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In vitro antibiotic susceptibility of bacterial pathogens and risk factors associated with culture positive neonatal sepsis in two hospitals, Katsina metropolis, Nigeria

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Abstract:

Background: Neonatal sepsis is one of the most important causes of morbidity and mortality among neonates, particularly in developing countries. This study aimed to determine the risk factors and *in vitro* antibiotic susceptibility patterns of bacterial pathogens associated with neonatal sepsis in Federal Medical Centre (FMC) and Turai Umaru Yar'adua Maternal and Children Hospital (TUYMCH), Katsina, Nigeria.
Methodology: A total of 60 hospitalized neonates evaluated for neonatal sepsis at the special care baby units (SCBU) of the two healthcare facilities whose parents gave informed consent were enrolled for the study between July and December 2020. Blood samples were aseptically collected from the neonates and cultured on BacT/Alert automated platform (BioMérieux, Mercy-Etoile, France) machine. Bacteria were identified from all positive cultures and *in vitro* susceptibility test was performed on the isolates to determine their minimum inhibitory concentrations (MICs) to eight selected antibiotics using the Vitek-2 compact system. Data were analyzed by SPSS version 22.0.
Results: A total of 60 neonates with clinical features suggestive of sepsis were enrolled. The mean age of the neonates is 1.35±0.48 days while the mean weight is 2.13±0.89 kg. Neonates with early onset sepsis (<3 days) constituted 65% while those with late-onset sepsis (>3 days) constituted 35%. Thirty-one (51.7%) neonates were culture positive while 29 (48.3%) were culture negative for bacterial pathogens. Gram-positive bacteria predominated, constituting 80.6% while Gram-negative bacteria constituted 19.4%. The most frequent Gram-positive bacteria were coagulase-negative staphylococci (51.6%, 16/31), with *Staphylococcus haemolyticus* 5 (16.1%) predominating, while the most frequent Gram-negative bacteria isolate was *Escherichia coli* 2 (6.5%). A high degree of antibiotic resistance (>50% rate) was exhibited by the isolates against most of the tested antibiotics including third generation cephalosporins and fluoroquinolones. Gentamicin was the only antibiotic effective, with 65.5% of all isolates sensitive to it; 68.0% Gram-positives and 50.0% Gram-negatives. Vancomycin was also effective against Gram-positive bacteria, with 68.0% of the isolates sensitive to it. Previous premature delivery (64.5%, 20/31) and baby delivery at home were respectively the only maternal and neonatal factors significantly associated with culture-positive neonatal sepsis (OR=2.975, 95% CI=1.040-8.510). There was no significant difference between culture positive and negative neonatal sepsis with respect to clinical manifestations such as refusal of feeds, fever, jaundice, fast breathing, convulsion and body temperature (p>0.05).
Conclusion: Neonatal sepsis is a substantial cause of mortality and morbidity among neonates admitted at the FMC and TUYMCH, Katsina, Nigeria. There is a need for regular surveillance of the risk factors, causative organisms, and antibiotic susceptibility patterns of isolated pathogens, to inform the choice of empirical antibiotic treatment pending the results of blood cultures.

Keywords: neonates, sepsis, risk factor, antibiotic, bacteria.

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Sensibilité aux antibiotiques in vitro des pathogènes bactériens et facteurs de risque associés à une septicémie néonatale à culture positive dans deux hôpitaux, métropole de Katsina, Nigeria

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Résumé:

Contexte: La septicémie néonatale est l'une des causes les plus importantes de morbidité et de mortalité chez les nouveau-nés, en particulier dans les pays en développement. Cette étude visait à déterminer les facteurs de risque et les schémas de sensibilité aux antibiotiques in vitro des agents pathogènes bactériens associés à la septicémie néonatale dans le Centre Médical Fédéral (FMC) et le Turai Umaru Yar'adua Hôpital de la Mère et de l'Enfant (TUYMCH), Katsina, Nigeria.
Méthodologie: Un total de 60 nouveau-nés hospitalisés évalués pour une septicémie néonatale dans les unités de soins spéciaux pour bébés (SCBU) des deux établissements de santé dont les parents ont donné leur consentement éclairé ont été inclus dans l'étude entre juillet et décembre 2020. Des échantillons de sang ont été prélevés de manière aseptique sur les nouveau-nés et cultivés sur la plate-forme automatisée BacT/Alert (BioMérieux, Mercy-Etoile, France). Les bactéries ont été identifiées à partir de toutes les cultures positives et un test de sensibilité in vitro a été effectué sur les isolats pour déterminer leurs concentrations minimales inhibitrices (CMI) à huit antibiotiques sélectionnés à l'aide du système compact Vitek-2. Les données ont été analysées par SPSS version 22.0.
Résultats: Au total, 60 nouveau-nés présentant des caractéristiques cliniques évoquant une septicémie ont été recrutés. L'âge moyen des nouveau-nés est de 1,35±0,48 jours alors que le poids moyen est de 2,13±0,89 kg. Les nouveau-nés atteints d'un sepsis précoce (<3 jours) représentaient 65% tandis que ceux atteints d'un sepsis tardif (>3 jours) représentaient 35%. Trente et un (51,7%) nouveau-nés étaient positifs à la culture tandis que 29 (48,3%) étaient négatifs à la culture pour les pathogènes bactériens. Les bactéries Gram-positives prédominaient, constituant 80,6% tandis que les bactéries Gram-négatives constituaient 19,4%. Les bactéries à Gram positif les plus fréquentes étaient les staphylocoques à coagulase négative (51,6%, 16/31), *Staphylococcus haemolyticus* 5 (16,1%) prédominant, tandis que l'isolat de bactéries à Gram négatif le plus fréquent était *Escherichia coli* 2 (6,5%). Un degré élevé de résistance aux antibiotiques (> 50% de taux) a été présenté par les isolats contre la plupart des antibiotiques testés, y compris les céphalosporines de troisième génération et les fluoroquinolones. La gentamicine était le seul antibiotique efficace, avec 65,5% de tous les isolats qui y étaient sensibles; 68,0% de Gram positifs et 50,0% de Gram négatifs. La vancomycine était également efficace contre les bactéries Gram-positives, 68,0% des isolats y étant sensibles. Un accouchement prématuré antérieur (64,5%, 20/31) et un accouchement à domicile étaient respectivement les seuls facteurs maternels et néonatals significativement associés à une septicémie néonatale à culture positive (OR=2,975, IC 95%=1,040-8,510). Il n'y avait pas de différence significative entre la septicémie néonatale positive et négative à la culture en ce qui concerne les manifestations cliniques telles que le refus de s'alimenter, la fièvre, la jaunisse, la respiration rapide, les convulsions et la température corporelle (p>0,05).
Conclusion: La septicémie néonatale est une cause importante de mortalité et de morbidité chez les nouveau-nés admis au FMC et TUYMCH, Katsina, Nigeria. Il est nécessaire de surveiller régulièrement les facteurs de risque, les organismes responsables et les schémas de sensibilité aux antibiotiques des agents pathogènes isolés, afin d'éclairer le choix d'un traitement antibiotique empirique en attendant les résultats des hémocultures.

Mots-clés: nouveau-nés, septicémie, facteur de risque, antibiotique, bactérie.

Introduction:

Sepsis is a significant cause of neonatal morbidity and mortality, especially in developing countries. The microbial organisms of sepsis and their antimicrobial susceptibility patterns are dynamic (1). Despite several attempts to lessen its effect, neonatal sepsis has remained a significant cause of morbidity and mortality in neonates. It remains one of the leading causes of death in neonates, both in the developed and developing countries (2). The neonatal period is the most endangered period of life due to vulnerability to infectious agents. Neonates are deficient in both cell-mediated and humoral immunity due to relative immaturity of their immune systems and lack of exposure to infectious pathogens, and the fact that they produce immunoglobulins at lower proportion compared to adults (2).

Neonatal sepsis is a clinical condition comprising of non-specific signs and symptoms of infection, accompanied by bacteremia in the first 28 days of life. It is characterized by systemic signs of circulatory compromise such as decrease peripheral perfusion, pallor, hypothermia, and poor responsiveness (3). Neonatal sepsis and mortality risk increase with decreasing birth weight and gestational age (4). Neonatal sepsis may also refer to neonatal systemic infection, including septicemia, pneumonia, meningitis, urinary tract infection, arthritis, and osteomyelitis (5). Neonatal sepsis could be early-onset in the first 72 hours of life and known to be acquired through prenatal and intrapartum maternal transmission, while late-onset sepsis starts from the fourth day to the fourth week of life (5).

Globally, about 40% of deaths in under-five children occur in the neonatal period resulting in about 2.9 million neonatal deaths each year (6). The highest mortality rates for neonates occur in the developing and poorest countries and a third of these deaths are ascribed to

infections acquired by the neonate during labor and delivery or immediately after birth (6).

Nigeria is said to account for the highest number of neonatal deaths in Africa and third in the world, after India and China, with sepsis responsible for about 30% to 50% of deaths (7). Prevalence of sepsis in neonates reported from earlier hospital-based studies ranges between 7.04 and 22.9 per 1000 live births (8). Sepsis-related case fatality rates are mostly preventable with appropriate antimicrobial use and aggressive supportive care. Nevertheless, neonatal sepsis has no pathognomonic features and the clinical presentations also varies (5). Poor or delayed laboratory services also make laboratory diagnosis problematic in resource-limited settings. As a result, neonatal healthcare providers in resource-poor settings make an uncertain diagnosis and empirical treatment of neonatal sepsis, using the new neonatal international management guidelines of the World Health Organization (3).

Certain pathogens implicated in neonatal sepsis have progressively developed increased resistance to frequently used antibiotics due to the selection burden, which is an unavoidable phenomenon in antimicrobial use and thus makes treatment of neonatal sepsis very difficult (2). However, the variety of organisms causing neonatal sepsis differs significantly across different regions and countries, and changes over time, even in the same place. This variation may affect the achievement of empirical management (3). In the developed countries, the most common organisms of neonatal sepsis are Group B streptococci (GBS), *Escherichia coli,* and *Listeria monocytogenes* while Gram-negative bacteria and coagulase-negative staphylococci are the commonest in the developing countries (9).

It is notable that the rising incidence of drug-resistant bacteria has also made treatment more problematic and expensive (10). It is important, therefore, that the epidemiology of neonatal sepsis should be regularly updated to provide information required for regular review of the choice of antimicrobials most appropriate for the treatment of sepsis in neonates, in different places and at different times (11).

The choice of antibiotic therapy for the treatment of neonatal sepsis is still challenging due to the emergent antibiotic resistance to the common antibiotics used in the treatment of infection. The aim of this study therefore is to determine the risk factors and *in vitro* antibiotic susceptibility of bacterial pathogens associated with neonatal sepsis in Katsina metropolis of Nigeria.

Materials and method:

Study area

The study was carried out at the special care baby units (SCBU) of the Federal Medical Centre, Katsina, a tertiary health facility, and Turai Umar Yar'adua Maternity and Children Hospital, Katsina, northwest Nigeria. Katsina is situated about 260 kilometres east of the city of Sokoto and 135 kilometres northwest of Kano, and shares international border with the Niger Republic, and has a total area of 24,192 km^2 and geographical coordinates of $12^0$15'N and $7^0$30'E. As at 2016, the estimated population of Katsina metropolis was 505,000.

Katsina is predominantly an agrarian society with majority of the inhabitants being of the Hausa-Fulani tribe. The two hospitals were preferred as a study area due to availability of the study population and laboratory facilities required for the research. The hospitals provide newborn healthcare services to the population of Katsina metropolis and the surrounding local government areas.

Study design and duration

This was a hospital-based descriptive observational study of neonates with clinical features of sepsis, and involved collection and microbiological analysis of blood specimens as well as administration of structured questionnaire on each enrolee to collect relevant socio-demographic and clinical information. The study was carried out between July and December 2020.

Ethical consideration

Ethical approvals were obtained from the Ethic and Research Committees of the Federal Medical Centre, Katsina (FMCNHREC.REG. N003/082012) and the Katsina State Ministry of Health (MOH/ADM/SUB/1152/1/350). The study was carried out in line with the WHO guidelines for research conduct on human subjects. Participant information sheet was issued and consent form was signed by the parents of selected neonates.

Sample size, selection criteria, and sampling method

A total of 60 neonates admitted into the special care baby units (SCBU) of the two hospitals being evaluated for sepsis during the period of the study were included**.** Neonates whose parents or guardians declined consent, and those already commenced on antibiotics were excluded from the study.

Data and sample collection

The socio-demographic and clinical information of each enrolee were collected using a structured and interviewer-administered questionnaire, consisting of variables such as gender, age (in days), gestational age at birth, birth weight, place of delivery, presenting symptoms, onset of symptom, and clinical signs as well as maternal variables such as history of maternal fever during pregnancy, age, weight, ethnicity, and antenatal care during pregnancy. The questions were interpreted verbally into Hausa language where necessary.

About 2 millilitres of blood samples were collected from each neonate enrolee before administration of antibiotic therapy, from a peripheral vein using aseptic technique. The blood was inoculated immediately into a properly labeled Bact/Alert sample bottle, which was then transferred to a closed container and immediately transported to the laboratory.

Bacterial detection using the BacT/Alert blood culture detection system

The BacT/Alert microbial detection system and culture bottle offer both microbial detection system and culture media with appropriate nutritional and environmental conditions for microorganisms that may be present in the test samples. Inoculated bottles are placed into the device where they are incubated and continuously observed for the presence of microorganisms that will grow in the Bact/Alert bottles (12). The system uses a colorimetric sensor and reflected light to monitor the existence and production of carbon dioxide (CO_2) which is dissolved in the culture medium. With the presence of microorganisms in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium, which cause the color of the gas-permeable sensor at the bottom of the culture bottle to change to yellow (12).

The BacT/Alert disposable culture bottle contains 40 ml pancreatic digest of casein (1.7% w/v), papain digest of soybean meal (0.3% w/v), sodium polyanethol sulfonate (SPS) (0.035% w/v), pyridoxine HCl (0.001% w/v), supplementary complex amino acid and carbohydrate substrates in purified water. The inoculated culture bottles were loaded into the BacT/Alert machine for aerobic incubation for 5 days or until culture positive bottles, recognized by color change to yellow, are acknowledged and removed for bacterial identification through Gram stain smears and sub-cultures on blood and MacConkey agar plates. Negative cultures were checked by smear and subculture at interval prior to discarding them as negative if there were no growth on sub-cultures. The procedures for loading and unloading the culture bottles into the BacT/Alert machine were done in line with the manufacturers' user-instruction manual.

Bacterial identification and antibiotic susceptibility test

The Vitek-2 compact system (30 card capacity), a computerized microbiology device that utilizes growth-based technology (13,14) was used for bacterial identification and susceptibility testing of the isolates. The machine uses a fluorogenic procedure for microbial identification and a turbidimetric technique for susceptibility testing to generate minimum inhibitory concentration (MIC) data, using a 64 well card which is barcoded with data on card type, expiration date, lot number, and unique card identification number. The test kits used were ID-GN (for Gram-negative bacilli identification), ID-GP (for Gram-positive cocci identification), AST-GN (for Gram-negative susceptibility) and AST-GP (for Gram-positive susceptibility). The Vitek-2 ID-GN card recognizes 154 species of *Enterobacteriaceae* and a select group of glucose non-fermenting Gram-negative bacteria within about 10 hours. The Vitek-2 ID-GP card recognizes 124 species of staphylococci, streptococci, enterococci, and a group of Gram-positive bacteria within about 8 hours. The AST results were available for bacteria in less than 18 hours.

The identification cards were inoculated with bacterial suspensions by means of an integrated vacuum device. A test tube holding the suspension was placed in a distinct rack (called cassette) and the identification card was placed in the adjacent slot while implanting the transfer tube into the corresponding suspension tube. The filled cassette was then placed manually into a vacuum chamber station. The Vitek-2 card and sample were connected via barcode. Once the cassette was loaded, the device handles all successive steps for inoculation and interpretation with regards to identification and susceptibility test. Eight pre-installed antibiotics (gentamicin, ciprofloxacin, cefuroxime, ceftriaxone, ceftazidime, augmentin, meropenem, and vancomycin) were tested for each isolate, except vancomycin for only Gram-positive isolates. The manufacturer's instructions were strictly followed during bacterial identification and antibiotic susceptibility test.

Data processing and statistical analysis:

All demographic and clinical data were analyzed using SPSS version 22.0. The Chi-square (x^2) test and Odds ratio (with 95% confidence interval) were used to determine associa-

tion of risk factors with culture-positive and culture-negative sepsis, and *p* value less than 0.05 was considered statistically significant.

Results:

A total of 60 hospitalized neonates evaluated for suspected neonatal sepsis at the special care baby units (SCBU) of the Federal Medical Centre (FMC) and Turai Umaru Yar'adua Maternal and Children Hospital (TUYMCH), Katsina, Nigeria, were enrolled into study between July and December 2020. The clinical parameters and laboratory culture results of neonates are presented in Table 1. The mean age of the neonates is 1.35±0.48 days, while the mean weight is 2.13±0.89 kg. Neonates with early onset sepsis (<3 days) constituted 65.0% while those with late-onset sepsis (>3 days) were 35.0%. Of the 60 blood samples collected from the neonates, 31 (51.7%) were culture positive while 29 (48.3%) were culture negative.

Table 2 shows the distribution of bacteria associated with neonatal sepsis among the neonates. Gram-positive bacteria predominated, constituting 80.6% (25/31) while Gram-negative bacteria constituted just 19.4% (6 of 31). The most frequent Gram-positive bacteria were coagulase-negative staphylococci (51.6%, 16/31), with *Staphylococcus haemolyticus* 5 (16.1%), *Staphylococcus epidermidis* 4 (12.9%) and *Staphylococcus hominis* 2 (6.5%). *Staphylococcus aureus* constituted 12.9% (4/31) while *Enterococcus* spp constituted 9.7% (3/31). The most frequent Gram-negative bacteria isolates was *Escherichia coli* 2 (6.5%).

Table 3 shows the susceptibility profile of the isolated bacteria pathogens. A high degree of antibiotic resistance was exhibited by the isolates against the eight commonly used antibiotics tested. Gentamicin and vancomycin were the only antibiotics that exhibited some *in vitro* inhibitory actions against Gram-positive bacteria, with 68% (17/25) of the isolates sensitive to them, while ciprofloxacin and meropenem exhibited inhibitory actions against the Gram-negative bacteria tested, with 100% (4/4) and 75% (3/4) susceptibility rates respectively.

Table 1: Clinical parameters and laboratory culture results of neonates with suspected sepsis at FMC and TUYMCH, Katsina

Clinical parameters and culture result	Frequency (%)
Mean age (days)	1.35±0.48
Mean weight (kg)	2.13±0.89
Onset of infection	
Early onset (< 3 days)	39 (65.0)
Late onset (> 3 days)	21 (35.0)
Culture result	
Culture positive	31 (51.7)
Culture negative	29 (48.3)

Table 2: Distribution of bacteria associated with neonatal sepsis among neonates at FMC and TUYMCH Katsina, Nigeria

Bacteria isolates	Frequency (%)
Gram positive	**25 (80.6)**
Staphylococcus aureus	4 (12.9)
Staphylococcus haemolyticus	5 (16.1)
Staphylococcus epidermidis	4 (12.9)
Staphylococcus hominis	2 (6.5)
Staphylococcus lentus	1 (3.2)
Staphylococcus gallinarum	1 (3.2)
Staphylococcus saprophyticus	1 (3.2)
Staphylococcus xylosus	1 (3.2)
Staphylococcus pseudointermedius	1 (3.2)
Enterococcus faecalis	2 (6.5)
Enterococcus faecium	1 (3.2)
Streptococcus agalactiae	1 (3.2)
Globicatella sanguinis	1 (3.2)
Gram negative	**6 (19.4)**
Escherichia coli	2 (6.5)
Klebsiella pneumoniae	1 (3.2)
Acinetobacter baumannii	1 (3.2)
Sphingomonas paucimobilis	1 (3.2)
Ralstonia mannitolilytica	1 (3.2)
Total	**31 (100.0)**

Table 3: Percentage susceptibility of bacteria associated with neonatal sepsis among neonates at FMC and TUYMCH, Katsina, Nigeria

Isolates/antibiotics		**GEN (%)**	**CIP (%)**	**CEF (%)**	**CTR (%)**	**CFZ (%)**	**AUG (%)**	**MER (%)**	**VAN (%)**
Gram positive (n=25)	S	17 (68)	8 (32)	4 (16)	6 (24)	4 (16)	3 (12)	1 (4)	17 (68)
	R	8 (32)	17 (68)	21 (84)	19 (76)	21 (84)	22 (88)	24 (96)	8 (32)
S. aureus (n=4)	S	3 (75)	0	1 (25)	2 (50)	2 (50)	0	1 (25)	1 (25)
	R	1 (25)	4 (100)	3 (75)	2 (50)	2 (50)	4 (100)	3 (75)	3 (75)
S. haemolyticus (n=5)	S	3 (60)	1 (20)	0	0	0	0	0	5 (100)
	R	2 (40)	4 (80)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	0
S. epidermidis (n=4)	S	3 (75)	2 (50)	1 (25)	1 (25)	1 (25)	1 (25)	0	2 (50)
	R	1 (25)	2 (50)	3 (75)	3 (75)	3 (75)	3 (75)	4 (100)	2 (50)
S. hominis (n=2)	S	2 (100)	1 (50)	0	0	0	0	0	2 (100)
	R	0	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0
S. lentus (n=1)	S	1 (100)	1 (100)	0	0	0	0	0	1 (100)
	R	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
S. gallinarum (n=1)	S	1 (100)	0	1 (100)	1 (100)	0	1 (100)	0	0
	R	0	1 (100)	0	0	1 (100)	0	1 (100)	1 (100)
S. saprophyticus (n=1)	S	1 (100)	1 (100)	0	0	0	0	0	1 (100)
	R	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
S. xylosus (n=1)	S	1 (100)	0	0	0	0	0	0	1 (100)
	R	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
S. pseudointermedius (n=1)	S	1 (100)	0	0	0	0	0	0	1 (100)
	R	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
Streptococcus agalactiae (n=1)	S	0	0	0	0	0	1 (100)	0	0
	R	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)
Enterococcus (n=2) (*E. faecalis* and *E. faecium*)	S	0	2 (67)	0	1 (33)	0	0	0	3 (100)
	R	3 (100)	1 (33)	3 (100)	2 (67)	3 (100)	3 (100)	3 (100)	0
Globicatella sanguinis (n=1)	S	1 (100)	0	1 (100)	1 (100)	1 (100)	0	0	0
	R	0	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)
Gram negative (n=4)	S	2 (50)	4 (100)	1 (25)	1 (25)	1 (25)	2 (50)	3 (75)	NA
	R	2 (50)	0	3 (75)	3 (75)	3 (75)	2 (50)	1 (25)	NA
Escherichia coli (n=2)	S	2 (100)	2 (100)	0	0	1 (50)	2 (100)	2 (100)	NA
	R	0	0	2 (100)	2 (100)	1 (50)	0	0	NA
Klebsiella pneumoniae (n=1)	S	0	1 (100)	0	0	0	0	1 (100)	NA
	R	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0	NA
Ralstonia mannitolilytica (n=1)	S	0	1 (100)	1 (100)	1 (100)	0	0	0	NA
	R	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)	NA
Total bacterial (n=29)*	S	19 (65.5)	12 (41.4)	5 (17.2)	7 (24.1)	5 (17.2)	5 (17.2)	4 (13.8)	NA
	R	10 (34.5)	17 (58.6)	24 (82.8)	22 (75.8)	24 (82.8)	24 (82.8)	25 (86.2)	NA

S-Sensitivity, R-Resistance, GEN-Gentamicin, CIP-Ciprofloxacin, CEF-Cefuroxime, CTR-Ceftriaxone, CFZ-Ceftazidime, AUG-Augmentin, MER-Meropenem, VAN-Vancomycin; NA=Not applicable; *=*Acinetobacter baumannii* (n=1) and *Sphingomonas paucimobilis* (n=1) were not included

Table 4 shows the association between sociodemographic characteristics of the neonates in relation to culture positive and negative sepsis. There was no statistically significant association between culture positive and negative neonatal sepsis with respect to gender (OR= 1.014, 95% CI=0.3662-2.805, *p*=1.000), age of sepsis onset (OR=0.527, 95% CI=0.1786-1.558, *p*=0.3715), gestational age at birth (OR=0.5102, 95% CI=0.1789-1.455, p=0.3164), birth weight (OR=0.5464, 95% CI=0.1897-1.574, *p*=0.3903) and mode of delivery (OR = 07788, 95% CI= 0.1586 - 3.824, *p*=0.7577). However, with respect to place of delivery, neonates delivered at home were almost 3 times more likely to have culture positive sepsis than children delivered in the hospital (OR=2.975, 95% CI=1.040-8.510, *p*=0.0717).

Table 4: Neonatal risk factors associated with culture positive neonatal sepsis

Risk factors		Neonatal sepsis		x^2	OR (95% CI)	*p* value
		No positive (%)	No negative (%)			
Gender	Male	14 (51.9)	13 (48.1)	0.000	1.014 (0.3662 - 2.805)	1.000
	Female	17 (51.5)	16 (48.5)			
Age (days)	≤ 3 (EOS)	18 (46.2)	21 (53.8)	0.7987	0.527 (0.1786 - 1.558)	0.3715
	>3 (LOS)	13 (61.9)	8 (38.1)			
Gestational age at birth (weeks)	<37 (Preterm)	10 (41.7)	14 (58.3)	1.004	0.5102 (0.1789 - 1.455)	0.3164
	>37 (Term)	21 (58.3)	15 (41.7)			
Birth weight (kg)	< 2.5 (LBW)	17 (45.9)	20 (54.1)	0.738	0.5464 (0.1897 - 1.574)	0.3903
	>2.5 (NBW)	14 (60.9)	9 (39.1)			
Mode of delivery	Vaginal	27 (50.9)	26 (49.1)	0.09516	0.7788 (0.1586 - 3.824)	0.7577
	Caesarean section	4 (57.1)	3 (42.9)			
Place of delivery	Home	20 (64.5)	11 (35.5)	3.243	2.975[+] (1.040 - 8.510)	0.0717[+]
	Hospital	11 (37.9)	18 (62.1)			

x^2 = Chi square; OR = Odds ratio; CI = Confidence interval; EOS = early-onset sepsis; LOS = late-onset sepsis; LBW = low birth weight; NBW = normal birth weight; [+] = Although the *p* value here is >0.05, the 95% CI of the OR indicated that culture positive neonatal sepsis occurred significantly more (by a factor of 2.975) following home delivery (64.5%) than following hospital delivery (37.9%);

Table 5: Maternal risk factors associated with culture positive neonatal sepsis

Risk factors		Neonatal sepsis		x^2	OR (95% CI)	*p* value
		No positive (%)	No negative (%)			
Application of traditional substances to the umbilicus	Yes	16 (61.5)	10 (38.5)	1.161	2.027 (0.7158 - 5.738)	0.2813
	No	15 (44.1)	19 (55.9)			
Premature rupture of membrane	Yes	10 (43.5)	13 (56.5)	0.540	0.5861 (0.2050 - 1.675)	0.4623
	No	21 (56.8)	16 (43.2)			
Mothers' level of education	None	20 (66.7)	10 (33.3)	7.167	NA	0.0668
	Primary	3 (25.0)	9 (75.0)			
	Secondary	5 (38.5)	8 (61.5)			
	Tertiary	3 (60.0)	2 (40.0)			
Mothers' residence	Rural	8 (57.1)	6 (42.9)	0.585	NA	0.7465
	Semi-urban	15 (53.6)	13 (46.4)			
	Urban	8 (44.4)	10 (55.6)			
Mothers' occupation	Housewife	28 (50.9)	27 (49.1)	1.517	0.6914 (0.1070 - 4.468)	0.6969
	Civil servant	3 (60.0)	2 (40.0)			
Previous premature delivery	Yes	20 (64.5)	11 (35.5)	3.243	2.975[+] (1.040 - 8.510)	0.0717[+]
	No	11 (37.9)	18 (62.1)			

x^2 = Chi square; OR = Odds ratio; CI = Confidence interval; [+] = Although the *p* value here is >0.05, the 95% CI of the OR indicated that culture positive neonatal sepsis occurred significantly more (by a factor of 2.975) following previous premature delivery (64.5%) than when there was no previous premature delivery (37.9%); NA = Not applicable

Table 5 shows association between maternal risk factors in relation to culture positive and negative neonatal sepsis. There was no statistically significant association between culture positive and culture-negative neonatal sepsis with respect to maternal factors such as application of traditional substance for umbilical care (OR = 2.027, 95% CI = 0.7158 - 5.738, p=0.2813), premature rupture of membranes (OR=0.5861, 95% CI = 0.2050 - 1.675, p=0.4623), mother's educational level (x^2=7.167, p=0.0668), mother's place of residence (x^2=0.585, p=0.7465) and mother's occupation (OR = 0.6914, 95% CI = 0.1070 - 4.468, p=0.6969). However, mothers with previous history of premature delivery are about 3 times more likely to have culture positive neonatal sepsis compared to mothers who had no previous history of premature delivery (OR=2.975, 95% CI=1.040-8.510, p=0.0717).

Table 6 shows the clinical manifestations of culture-positive and culture-negative neonatal sepsis. The commonest presentations of neonatal sepsis were fever (76.7%, 46/60), pyrexia with temperature > 36.2°C (50%, 30/60), refusal to suck (35.0%, 21/60), fast breathing (26.7%, 16/60) and hypothermia (23.3%, 14/60). However, there was no significant difference in any of the clinical manifestations between culture-positive and culture-negative neonatal sepsis (p>0.05).

Discussion:

In this study, 63.3% of the neonates studied had early onset sepsis while 36.7% had late-onset sepsis, indicating that early onset sepsis was more common than late-onset sepsis in agreement with the findings of Dedeke et al., (1) in Abeokuta, Nigeria and from similar studies conducted in Cameroon (18) and Bangladesh (19). However, this contradicts the findings of Mokuolu et al., (15) in Ilorin, Nigeria (15), and of some studies from other developing countries including Pakistan (16) and Libya (17), where late-onset sepsis was reported to be commoner than early onset sepsis.

The prevalence of culture-positive neonatal sepsis (51.7%) in our study is remarkably higher compared to 30.8% reported by Mokuolu et al., (15) in Ilorin and 34.0% by Shobowale et al., (2) in Lagos, Nigeria. The high prevalence in our study may be due to the seasonality of bacteria aetiology of neonatal sepsis but the small sample size in our study requires us to cautiously interpretate our findings. Gram-positive bacterial pathogens predominated in our study, constituting 80.6% while Gram-negative bacteria constituted just 19.4%. The most frequent Gram-positive bacteria were coagulase-negative

Table 6: Clinical presentations of the study subjects in relation to neonatal sepsis

Clinical presentation		**Neonatal sepsis**		**x^2**	**OR (95% CI)**	***p* value**
		No positive (%)	**No negative (%)**			
Fever	Yes	23 (52.3)	21 (47.7)	0.02427	1.095 (0.3485-3.442)	0.8762
	No	8 (50.0)	8 (50.0)			
Jaundice	Yes	5 (45.5)	6 (54.5)	0.01498	0.7372 (0.1983-2.741)	0.9026
	No	26 (53.1)	23 (46.9)			
Fast breathing	Yes	8 (50.0)	8 (50.0)	0.02427	0.9130 (0.2906-2.869)	0.8762
	No	23 (52.3)	21 (47.7)			
Refusal to suck	Yes	13 (61.9)	8 (38.1)	0.7987	1.896 (0.6420-5.599)	0.3715
	No	18 (46.2)	21 (53.8)			
Convulsion	Yes	5 (71.4)	2 (28.6)	0.5053	2.596 (0.4619-14.592)	0.4772
	No	26 (49.1)	27 (50.9)			
Temperature	< 36.6°C (hypothermia)	9 (64.3)	5 (35.7)	3.864	NA	0.1449
	36.6 - 37.2°C (normal)	5 (31.3)	11 (68.8)			
	> 37.2°C (pyrexia)	17 (56.7)	13 (43.3)			

x^2 = Chi square; OR = Odds ratio; CI = Confidence interval; NA = Not applicable

staphylococci (51.6%) consisting of mainly *S. haemolyticus* (16.1%), *S. epidermidis* (12.9%) and *S. hominis* (6.5%). *Staphylococcus aureus* constituted 12.9% of the isolates while *Enterococcus* spp constituted 9.7%, with the most frequent Gram-negative bacteria isolate being *E. coli* (6.5%). These findings are in consonance with those of Mokuolu et al., (15) where Gram-positive bacteria particularly *Staphylococcus* spp were the commonest pathogens associated with neonatal sepsis. In a similar study by Labi et al., (21), *Staphylococcus* spp were also the most common bacteria causing neonatal sepsis. Our study however contradicted the findings of Dedeke et al., (1) and Shobowale et al., (2), where *K. pneumoniae* was the predominant bacterium causing neonatal sepsis in their studies. Even though some of the isolated bacteria in our study were normal commensals of the skin and gastrointestinal tract, they are pathogenic in neonates due to their weak and immature immune system.

Gentamicin and vancomycin were the only antibiotics that exhibited above average *in vitro* inhibitory actions against Gram-positive bacteria in our study, with 68% of the isolates sensitive to them. This is similar to the findings of Yadav et al., (23), who reported that the antibiotic with *in vitro* inhibitory activity against mostly Gram-positive bacterial isolates in their study was gentamicin (93% sensitivity). Vinodkumar et al., (24) also reported low resistance of their isolates to gentamicin. Our finding however contrasted the study conducted by Mustafa and Ahmed (22), who reported low sensitivity of their isolates to gentamicin. The sensitivity rate of 68% for vancomycin against the Gram-positive isolates in our study is similar to the findings of Singh et al., *(*32). Gentamicin and vancomycin still remains the drug of choice for empirical treatment of neonatal sepsis, but recently resistance to these drugs has been reported especially in the developing countries due to decreesing costs of these drugs and increased availability, which may have led to their overuse in empirical therapy of sepsis and other life-threatening infections.

The high degree of resistance exhibited by the isolates to commonly used antibiotics including broad-spectrum cephalosporins, reported in our study is similar to reports of other studies (18). Rizwan et al., (27) and Kayange et al., (28) reported only moderate sensitivity to ciprofloxacin by the isolates in their study while the sensitivity of the isolates in our study to ceftriaxone (a third-generation cephalosporin) is below 50%. However, the study by Abdelsalam et al., (29) reported overall high sensitivity of the bacterial isolates in their study to ciprofloxacin, which contrasted the findings of our current study. The studies by Dedeke et al., (1) and Aku et al., (25) reported low sensitivity of the bacterial isolates to cefuroxime, which agrees with our current study of 17.2% sensitivity to cefuroxime. Reduced susceptibility of bacterial isolates to cephalosporins and fluoroquinolones is a big challenge in the empirical treatment of neonatal sepsis (30,31).

Reports of multi-drug resistant bacteria causing sepsis in neonates, especially in developing countries are on the increase (20). Downie et al., (26) reported that over 40% of cases of neonatal sepsis were due to bacteria that were resistant to the antibiotic combination of ampicillin and penicillin as well as gentamicin, or the commonly used alternative third-generation cephalosporins. These reports support the need for continuous review of empirical antibiotics used in the treatment of sepsis in neonates, to ensure optimal antimicrobial use. The high resistance rate against many of the antibiotics was not surprising because inappropriate and overuse of antibiotics in both humans and animals in Nigeria might have accounted for the high resistance rates detected in our study.

In this study, 51.7% (31/60) of the neonates were delivered at home while 48.3% (29/60) were delivered in hospital, but neonates delivered at home (64.5%) were about 3 times more likely to have culture-positive neonatal sepsis compared to those delivered in hospital (35.5%) (OR=2.975, 95% CI=1.040-8.510). This is in agreement with the findings of Shobowale et al., (2), who reported that babies who were not delivered in hospital were more likely to develop sepsis when compared to babies delivered in hospital. Previous history of premature delivery was significantly associated with culture-positive neonatal sepsis in this study (OR= 2.975, 95% CI = 1.040 - 8.510). Studies have shown that previous premature delivery is a risk factor for another premature delivery, while prematurity is a risk factor for neonatal sepsis. This is because underdeveloped immune system, common in premature babies, is associated with higher risk of infection (33). Darmstadt et al., (34) reported that premature neonates have 3 to10 fold higher occurrence of infections than term and normal birth weight infants.

The most common clinical manifestations of neonatal sepsis in our study were fever (76.7%), refusal to suck (35.0%), fast breathing (26.7%) and hypothermia (23.3%), which agree with some of the findings of Dedeke et al., (1), who reported the common clinical presentations among babies with sepsis to be fever, respiratory distress, and refusal to suck. However, a similar study by Karthikeyan and Prem-

kumar (20) in India reported respiratory distress to be the major presenting feature of neonatal sepsis among their patients. With respect to culture positivity, there was no significant difference in the clinical manifestations of sepsis between culture-positive and culture-negative neonates in our study, which implies that clinical diagnosis of neonatal sepsis is very vital in the initial critical period in the management of neonatal sepsis.

Conclusion:

Neonatal sepsis is common among newborns in Federal Medical Centre (FMC) and Turai Umaru Yar'adua Maternal and Children Hospital (TUYMCH), Katsina, Nigeria, with early onset more common than late-onset sepsis. Gram-positive bacteria are the major pathogens with coagulase-negative staphylococci dominating, while the most frequent Gram-negative bacteria are *E. coli*. The isolates exhibited high resistance (>50% rate) to most of the tested antibiotics including third generation cephalosporins and fluoroquinolones. Gentamicin and vancomycin were the only antibiotics effective, with 65.5% of all isolates sensitive to gentamicin, and 68% of Gram-positive isolates sensitive to vancomycin.

Previous premature delivery and delivery at home were factors significantly associated with culture-positive neonatal sepsis, but there is no significant difference between culture-positive and culture-negative neonatal sepsis with respect to clinical manifestations. There is need for regular surveillance of causative organisms of neonatal sepsis and monitoring of their susceptibility patterns, to inform the choice of empirical antibiotic treatment pending results of blood culture tests.

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Contributions of authors:

OHK, AB and AS conceived idea and developed the study concept. OHK performed the laboratory analysis, while AB and AS supervised the study. All authors discussed the results and contributed to the final manuscript draft for submission.

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