abdullahi Wase Teaching Hospital and 61 were collected from Khalifa Isyaku Rabi'u Pediatric Hospital. Within hospitals, systematic sampling was applied to enroll the 231 neonates who meet the inclusion criteria during the study period.

Questionnaire Administration

A structured questionnaire and clinical checklist was administered to mothers and healthcare records reviewed. The data was collected from the study hospitals one after the other in weeklybasis. Information included: maternal age, parity, mode of delivery, historyof PROM (premature rupture of membranes), UTI during pregnancy (Musicha, 2021). Neonatal factors: prematurity, birth weight, Apgar score, place of delivery (Bello *et al*., 2022).

Isolation and Identification of Bacterial isolates

Blood samples (2ml) were aseptically collected from neonates before antibiotic administration. Blood sample were incubated and monitored for the ring alarm over 5–7 hours using an automated blood culture system (BACTEC automate method) where the suspected sample was determined by culture manual methods on blood agar, MacConkey agar, and chocolate agar, then incubated at 35–37°C (Musicha, 2021). Bacterial identification were carried out using Gram staining where the sampleswere streak in a glass slide and flood with Crystal violet for 60s, wash and fix with Lugol's iodine and wash, decolorized with 75% acetone and wash, and finally reflood with Safranin for 30s, air dried and view with 100X objective lens; biochemical tests and confirmed using VITEK systems (CLSI, 2020).The standard laboratory procedure for detecting neonatal sepsis using an automated system typically involves the following steps.

Automated System Procedure

The blood samples were inoculated into a culture bottle (aerobic and anaerobic) and the culture bottles was incubated into the automated system. The system was allowed to monitor the culture for microbial growth (e.g., CO2 production, pH changes) and provide a preliminary result (e.g., positive or negative) based on the detection of microbial growth (CLSI, 2020).

Gram Staining and Microscopy

Once the automated system flags a positive result, a Gram stain was performed from the culture broth and identifies the type of microorganism base on its morphology (CLSI, 2020).

Data Analysis

Data was analyzed using SPSS version 25. Descriptive statistics (frequency, mean, standard deviation) was summarizing socio-demographic and clinical data. Chi-square or Fisher's exact test was assessing associations between risk factors and culture positivity. Antibiotic susceptibility patterns were summarized using percentages. Logistic regression was used to determine predictor of sepsis and antimicrobial resistance.

Results

Distribution of Bacterial Infection and among Neonates based on Gender

The result of the study as shown in Table 4.1 revealed that out of the 231 neonates studied in Kano Metropolis 88 (38.10%) had positive bacterial infection. The result show that among the three hospitals studied, Murtala Muhammad Specialist Hospital had the highest number of neonates with positive bacterial infection with 40 (42.60%) while Khalifa Isyaku Rabi'u Pediatric Hospital had the least with 20 (33.33%).

Table 1: Distribution of Bacterial Infection and among Neonates in some Hospitals in Kano Metropolis based on Gender

Hospital	No. Tested (%)	Neonates with + growth (%)	Neonates with bacterial infection		Neonates withoutInfection (%)	Neonates withoutinfection	
			Male No (%)	Female No (%)		Male (%)	Female (%)
MMSH	92 (39.8)	40 (17.3)	24 (10.4)	16 (6.9)	52 (22.5)	22 (9.5)	30 (12.9)
MAWTH	78 (33.8)	28 (12.1)	16 (6.9)	12 (5.2)	50 (21.6)	24 (10.4)	26 (11.3)
KIRPH	61 (26.4)	20 (8.7)	10 (4.3)	10 (4.3)	41 (17.7)	20 (8.7)	21 (9.0)
Total	231 (100)	**88 (38.10)**	**50 (21.6)**	**38 (16.4)**	**143 (61.9)**	**66 (28.6)**	**77 (33.3)**

Key: MMSH: Murtala Muhammad Specialist Hospital, MAWTH Muhammad Abdullahi Wase Teaching Hospital and KIRPH Khalifa Isyaku Rabiu Pediatric Hospital

Characteristics of Bacterial Isolates from Infected Neonates in Kano metropolis

The results presented in Table 2 reveals a striking dominance of *Staphylococcus aureus*, which accounted for 91.3% (80/88) of all bacterial isolates recovered from neonates with suspected sepsis. This exceptionally high proportion signals a critical role of *Staphylococcal* transmission in both hospital and community environments within Kano Metropolis. *S. aureus* is well known for its ability to colonize neonatal skin and mucosa, especially under conditions of frequent handling, shared equipment, and suboptimal hygiene.

Table 2: Characteristics of Bacterial Isolates from Infected Neonates in Kano Metropolis

Colony Appearance	Gram reaction	Coagulase	Citrate	Oxidase	Catalase	Suspected organisms	rcentage (%)
Golden-yellow conies on blood agar, often bête-hemolytic	Positive (cocci)	+	+/-	-	+	*Staphylococcus aureus*	80 (90.9%)
Small gray colonies on blood agar, may be alpha-hemolytic (green zone) beta-hemolytic (clear zone) or non-hemolytic	Positive (cocci)	-	-	-	-	*Streptococcus*spp	1 (1.1%)
Large lactose-fermenting colonies on MacConkey agar	Negative (rod)	-	+/-	-	+	*Escherichiacoli*	4 (4.5%)
Large mucoid lactose-fermenting colonies on MacConkey agar	Negative (rod)	-	+	-	+	*Klebsiella*spp	1 (1.1%)
Greenish-blue colonies on nutrient agar andgrape odor	Negative (rod)	-	+	+	+	*Pseudomonasaeruginosa*	2 (2.3%)

Distribution of Bacterial Infection (Neonatal Sepsis) among Neonates according to their Ages and Gender

The age-based distribution of culture-positive neonates presented Table 3 provides a clear epidemiological pattern distinguishing between early-onset neonatal sepsis (EONS) which is usually be detected from 1-3 days and late-onset neonatal sepsis (LONS) that are over 3 days of delivery. EONS accounted for only positive cases (14.8%), while LONS accounted for positive cases (85.2%). It is evident from the study findings that EONS accounted for only 4 out of 27 positive cases (14.8%), while LONS accounted for 63 out of 78 positive cases (85.2%) when combining the 8–11 days and 12–15 days age groups.

Table 3: Distribution of Bacterial Infection (Neonatal Sepsis) among Neonates according to their Ages and Gender

Age (days)	Number (n)	Male (n)	Female (n)	Positive (%)	Negative (%)
1-3	27	15	12	4(1.7)	16 (6.9)
4- 7	49	29	20	9(3.9)	25(10.8)
8- 11	51	27	24	25(10.8)	24(10.4)
12- 15	32	21	11	18(7.8)	14(6.0)
16- 19	20	12	8	11(4.8)	9(3.9)
20 – 23	27	12	15	8(3.4)	27 (11.7)
24 – 27	17	9	8	10(4.3)	17(7.4)
28- 30	8	3	5	3(1.3)	11 (4.8)
Total	**231(100%)**	**128**	**103**	**88 (38.0)**	**143 (61.9)**

Discussion

The result of the study as shown in Table 1 revealed that out of the 231 neonates studied in Kano Metropolis 88 (38.10%) had positive bacterial infection and this finding is in line with the research reported by Abdullahi *et al.*, (2023) and Zhang and Xue (2020). The result show that among the three hospitals studied, Murtala Muhammad Specialist Hospital had the highest number of neonates with positive bacterial infection with 40 (42.60%) while Khalifa Isyaku Rabi'u PediatricHospital had the least with 20 (33.33%). And the rationale behind the highest occurrence in Specialist hospital is due to the fact that any serious infections are expected to be reported to the consultant for rapid assessment and treatment (Abdullahi *et al.,* 2023).

The result of the study as exhibited Table 2 show that 51 (57.95%) of the 88 neonates with bacterial infection were males while 64 (40.54%) were females. Murtala Muhammad Specialist Hospital has the highest percentage of the positive occurrence with 54.54% males and 45.46% Females and the highest occurrence in Specialist hospital is due to the fact that any serious infections are expected to be reported to the consultant for rapid assessment and treatment (Abdullahi *et al.,* 2023).

The results presented in Table 3 reveals a striking dominance of *Staphylococcus aureus*, which accounted for 91.3% (80/88) of all bacterial isolates recovered from neonates with suspected sepsis. This exceptionally high proportion signals a critical role of *Staphylococcal* transmission in both hospital and community environments within Kano Metropolis. *S. aureus* is well known for its ability to colonize neonatal skin and mucosa, especially under conditions of frequent handling, shared equipment, and suboptimal hygiene. Nigerian neonatal surveillance studies have similarly documented high *Staphylococcal* prevalence in crowded special-care baby units where hand hygiene compliance remains a persistent challenge (Bello and Nuhu, 2022). Therefore, the bacteriological landscape observed in this study strongly implicates human-contact transmission as a major contributor to neonatal sepsis.In addition to its high occurrence, the predominance of *S. aureus* raises serious clinical concerns due to its known potentials for developing resistance to multiple antibiotic classes. Even when not methicillin-resistant, *S. aureus* is frequently associated with severe systemic infection in neonates because of its virulence factors such as toxins and adhesion molecules. The overwhelming representation of this organism in the current study findings suggests that infection-prevention strategies should prioritize interventions that reduce person-to-person spread, particularly ensuring consistent hand washing, sterilization of newborn instruments, and minimizing unnecessary handling of neonates. This aligns with findings from northwest Nigeria where similar patterns prompted hospitals to institute aggressive infection-control programs (Bello *et al.*, 2021).

The presence of Gram-negative organisms, though proportionally smaller, remains clinically significant. *Escherichia coli* accounted for 4.3% of isolates, while *Pseudomonas aeruginosa* and *Klebsiella* species contributed 2.2% and 1.09%, respectively (Table 3). These organisms, despite their lower frequency, are often associated with rapid deterioration, septic shock, and high mortality in newborns due to their strong virulence potential and multidrug resistance tendencies. Several Nigerian studies have cautioned that even a modest representation of Gram-negative rods in neonatal cultures is concerning, as they are frequently implicated in outbreaks driven by contaminated fluids, surfaces, or neonatal equipment (Abubakar *et al.*, 2023). Thus, their detection in this study underscores the need for rigorous sterilization practices.Furthermore, the detection of *Streptococcus* species at 1.09% (Table 3) suggests a low occurrence of these organisms in neonatal sepsis within Kano, although their contribution to early-onset sepsis in other regions is well recognized. Their relatively low occurrence may be attributed to improved intrapartum practices, maternal screening, and the declining prevalence of vertical transmission routes. Collectively, the study findings highlight an organism profile heavily dominated by *S. aureus*, supported by smaller but clinically important Gram-negative pathogens, thereby emphasizing the dual need for skin-contact hygiene and environmental sanitation in the study area.

The age-based distribution of culture-positive neonates presented Table 4 provides a clear epidemiological pattern distinguishing between early-onset neonatal sepsis (EONS) which is usually be detected from 1-3 days and late-onset neonatal sepsis (LONS) that are over 3 days of delivery. EONS accounted for only positive cases (14.8%), while LONS accounted for positive cases (85.2%). It is evident from the study findings that EONS accounted for only 4 out of 27 positive cases (14.8%), while LONS accounted for 63 out of 78 positive cases (85.2%) when combining the 8–11 days and 12–15 days age groups. This disparity reveals a striking predominance of LONS in Kano Metropolis. Such a high burden of LONS frequently reflects environmental exposure after birth including suboptimal hygiene practices, poor cord care, and inadequate infection-prevention measures in both home and hospital environments. Similar dominance of late-onset infections has been reported in other studies conducted in northern Nigeria, where environmental factors play a major role in neonatal morbidity (Auwal and Abdullahi, 2022).

Late-onset sepsis was particularly pronounced in the 8–11 days age group, where 29 out of 51 neonates (56.9%) tested positive. This age group alone constituted nearly half of all late-onset cases. This spike corresponds with increased handling of infants, exposure to feeding bottles, and traditional practices involving the umbilical stump. Previous studies in Nigerian report that the second week of life is an especially vulnerable period because maternal antibodies begin to decline, leaving neonates more susceptible to community-acquired pathogens (Balogun and Abba, 2020). The data in Table 2 therefore strongly emphasize the need for targeted educational interventions for mothers immediately after delivery. The second major concentration of LONS cases occurred among neonates aged 12–15 days, where 18 out of 32 neonates (56.3%) were culture-positive. This continued high prevalence reinforces the sustained vulnerability during the second week of life. In addition to declining maternal immunity, improper storage of breast milk, contaminated feeding utensils, and overcrowded households have been described as important contributors to infection during this period (Abubakar and Maina, 2023).

In contrast to the high LONS figures, early-onset sepsis (defined as 0–72 hours after birth) remained comparatively low. Only 4 out of 27 neonates (14.8%) tested positive within this early neonatal period, suggesting improving maternal care, better intrapartum prophylaxis,

and enhanced aseptic delivery techniques in the hospitals studied. Studies in tertiary facilities across Nigeria have recorded similar reductions in EONS due to strengthened obstetric protocols and improved antenatal infection screening (Oladipo and Hamza, 2021). The reported low EONS rate in the current study reflects progress in reducing vertical transmission from mother to child.Notably, the lowest positivity rate was seen among neonates aged 28–30 days, where only 3 out of 78 neonates (3.8%) tested positive. This steep decline demonstrates the gradual maturation of the neonatal immune system and reduced reliance on hospital care after the initial weeks of life. Nevertheless, the presence of any infection at this stage highlights ongoing concerns about community-acquired pathogens, particularly in environments where hygiene standards may be inadequate. Overall, the study findings as clearly presented in Table 2 clearly establishes 8–15 days of life as the most critical window for neonatal sepsis, with LONS with over 85% of all positive cases, underscores the need for strengthened hygiene practices, maternal education, and enhanced surveillance during this high-risk period.

Conclusion

Based on the findings of this study, the first objectiveto isolate and identify bacteria from neonates clinically diagnosed with sepsis in Kano Metropoliswas successfully achieved. Bacteriological analysis revealed a substantial burden of neonatal sepsis across the studied hospitals, with an overall culture positivity of 43.1%. The isolated organisms included *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, and *Streptococcus species*, indicating the involvement of both Gram-positive and Gram-negative bacteria in neonatal sepsis. Among these, *Staphylococcusaureus* was the predominant isolate, suggesting that contact- and environment-related transmission plays a significant role in neonatal infections within Kano Metropolis.

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