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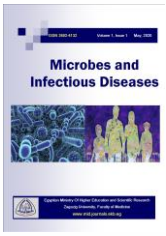

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Original article

Bacteriology of neonatal sepsis in a hospital in southwest Nigeria

Patricia I. Eniowo [1], Abiodun J. Kareem [*2], Akinbowale R. Eniowo [3], Ebunoluwa A. Adejuyigbe [4]

1- *Department of Paediatrics. Mother and Child Hospital, Akure, Ondo-State, Nigeria.*
2- *Department of Paediatrics. Federal Medical Centre, Owo, Ondo State, Nigeria.*
3- *Department of Obstetrics and Gynaecology. Federal Medical Centre, Owo, Ondo State, Nigeria.*
4- *Department of Paediatrics and Child health. Obafemi Awolowo University, Ile-Ife, Osun-State, Nigeria.*

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ABSTRACT

Background: Sepsis is a common diagnostic challenge in newborns with clinical features which are protean, subtle, and non-specific. There are varying incidences of neonatal sepsis, etiological agents, and antimicrobial sensitivity pattern. However, to adopt an empirical antibiotic in a region, there is a need to determine the etiological agent and antimicrobial sensitivity pattern in that region. This study aimed to determine the common organism responsible for neonatal sepsis and the antibiotics sensitivity pattern. **Methods:** This was a prospective cross-sectional study. Consecutive neonates with features of sepsis were recruited. Blood culture and sensitivity patterns were carried out. Data were analyzed and p-value ≤ 0.05 was considered significant. **Results:** A total of 180 neonates comprising 106 (58.9%) males with a male to female ratio 1.4:1. The incidence of neonatal sepsis was 17.8%. Majority of neonates with sepsis were out-born (56.3%) and fever (62.5%) was the most common presenting feature. The most common organism isolates were *Staphylococcus aureus* accounting for (70.5%), followed by Klebsiella (11.7%) while coagulase negative staphylococcus (2.9%) was the least. The overall sensitivity of organisms isolated showed a sensitivity of 90.9% to ciprofloxacin, 86.4% to ceftriaxone, 77.3% to ofloxacin, and 72.8% to cefuroxime. While there was resistance to genticin 54.5%, ampicillin 40.9%, and cloxacillin 41.7%. **Conclusion:** There was high resistance to the commonly used empirical antibiotics and overall sensitivity to ciprofloxacin. Therefore, there is a need to review the present antibiotic protocol.

Introduction

Bacterial sepsis is the clinical syndrome resulting from systemic infection and proven by positive blood culture or other central culture [1,2]. It is one of the main reasons for admission into the neonatal unit and it remains a major contributor to infants and under-five mortality in Nigeria. Neonates are particularly vulnerable to infection because of the immaturity of the various immune protective mechanisms as well as the various invasive procedure which some are subjected to at birth [3,4]. The case fatality ranges from 5-60% despite antibiotic usage [5].

According to the World Health Organization (WHO) about five million neonatal deaths occur each year and 98% of this occurs in the developing countries [6]. Deaths recorded from neonatal sepsis vary from 5% in the developed world and up to 60% in the developing world [3,6-

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* *Corresponding author:* Abiodun John Kareem
E-mail address: biodunkareem13@gmail.com

8]. The reported incidence varies from 6.5 to 23 per 1000 live births in Africa and from 7.1 to 38 per 1000 live births in Asia [9-11]. In Nigeria, **Adejuyibge et al**. [8] in Ile Ife reported an incidence of 22.9 per 1000 live births while **Ojukwu et al**. [12], in Abakaliki, and **Mokuolu et al.** [13], in Ilorin, reported an incidence of 7.98% and 7.04% respectively. A study in Sagamu by **Ogunlesi et al.** [14] reported an incidence of 33.5 per 1000 live births and 39.5 per 1000 live birth by **West et al.** [15] in Port Harcourt. The developed countries have made remarkable progress in reducing the incidence of neonatal sepsis by providing access to hygienic skilled delivery for all women, risk based intrapartum antibiotic prophylaxis, and high-quality intensive care for newborns, this is not so in the developing countries.

The etiological agents responsible for bacterial sepsis in the neonates vary from time to time and from place to place over a given period [2,10,16]. These changes have been observed both in the developing and developed countries over the past years [9]. Group B Streptococcus and *Escherichia coli* predominate in the United States while *Staphylococcus aureus* and Gram-negative bacilli are the commonly isolated organisms in the developing countries [17]. This is further supported by **Adejuyigbe et al.** [8], and **Chiabi et al**. [18] where *Staphylococcus aureus*, Klebsiella species and Pseudomonas were the common isolated organisms. On the contrary, studies by **Dawodu et al.** [19], and **Alausa et al.** [20] in Ibadan, Nigeria reported *Escherichia coli* as the most common organism. The controversy continues, therefore, there is need for further research into the bacteriology of neonatal sepsis and the antibiotics sensitivity.

It is recommended that antibiotic therapy should be initiated immediately after completion of diagnostic evaluation and the choice of the antibiotics is determined by the prevalent of the organisms and the sensitivity pattern in the environment [8,13]. Antibiotic administered are adjusted based on susceptibility of microorganism isolated from culture and clinical response. This warrant regular research into the susceptibility pattern of the microorganism isolated from the culture and the administration of the antibiotics as an empirical drug. Therefore, this prospective study aimed to determine the common organism responsible for sepsis in the neonate and the antibiotics sensitivity pattern of the organism in a hospital in southwest, Nigeria.

Materials and Methods

Study design: This was a prospective cross-sectional study.

Study location: The study was carried out at the neonatal ward of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife. Ile-Ife is an ancient town which lies on longitude 4^0 $69^{'}$ E and latitude 7^0 $50^{'}$ N with a population of about 355,281 [21,22]. The neonatal ward is a 25-bed ward that admits both inborn and out-born neonates into two separate wings on the ward. Between 40 and 50 babies are managed in the neonatal ward in a month.

Study strategy: One hundred and eighty (180) consented consecutive term and preterm neonates with weight greater than 1500 grammes delivered both in and out of the hospital who were admitted into the ward for presumed or risk of sepsis were recruited. Newborn who had prior antibiotics therapy before admission and history of antepartum antibiotics therapy in the mother within a week before delivery were excluded from the study.

Ethical consideration: Ethical approval with number IRB/IEC/0004553 was obtained from the Research and Ethics Committee of the Hospital. A written informed consent was obtained from the mother or guardian of all recruited neonates.

Data collection: Detailed history of each recruited neonate was done and relevant maternal data including the socio-demographic information, last menstrual period (LMP), estimated gestational age (EGA), and place of birth were documented. The maternal risk factors such as maternal peripartal pyrexia, prolonged rupture of membranes, foul smelling or meconium-stained liquor, chorioamnionitis, prolonged labor, multiple vaginal examination, and antepartum hemorrhage were documented. The infant gender, age at admission, age at onset of symptoms, and Apgar score if known were documented. Symptoms like fever, poor activity, irritability, excessive crying, poor feeding, vomiting, apnea, lethargy, respiratory distress, cyanosis, jaundice, and abdominal distension were obtained and documented.

Thorough physical examination was carried out on each of the neonates at admission. Presence of clinical signs such as pallor, jaundice, cyanosis,

respiratory distress, hepatomegaly, splenomegaly, and bulging fontanel were documented.

Symptoms occurring within 72 hours of life was termed early onset sepsis (EOS), while symptoms occurring later than 72 hours of life was termed late onset sepsis (LOS) [2].

The weight was measured using a Seca; Hamburg, Germany, Model 3857017099 weighing scale to the nearest 0.01 kilogram. The occipito-frontal circumference was measured using an inelastic tape measure to the nearest 0.1centimeter and the full length was measured using an infanto-meter (Seca; Germany, Model 210 1821009) measured to the nearest 0.1cm.

Sample collection: Peripheral venous blood was obtained from the dorsum of the hand or antecubital fossa after a thorough cleaning of the site with methylated spirit (70% alcohol) until the site is clean and Povidone-iodine applied to the site. Then 1ml of blood was collected into the Bactec peds plus blood culture broth (BD BACTEC PEDS PLUS^{TM}/F 1-3ml manufactured by Becton, Dickinson and company USA, lot numbers 3081063 and 3267034) for blood culture. Blood culture samples were immediately taken to the laboratory and incubated using the BACTEC 9050 Series which is an automated culturing machine, which has the ability to detect organism growth at temperature between 35-37^{0}C using a sensor that detects increase in $C0_2$ production inside the bottles being incubated which is a signal that there is microbial growth. Positive culture bottles were then sub-cultured for isolation of micro-organisms into the differential media (Chocolate and MacConkey media) and incubated at 35-37^{0}C for 24 hours.

Sample analysis: Bacteria colonies that were Gram positive and those that were Gram-negative were characterized by employing standard bacteriological techniques as described by **Cowan and Steel** [23]. The organism suspected to be Staphylococci were thereafter inoculated on the mannitol-salt agar (MSA) plate and those colonies that ferment mannitol on MSA plate were further characterized. Confirmation as to whether they were pathogenic staphylococci was based on coagulase production on slide and tube coagulase tests. Coagulase negative staphylococcus was speciated by the methods of **Schleifer and Kloos** [24]. Other bacterial colonies that were Gram-negative were studied and characterized employing standard bacteriological technique.

Antibiotics sensitivity test was done using the Mueller-Hinton agar. Gram positive and Gram-negative isolates were tested against the appropriate antibiotics bearing in mind the susceptibility and resistance pattern in this environment using the Kirby-Bauer disc diffusion method. The general-purpose differential and selective media employed in this study include: the BACTEC ped plus culture broth, chocolate agar, MacConkey agar, citrate agar, urease agar, and Mueller-Hinton agar. The stains and reagents employed include the Gram's stain, Kovac's reagent and hydrogen peroxide.

Each isolate was processed and characterized using standard laboratory procedure. Preliminary identification of isolates was from Gram's reaction and morphological characteristics.

Other investigations carried out included serum chemistry, suprapubic aspirate for urine microscopy, culture and sensitivity; lumber puncture for cerebrospinal fluid (CSF) analysis and chest radiograph in subjects with respiratory symptoms and signs.

Data analysis: Data were analyzed using the Statistical Package for Social-Sciences (SPSS) version 22.0. The mean, ranges and standard deviations of continuous variables were computed. The difference between means in continuous variables was determined using Student t-test while difference between proportions in categorical variables was determined using Chi-square test. Statistical significance was assumed at $p<0.05$ at 95% confidence interval.

Results

A total of 180 neonates were recruited into the study of which the male to female ratio was 1.4:1. There were 106 (58.9%) males. Seventy-nine (43.9%) were inborn, while 101(56.1%) were out-born. The age of the subjects ranged between 0 to 26 days with a mean (±SD) age of 89.39 ±128.9 hours. One hundred and nine (60.6%) were less than 72 hours old. One hundred and fifty-four (85.5%) were term and the mean gestational age was 38 ±1.6 weeks. The weight ranged from 1.62 to 4.60kg with a mean of 2.92 ±0.62kg. **Table 1** showed the age and weight at admission, sex and gestational age of the subjects. Eighty-seven (48.3%) of the mothers were primipara, 85(47.2%) were multipara while 8(4.4%) were grand-multipara.

Of the 101 out-born subjects, 35(19.4%) were delivered in mission homes, 34(18.9%) in private hospital and maternity centers, 27(15.0%) by

the traditional birth attendants (TBAS) and 5(2.8%) were delivered at home. One hundred and eighteen (65.5%) of the subjects were delivered via spontaneous vaginal delivery **(Table 2)**.

One hundred and fifty-one mothers had identifiable risk factors for sepsis. About two-third 100 (66.3%) had prolonged rupture of membranes for more than 24 hours, while 1(0.6%) had culture proven urinary tract infection. There was no identifiable risk factor for sepsis in 29 (16.1%) of the mothers **(Table 3)**.

Table 4 revealed the common signs demonstrated by subjects with culture proven sepsis. Fever was present in 20 (62.5%) followed by respiratory distress in 18 (56.3%), and 3 (9.4) presented with pallor.

Of the 180 subjects recruited, 32 (17.8%) of them had culture proven sepsis while 148 (82.2%) had negative culture results. Out of the 32 subjects with culture proven sepsis, 21(65.6%) were male while 11(34.4%) were female putting the male to female ratio for subjects with culture proven sepsis at 1.9:1. The higher rate of sepsis found in the male in this study was not statistically significant (p=0.182). Eighteen (56.3%) of the 32 subjects had late onset sepsis while 14 (43.8%) had early onset sepsis.

Four neonates with positive blood cultures had associated co-morbidity. One of the subjects had obstructive uropathy with positive urine microscopy and culture, one was HIV INFECTED baby with pyoderma, another had omphalitis, and one had plural effusion.

Duration of admission: One hundred and three (57.2%) of the babies were on admission for 2-7 days, 66 (36.7%) stayed between eight to 14 days while 11(6.1%) were on admission for more than 14 days. The mean duration of admission of subjects recruited for this study was 7.6 ±3.2 days. None of the subject was readmitted during follow-up.

Outcome of management: One hundred and sixty-seven (92.8%) of the subjects were discharged; four (2.2%) discharged against medical advice, while there were nine (5.0%) mortalities. Eight of the nine mortalities occurred within 72 hours of admission. Five of the nine mortalities had culture proven sepsis, three of them were male while two were female. The male to female ratio amongst the subjects with culture proven sepsis that died was 1.5:1 but this was not statistically significant (p=0.67). The percentage mortality among subjects with culture proven sepsis in this study was 15.6%. About 88.9% of the mortalities were recorded among the subjects that were out-born. Four of the subjects with culture proven sepsis that died were delivered in mission home and they all had late onset sepsis while the fifth who was delivered in OAUTHC via emergency Caesarean section following prolonged obstructed labor from mission home had early onset sepsis. Of the 32 subjects with culture proven sepsis 24(75.0%) were out-born, while 8(25.0%) of them were inborn. Seventeen (70.8%) of the subjects with sepsis were out-born and were more than 72 hours at admission while 7(29.1%) of them were less than 72 hours. The prevalence of culture proven sepsis among the inborn subjects was 10.1% while the prevalence among the out-born was 23.8%. Although a higher percentage of the out-born had sepsis this was not statistically significant when compared with the inborn subjects (p=0.250).

There were 34 bacterial isolated from 32 subjects because two of the subjects had multiple bacterial isolates. Twenty-four (70.5%) of the isolates were Staphylococcus aureus, four (11.7%) were Klebsiella and three (8.82%) were *Escherichia coli*. *Proteus mirabilis*, *Pseudomonas aeruginosa* and Coagulase negative staphylococcus accounted for one (2.9%) each and they were isolated from subjects older than 72 hours at admission **(Figure 1)**. Of the 24 subjects from which *Staphylococcus aureus* were isolated, 13 (54.2%) were older than 72 hours while 11(45.8%) were less than 72 hours at admission. Four (11.7%) of the isolate were Klebsiella of which two were isolated from subjects less than 72 hours. Three (8.82%) of the isolates were *Escherichia coli*. of which 2(66.7%) were isolated in subjects that were less than 72 hours while one (33.3%) was isolated from a subject with obstructive uropathy.

One hundred and forty-eight (82.2%) had intravenous antibiotics for 2-7days, 30 (16.7%) had antibiotics for 8-14 days while two subjects, one with pyoderma and another with bronchopneumonia complicated by pleural effusion had antibiotics for more than 14 days. The mean duration of antibiotic used was 6.4 ±3.5 days.

Staphylococcus aureus has a sensitivity of 90.9%, 86.4%, 77.3%, 72.8% to ciprofloxacin, ceftriaxone, ofloxacin and cefuroxime respectively, while the sensitivity to genticin, ampicillin, cloxacillin and ceftazidime was 54.5%, 40.9%,

41.7% and 16.7% respectively. Klebsiella shows 100% sensitivity to ciprofloxacin and ofloxacin and 75% to genticin and 50% to ceftriaxone and ceftazidime and 25% to both cefuroxime and cloxacillin with 100% resistance to ampicillin. coagulase negative staphylococcus showed 100% sensitivity to ciprofloxacin, ofloxacin, cloxacillin and ceftazidime and showed 100% resistance to the other commonly use drugs **(Figure 2)**.

Table 1. Age and weight at admission, sex and gestational age of the subjects.

Parameters	Frequency	Percentage (%)
Age at admission in hours		
≤72 hours	109	60.6
>72 hours	71	39.4
Sex		
Male	106	58.9
Female	74	41.1
Gestation Age in weeks		
35 to Less than 37	26	14.5
37 to 42	154	85.5
Weight at admission		
1.5- <2.5	37	20.6
2.5-4.0	137	76.1
> 4.0	6	3.3

Table 2. Place of delivery and Mode of delivery of the subjects.

Variables	Frequency	Percentage
Place of delivery		
Inborn	79	43.9
Out-born	101	56.1
Mode of delivery		
Vaginal delivery	118	65.5
Emergency Caesarean section	58	32.2
Elective Caesarean section	4	2.2

Table 3. Maternal risk factors for sepsis.

Risk factor	Frequency	Percentage
***PROM**	100	66.3
Peripartal pyrexia	31	20.5
****Pre-labor ROM**	8	5.3
Multiple vaginal examination	7	4.6
Meconium-stained liquor	2	1.3
Antepartum hemorrhage	2	1.3
*****UTI**	1	0.7

***PROM-Prolonged rupture of membrane
**Pre-labor ROM-Pre-labor Rupture of membrane
***UTI-Urinary tract infection

Table 4. The clinical features observed in subjects with sepsis.

*Clinical signs	Frequency	Percentage (%)
Fever	20	62.5
Respiratory distress	18	56.3
Poor suck	17	53.1
Depressed primitive reflexes	13	40.6
Lethargy	13	40.6
Excessive crying	12	37.5
Irritability	12	37.5
Jaundice	9	28.1
Hypothermia	8	25.0
Convulsion	7	21.9
Vomiting	3	9.4
Pallor	3	9.4
Abdominal distension	3	9.4

*Multiple clinical signs

Figure 1. Pattern of organisms isolated.

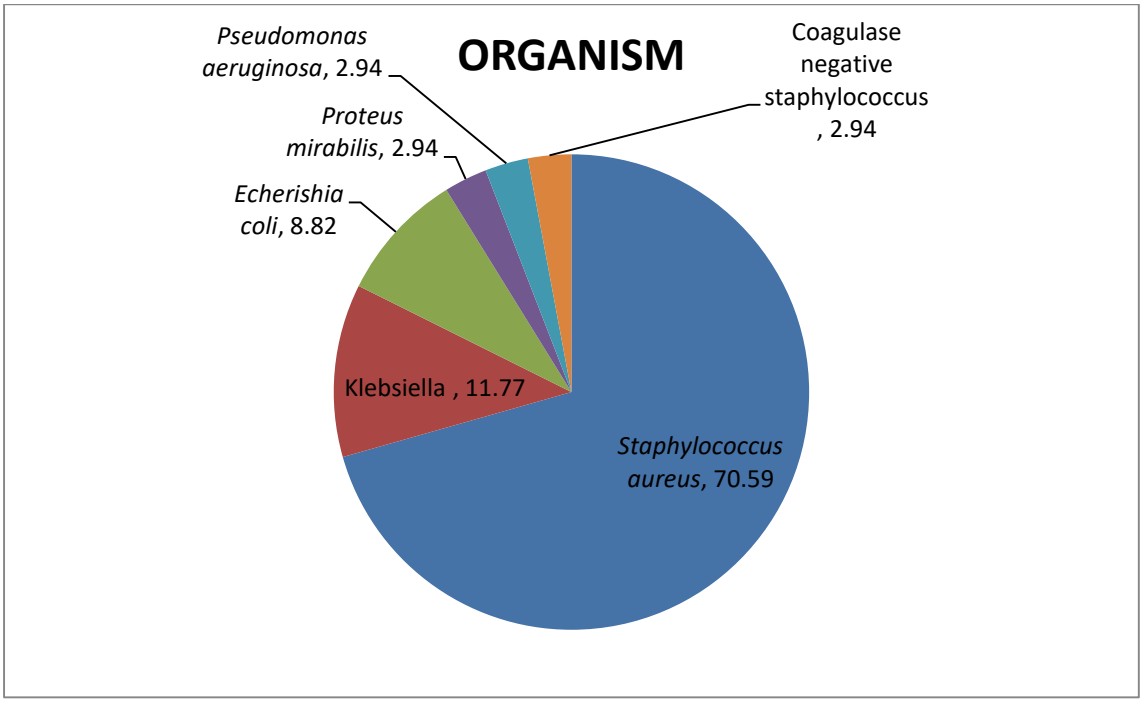
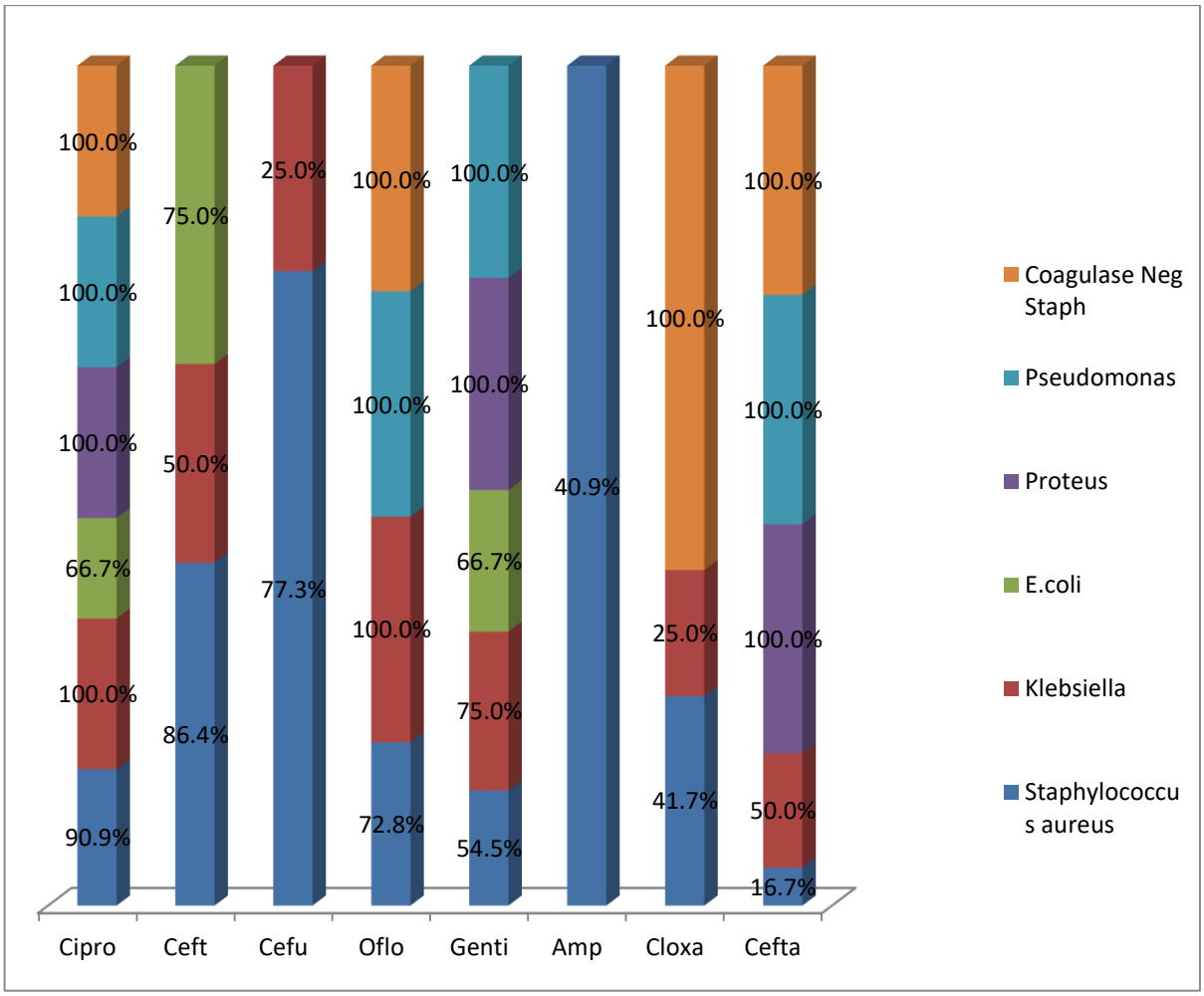


Figure 2. Antimicrobial sensitivity pattern of bacterial isolates.



Discussion

This study aimed to determine the bacteriology of neonatal sepsis. The prevalence of culture proven sepsis was 17.8% in the present study. This was comparable to 22.9%, 23.9% and 22.0% reported by **Adejuyigbe et al.** [8], **Ojukwu et al.** [12], and **Ambe et al.** [25] respectively in different studies in Nigeria. However, the prevalence observed in this study was higher than the 0.5- 8% reported by studies from the developed countries [11]. This showed that there has been no remarkable reduction in the prevalence of neonatal sepsis in this environment. The observed low prevalence in the developed countries has been attributed to provision of access to hygienic skilled delivery for all women, risked based intrapartum antibiotic prophylaxis and high-quality intensive care for newborn [26]. The observed prevalence was lower than 43.1%, and 50.4% reported by **West et al.** [27] in Port Harcourt and **Anah et al.** [28] in Calabar respectively.

The incidence of neonatal sepsis in the present study was 22.9 per 1000 live birth. This was similar to the 22.9/1000 live birth reported by **Adejuyigbe et al.** [8]. However, this was higher higher than 7.98/1000 live birth and 7.04/1000 live birth reported by **Ojukwu et al.** [12] in Abakaliki. and **Mokuolu et al.** [13] in Ilorin respectively. The low incidence found in Abakaliki and Ilorin was attributed to adoption of measures to control infection such as restriction of intravenous fluid, and proper hand washing techniques.

The observed incidence in this study was lower than 33.5/1000 live births reported at Sagamu by **Ogunlesi et al.** [14] and 54.9/1000 live birth reported by **Anah et al.** [28]. The high incidence reported in these various studies was attributed to high prevalence of delivery outside the hospital, lack of antenatal care, poor socioeconomic status and low birth weight. The difference could also be due to the prospective design of the present study as against the other studies that were retrospective study. Also, the

differences in the prevalence and incidences of these studies might be due to the variation in the methods of isolation of bacterial agents and the inclusion criteria into the various studies.

In the present study more male had culture proven sepsis than female with a male to female ratio of 1.9:1. This is comparable with reports from other studies [19,23], and is in keeping with Washburn hypothesis which suggested that the presence of the factors regulating the synthesis of immunoglobulin is located on X- chromosome, and the presence of double chromosome in female may produce greater genetic diversity in female immunologic defense compare to the single X chromosome found in male [2].

The out-born were older than the inborn and this might be due to poor health seeking behavior and delayed referral system in the setting where this study was carried out.

The overall mortality recorded in this present study was 5%, however the mortality amongst subjects with culture proven sepsis was 15.6% of which 88.9% of the mortality were recorded among the out-born subjects. The mortality rate of 15.6% recorded in this study was lower than 26.7%, 30.3% reported in some studies within Nigeria [8,12]. However, the mortality rate in the present study was comparable to 18.1% reported in Uganda [29].

In the present study, 56.3% of the subjects had late onset sepsis while 43.8% had early onset sepsis; this is not surprising since up to 75% of subjects with sepsis were out-born. The symptoms and signs of neonatal sepsis have been well documented and found to be vague and non-specific [30,31], a feature which was evidence in the present study. In this study, fever was the commonest symptoms (62.5%) among neonates presenting with sepsis followed by respiratory distress (56.3%). Similar finding was reported in Maiduguri by **Ambe et al.** [25], while **Ojukwu et al.** [12] reported respiratory distress as the commonest symptoms followed by fever. The variations in clinical features of sepsis in the various studies are not surprising giving the variation and non-specific nature of clinical features of sepsis. Though fever was the commonest symptom among the subjects with culture proven sepsis both in term and preterm, it was observed that five out of the seven preterm subjects with sepsis had hypothermia. This difference in temperature pattern observed has been attributed to the larger body surface area, smaller body mass and low subcutaneous fat in preterm neonates [19].

All the bacterial isolates in this present study have been previously documented as the causative agents of sepsis in neonates. In the present study Gram positive organisms predominate with *Staphylococcus aureus* accounting for 70.6% of the entire organisms isolated in both EOS and LOS. This was similar to the report from various studies in Nigeria [8,13,18,32]. However, this was at variance with the studies by **Dawodu et al.** [19] and **Omokhodion et al.** [33] from Ibadan, Nigeria, showed predominance of Gram-negative organisms as the causative agent for neonatal sepsis. These studies were reported over two decades ago which further confirms the variation of the etiological agents of neonatal sepsis over time within the same location and from place to place [5,9,16]. In this study, Klebsiella was the leading cause of gram-negative sepsis followed by *Escherichia coli*, similar to report by **Iregbu et al.** [34] from Abuja, Nigeria. Other Gram-negative organisms isolated in this study included Proteus spp. and *Pseudomonas aeruginosa.*

Coagulase negative staph was isolated from one of the subjects who was delivered in the mission home with history suggestive of asphyxia. There was no case of Group B beta hemolytic Streptococcal infection in the present study, confirming the observation of others that this organism is not a common cause of neonatal sepsis in Nigeria [19]. In the present study anaerobic organism was not isolated, this might be due to lack of appropriate media to isolate the organism in this study. *Staphylococcus aureus* and Klebsiella spp. were the predominant Gram positive and Gram-negative organisms isolated in the present study accounting for over 80% of the isolate hence the empirical antibiotic therapy must cover for these organisms.

All the isolates in this study were sensitive to ciprofloxacin and ofloxacin and have high invitro resistance to the commonly used antibiotics such as ampicillin, cloxacillin and genticin. There was moderate resistance to second and third generation Cephalosporin. This trend has also been reported in previous studies within Nigeria [8,12,13]. The organisms were sensitive to ciprofloxacin and ofloxacin. The findings in this present study showed high in-vitro resistant to the conventional empirical

antibiotics, genticin (54.5%), ampicillin (40.9%), and cloxacillin (41.7%) suggesting a high prevalence of methicillin resistance in the new born unit. There has been an increasing problem of multi-resistant bacteria causing neonatal sepsis in developing countries as shown by earlier reports confirming the changing pattern of bacterial sensitivity in neonatal sepsis over time [8,11-13,34].

The rational use of antibiotics may help to prevent emergence of resistance in the unit. The overall sensitivity of organisms isolated in this present study revealed a sensitivity of 90.9% to ciprofloxacin, 86.4% to ceftriaxone, 77.3% to ofloxacin and 72.8% to cefuroxime. Of the cephalosporins, ceftriaxone had the best sensitivity pattern followed by cefuroxime this was similar to report by **Mokuolu et al.** [13].

The limitation of this study was the inability of the Bactec culture broth to support the growth of anaerobic organisms.

In conclusion, there was no change in the commonest isolated organism in this environment over decades but there was change in the antibiotic's sensitivity pattern of the organism. There was high resistant to the commonly used empirical antibiotics and over all sensitivity to ciprofloxacin. This therefore called for the need to review the present antibiotic protocol. The high incidence of culture proven sepsis in this study showed the need for joint effort by the government, medical team and all concerned to curb the spread of infection and inculcation of infection prevention in the neonatal unit.

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Authors contribution

(A) **Patricia I. Eniowo** - Conception and design of the title, literature search, analysis and interpretation of the data, drafting of the article, revising the article. Approval of the version to be published.
(B) **Abiodun J. Kareem** - Conception and design of the title, literature search, interpretation of the data, drafting of the article and revising the article critically and corresponding author. Approval of the version to be published.
(C) **Adebowale R. Eniowo** - Conception and design of the title, literature search, interpretation of the data, drafting of the article and revising the article critically. Approval of the version to be published.
(D) **Ebunoluwa A. Adejuyigbe** - Conception and design of the title, literature search, analysis and interpretation of the data, drafting of the article, revising the article. Approval of the version to be published.

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