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Bacteriologic Profile, Antibiotic Regimen and Clinical Outcome of Neonatal Sepsis in a University Teaching Hospital in North Central Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author KIO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author FBT managed the literature searches and designed the patient recruitment protocol, author MOO performed part of the laboratory analyses. Author TOA designed and performed the epidemiological analysis and authors EBB with DZE managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aims: Neonatal sepsis is an important cause of morbidity and mortality in Nigeria and in most parts of the world. Consequently, we determined the prevalence of the common bacterial pathogens of neonatal sepsis, their antibiotic susceptibility profiles, antibiotic regimen used in treatment and their clinical outcomes in a resource limited environment.
Study Design: This was a prospective cross sectional study.
Place and Duration of Study: Study was conducted in the Special Care Baby Unit (SCBU),

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Department of Paediatrics and the Department of Medical Microbiology of Jos University Teaching Hospital (JUTH), Jos, Nigeria between May to December 2011.

Methodology: Biological samples were collected from 218 neonates suspected of sepsis (119 male, 99 female). The WHO and the Integrated Management of Childhood Illnesses (IMCI) criteria for suspicion of sepsis were used to select subjects into the study. Samples were processed and analyzed by standard methods in the microbiology laboratory. Antibiotic susceptibility testing was done. The antibiotic regimen used for therapy and subsequent clinical outcomes were documented.

Results: Prevalence of culture proven sepsis was 34.4% (75/218). The common isolates were *Klebsiella pneumoniae* (32%), *Staphylococcus aureus* (30.7%) and *Escherichia coli* (10.7%). More than 60% of the *K. pneumoniae* isolates were resistant to the antibiotics tested. The *E. coli* and *Enterobacter* isolates were 100% sensitive to meropenem. The Gram positive isolates were most sensitive to ciprofloxacin (85%). Resistance of *S. aureus* was 6% to cefotaxime and 61% to ampicillin. A total of 173 (79.4%) neonates were discharged home, 15 (6.8%) were discharged against medical advice and 30 (13.8%) died on admission. The antibiotic regimen with the least mortality was a combination of ciprofloxacin and gentamicin.

Conclusion: The cultures in this study showed variable antibiogram with complicated patterns of resistance. In all cases of suspected neonatal sepsis, we recommend culture and sensitivity tests to identify the causative pathogen and initiate specific antibiotic therapy. However, cefotaxime in combination with gentamicin is recommended as first line empirical therapy.

Keywords: Neonatal sepsis; Bacteriology; antibiotic regimen; outcome; Jos.

1. INTRODUCTION

The Millennium Development Goals include a reduction in child mortality by two-thirds between 1990 and 2015 [1]. This all important goal is still far from being reached in most parts of Africa. The World Health Organization (WHO) estimates that globally there are about 5 million neonatal deaths per year, ninety eight percent of them occurring in developing countries in the first week of life [2]. In Africa, neonatal sepsis is responsible for 10-69% of neonatal deaths [3-7]. In Nigeria, over 60% of neonatal admissions are for neonates suspected of having an infection or neonates at risk for infection [8,9].

Neonatal sepsis is an invasive infection occurring in the first twenty eight (28) days of life. It could be bacterial, viral, fungal or even toxin mediated [10]. Diagnosis is clinical, with extensive laboratory testing for confirmation and monitoring. In most developing countries, gram-negative bacteria remain as a major cause of neonatal sepsis [11,12]. However, *S. aureus* has been documented as the commonest bacterial aetiology of neonatal sepsis in several studies conducted in Nigerian hospitals [8,13-16]. These organisms have developed increased drug resistance over the last two decades and management of neonates with sepsis has become a major problem [17-27].

Because sepsis may manifest with nonspecific clinical signs and its effects may be devastating, rapid empirical antibiotic therapy is recommended. These signs could be multiple and include diminished spontaneous activity, less vigorous sucking, apnea, bradycardia, temperature instability (fever or hypothermia), respiratory distress, vomiting, diarrhoea, abdominal distention, jitteriness, seizures and jaundice [10]. Empirical therapy must not pre-empt the need to obtain all specimens for culture before therapy. Drugs are later adjusted according to antibiotic sensitivities and the site of infection. The mainstay of empirical therapy for early onset sepsis for both term and preterm infants in most centers is ampicillin and gentamicin, pending blood culture results [23-27]. The emergence of gentamicin resistant gram negative bacteria in some centers has heralded the search for other alternatives [8,13,14]. Resistance to ampicillin based formulations has also become a source of concern to most pediatricians. Moreover, spread of resistant organisms in hospitals is a recognized problem. The wide availability of over the counter antibiotics and the inappropriate use of broad spectrum antibiotics in our communities may be responsible for the spread of these resistant phenotypes.

In order to give neonates suspected of sepsis the best possible treatment, this study was designed to determine the prevalence of the common bacterial causes of neonatal sepsis in our environment, their antibiotic susceptibility profiles; the antibiotic regimen administered and

the clinical outcomes in the neonates in a university teaching hospital in Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

This study was a prospective cross sectional study carried out between May and December of 2011.

2.2 Study Area

The study was carried out in the Special Care Baby Unit (SCBU) of the Jos University Teaching Hospital, a 600 bed capacity health care institution serving a population of about 12 million people in North Central Nigeria. The SCBU is a 30 bed capacity unit where neonates in need of intensive and special care are managed. The hospital serves as a referral center for inhabitants of the region.

2.3 Ethical Considerations

Ethical clearance was obtained from the institutional research and ethics review board. Recruitment of neonates and participation in the study was subject to a signed written informed consent by parents or guardians of the neonates. This consent was obtained after adequate explanation has been given and the parent or guardian has agreed to sign or thumbprint in the consent form before recruitment and collection of samples from the neonates.

2.4 Recruitment of Participants

Specimens were collected from all neonates in the SCBU of the hospital who had a clinical suspicion of neonatal sepsis and whose parents or guardians consented to participate in the study. The WHO case definition for neonatal sepsis was used in conjunction with the Integrated Management of Childhood Illnesses (IMCI) criteria to select subjects for the study (Appendix I) [28]. A clinical diagnosis of neonatal sepsis was made if a neonate presented with at least one of the signs in the tool.

2.5 Sample Collection

Samples collected include blood, cerebrospinal fluid (CSF) and urine. Standard aseptic techniques were observed in both collection and processing of samples. These samples were collected before commencement of empirical antibiotic therapy. Blood for culture was obtained by venipuncture of any two peripheral veins after adequate aseptic preparations. These included wearing of sterile gloves and cleaning of intended puncture sites with tincture of iodine followed by an alcohol solution [29]. They were inoculated immediately at the site of collection into brain heart infusion broth at a ratio of 1:10 (Blood: Broth). CSF samples were collected by lumbar puncture between the 4^{th} and 5^{th} spinal arachnoid space after taking standard precautions and adequate aseptic preparation as previously mentioned [29]. About 2 ml of CSF was collected into a sterile universal bottle. Urine samples were obtained by suprapubic aspiration after ensuring that the neonate had not voided in the preceding 30 minutes to one hour. All samples were transported to the microbiology laboratory immediately and processed as soon as they were received.

2.6 Laboratory Methods

Samples collected from the neonates were processed in the microbiology laboratory by standard methods [29]. The samples collected from each neonate were inoculated on Blood agar, chocolate agar (Oxoid, Basingstoke, UK) and Mac Conkey agar (Fluka medica) plates using a sterile platinum wire loop. MacConkey and blood agar plates were incubated aerobically at a temperature of 35-37ºC for 24-48 hours, while chocolate agar plates were incubated in a candle extinction jar (to provide 5% CO2) to facilitate growth of fastidious organisms such as *Streptococcus* species and *Heamophilus influenza*.

Blood cultures were incubated at a temperature of 35-37ºC for a period of one to seven days. The broth cultures were examined macroscopically on a daily basis for gas formation, increased turbidity or clot formation which may indicate bacterial growth. If any of such macroscopic evidence was seen, an immediate subculture on Mac Conkey, chocolate and blood agar plate was carried out and incubated as previously mentioned. Blind subcultures were done from the blood culture broths on days 1, 3 and 7. Inoculated plates were examined the following day for evidence of growth. Isolates were identified by microscopy, culture and biochemical techniques [29,30]. Antibiotic susceptibility and resistance testing was carried out by the modified Kirby-Bauer disc diffusion method [31]. The following antibiotic discs (Oxoid, Basingstoke, UK) were applied on two different 90 mm petri dishes, allowed to pre-diffuse for about 20 minutes and incubated at 37ºC overnight: ampicillin (25 µg), oxacillin

(1 µg), amoxicillin-clavulanic acid (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), meropenem (30 µg), gentamicin (10 µg), ciprofloxacin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), penicillin (10 units) and vancomycin (30 µg). Antibiotic selections for testing and results determination were based on the Clinical Laboratory Standards Institute (CLSI) protocols [31]. American Type Culture Collection (ATCC) control strains were used for quality control [31]. The neonates were followed up and clinical outcomes documented.

2.7 Considerations for Selection of a True Isolate

An isolate was considered to be the true cause of the sepsis in the neonates when it has fulfilled any of the following criteria:

1. The isolate has been cultured from two blood culture bottles
2. An isolate cultured from one blood culture bottle in the three subcultures in a neonate with frank clinical features of neonatal sepsis and not a known contaminant such as Coagulase Negative Staphylococci (CoNS), *Pseudomonas aeruginosa*, *Corynebacterium* spp, *Fusobacterium* spp, *Propionibacterium* spp and *Bacillus* spp.
3. An isolate that was cultured from more than one specimen taken from the same neonate (e.g. blood, CSF and/or urine)
4. All culture bottles with mixed growth (defined as more than two types of bacteria) were discarded and no isolate documented.
5. Culture bottles with two growths where one of the isolates is a known contaminant (as listed in ii above) is processed further with a pure colony obtained by purity plating of the other non contaminant isolate. Where both isolates were possible contaminants, they were discarded.

2.8 Statistical Analyses

Statistical analyses were done using epi-info 3.5.3 CDC Atlanta, Georgia software. Continuous variables were expressed as means±standard deviation (SD), while categorical variables were expressed as absolute and relative frequencies. Chi-square test was used to compare categorical variables. Mantel-Henszel test was used for cell values greater than 5, while Fisher's exact test was used for expected cell values less than five. P value <0 .05 was considered significant.

3. RESULTS AND DISCUSSION

Two hundred and eighteen neonates (218) were evaluated. This population of neonates had 107 (49.1%) and 111 (50.9%) of them aged less than or equal to three days and above three days respectively. Male neonates were 119 (54.6%) and females 99 (45.4%) as shown in Table 1. In this study, 75 (34.4%) of the 218 neonates studied had positive culture results. The commonest isolate was *K. pneumoniae* with 24 isolates and a prevalence of 32%, followed by *S. aureus* with 23 isolates and a prevalence of 30.7%. Others were as shown in Fig. 1.

More than 60% of the *K. pneumoniae* isolates were resistant to most of the antibiotics tested as shown in Table 2 However, *K. pneumoniae* was least resistant to meropenem (17%). The Citrobacter species were completely (100%) resistant to the beta lactams and cephalosporins including cefepime (4^{th} generation) except for resistance to augmentin (67%) and cefoxitin (67%). The *E. coli* and *Enterobacter* isolates showed 0% resistance to meropenem but 50% were resistant to most other antibiotics. *S. aureus* had excellent sensitivity to the cephalosporins at a percentage greater than 70% except for ceftazidime (60%). Ampicillin and penicillin resistance in S aureus were 61% and 65% respectively. Thirty seven percent (37%) of the *S. aureus* isolates were resistant to vancomycin by disc diffusion. *Staphylococcus aureus* was most sensitive to cefotaxime (94%). Coagulase negative Staphylococci isolates had significant resistance to ampicillin, augmentin and penicillin (80%). The Gram positive isolates were most sensitive to ciprofloxacin (85%), followed closely by gentamicin (82%). Others susceptibility profiles were as documented in Table 2.

The clinical outcomes of the neonates studied were tabulated against the antibiotic regimen administered to the neonates. A total of 173 (79.4%) neonates were discharged home, Fifteen (6.8%) were discharged against medical advice and 30 (13.8%) died on admission. The antibiotic regimen with the least mortality was a combination of ciprofloxacin and gentamicin where five (11.9%) of the 42 neonates who received the combination died. However, the combination with the highest mortality was ampicillin plus cloxacillin and gentamicin combination which contributed 73.3% (22 out of 30) to the neonatal deaths in this study (Tables 3 and 4).

Table 1. Demographic characteristics of the neonates suspected of sepsis

Demographic characteristics	Frequency n = 218	Percent
Age (days)		
≤ 3 (Early onset)	107	49.1
> 3 (Late onset)	111	50.9
Mean age 7.08±7.69 days		
Sex		
Male	119	54.6
Female	99	45.4
Male : Female ratio = 1.2 : 1		
Weight at birth (Kg)		
< 2.5	99	45.5
≥2.5	108	49.5
Not known	11	5.0
Mean birth weight 2.59±0.87 Kg		
Gestational age at birth (weeks)		
28-32	22	10.1
33-36	41	18.8
≥ 37	155	71.1

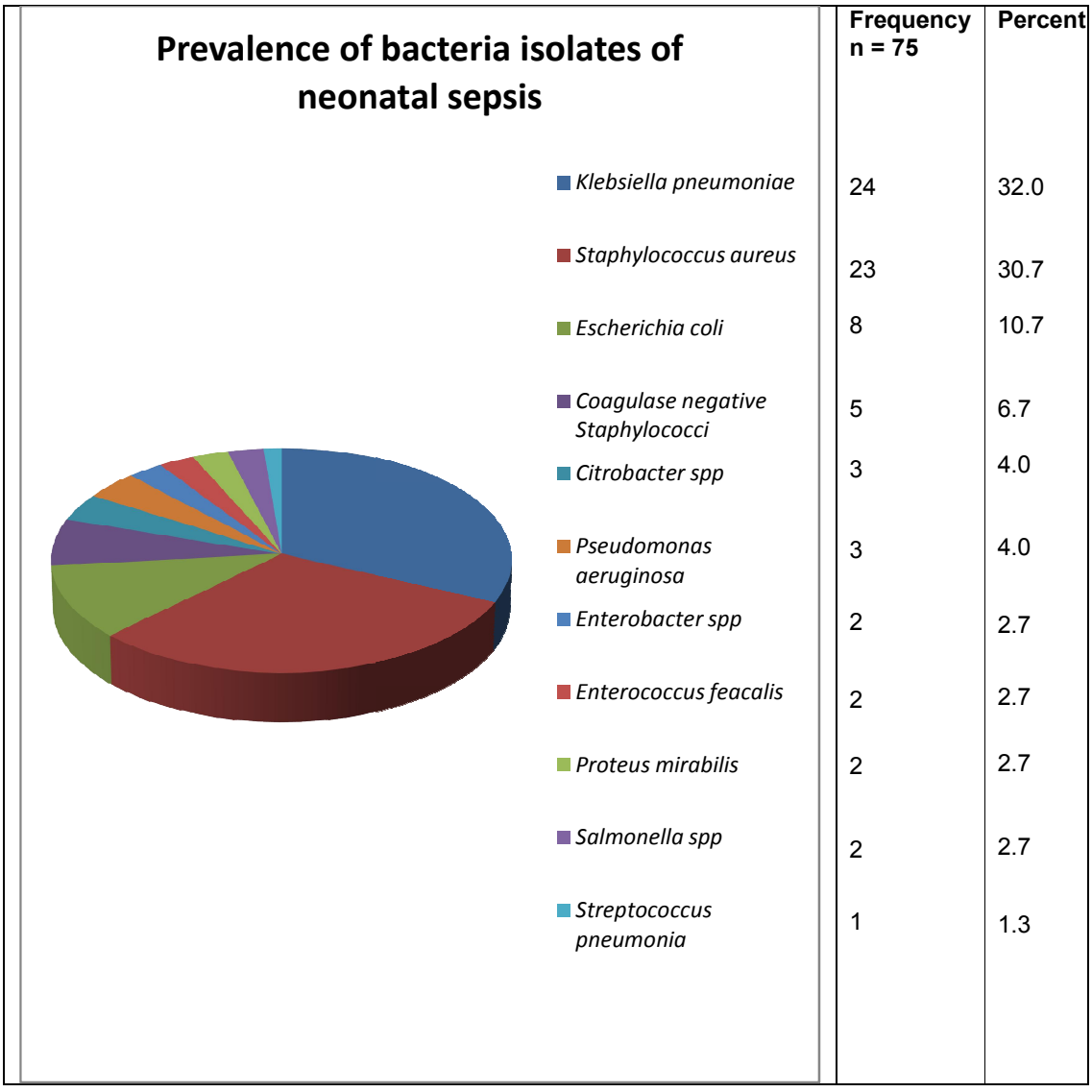
4. DISCUSSION

The most common causes of death in the neonatal period are neonatal infections [20]. The prevalence of neonatal sepsis in any environment is an important aspect of its overall health indices. We found a prevalence of 34.4% for culture proven neonatal sepsis which is similar to the findings in studies conducted in Sagamu, South-western, Nigeria (33.3%) [32] and in East Africa (37%) [9]. It is however lower than the 54% prevalence of neonatal sepsis documented in an earlier study in Jos, North Central, Nigeria [8]. In 2010, the hospital moved to its purpose built permanent site which has a bigger and better equipped nursery with better infection control facilities than in the old site. The previous SCBU had only six old rusty metal cots and four old fashioned incubators as opposed to the new nursery which is a 30 bed unit with new well equipped incubators, two resuscitaires, a dedicated exchange transfusion room, different units for inborn and outborn neonates and separate units for neonates suspected of infection and those uninfected. These factors could have contributed to the lower prevalence of neonatal sepsis in the current study. Another factor is the issue of distinguishing which isolate is a contaminant and which is not as more contaminants characterized as causative pathogens will eventually lead to a higher prevalence. On the other hand, related studies carried out in Abuja, Nigeria; had a lower prevalence of 22% [33]. The lower prevalence may be due to collection of samples after antibiotics must have been commenced in the neonate or better hand hygiene and equipment disinfection practices in the hospital.

There was a predominance of gram negative over gram positive isolates in this study and is comparable to the findings in previous studies in north central Nigeria [8,33] and in some centers in south western Nigeria [34,35]. *Klebsiella pneumoniae* emerged as the commonest bacterial aetiology of neonatal sepsis in this study; a finding which is in keeping with related studies carried out in Nigeria [32,33] and in other parts of the developing world [21-24,36]. This was not the case in another Nigerian study [8] where E. coli was reported as the commonest gram negative isolate in neonatal sepsis.

In contrast, some studies show a predominance of gram positive isolates over gram negative isolates as seen in Osogbo in the Southwest of Nigeria [14] as well as in Uganda [9] and South Africa [37]. Several studies in the developing world have found *S. aureus* to be the commonest pathogen in neonatal sepsis [13-16,34]. However, *S. aureus* was the second commonest organism causing neonatal sepsis in this study. The dominance of *S. aureus* in these studies may be related to contamination of the instruments used during clinical procedures at birth and in the nursery and from hands of mothers and caregivers both at home and in the nursery. The common African tradition of visiting

and playing with a newborn by friends, colleagues, relatives and neighbors and the exposure of the newborn to traditions such as naming ceremonies could also contribute to the increased rate of transmission and isolation of *S. aureus* in these neonates. Other factors that may be responsible for the higher rate of isolation of *S. aureus* in other studies include high rate of contamination during sample collection or increased use of invasive devices such as indwelling central catheters as seen in a South African study [37]. Group B *Streptococcus* (GBS) was not found in any of the neonates. More studies are required to ascertain whether this represents paucity of the organism as a neonatal pathogen in our environment or technical difficulties in isolating the organism.



	Frequency n = 75	Percent
Klebsiella pneumoniae	24	32.0
Staphylococcus aureus	23	30.7
Escherichia coli	8	10.7
Coagulase negative Staphylococci	5	6.7
Citrobacter spp	3	4.0
Pseudomonas aeruginosa	3	4.0
Enterobacter spp	2	2.7
Enterococcus feacalis	2	2.7
Proteus mirabilis	2	2.7
Salmonella spp	2	2.7
Streptococcus pneumonia	1	1.3

Fig. 1. Prevalence of bacteria isolates of neonatal sepsis in Jos University Teaching Hospital

Table 2. Antibiotic susceptibility patterns of the isolates from the neonates studied in Jos University Teaching Hospital in percentage

Isolates antibiotics (%)	Amp	Aug	Cep	Cft	Cfu	Cox	Ctz	Ctx	Chl	Cip	Ery	Gen	Mep	Oxa	Pen	Van
S. aureus **(n=23)**																
Sensitive	39	52	87	94	77	74	60	83	82	83	62	87	-	45	35	63
Resistant	61	48	13	6	23	26	40	17	18	17	38	13	-	55	65	37
CN Staphylococci **(n=5)**																
Sensitive	20	20	80	25	60	40	0	40	60	80	60	60	-	40	20	80
Resistant	80	80	20	75	40	60	100	60	40	20	40	40	-	60	80	20
Enterococcus **spp (n=2)**																
Sensitive	0	50	0	0	0	0	0	0	100	100	50	50	-	0	50	100
Resistant	100	50	100	100	100	100	100	100	0	0	50	50	-	100	50	0
S. pneumoniae **(n=1)**																
Sensitive	100	100	100	0	100	100	100	100	0	100	100	100	-	-	100	-
Resistant	O	0	0	100	0	0	0	0	100	0	0	0	-	-	0	-
K. pneumoniae **(n=24)**																
Sensitive	4	13	38	33	25	42	25	21	54	67	-	50	83	0	-	-
Resistant	96	87	62	67	75	58	75	79	46	33	-	50	17	100	-	-
E. coli **(n=8)**															-	-
Sensitive	25	50	50	43	28	37	25	37	50	75	-	75	100	25	-	-
Resistant	75	50	50	57	72	63	75	63	50	25	-	25	0	75		
Citrobacter **spp (n=3)**																
Sensitive	0	33	0	0	0	33	0	0	33	67	-	33	67	0	-	-
Resistant	100	67	100	100	100	67	100	100	67	33	-	67	33	100	-	-
Enterobacter **spp (n=2)**																
Sensitive	0	50	50	50	50	100	50	50	50	50	-	50	100	-	-	-
Resistant	100	50	50	50	50	0	50	50	50	50	-	50	0	-	-	-
P. aeruginosa **(n=3)**																
Sensitive	0	0	33	0	0	0	67	0	33	67	-	67	-	0	-	-
Resistant	100	100	67	100	100	100	33	100	67	33	-	33	-	100	-	-
P. mirabilis **(n=2)**																
Sensitive	100	100	0	100	100	100	0	100	100	100	-	100	-	0	-	-
Resistant	0	0	100	0	0	0	100	0	0	0	-	0	-	100	-	-
Salmonella **spp (n=2)**																
Sensitive	0	0	100	100	100	0	100	100	100	100	-	100	-	-	-	-
Resistant	100	100	0	0	0	100	0	0	0	0	-	0	-	-	-	-

Key: Amp = Ampicillin; Aug = Augmentin; Cep = Cefepime; Cft = Cefotaxime; Cfu = Cefuroxime; Cox = Cefoxitin; Ctz = Ceftazidime; Ctx = Ceftriaxone; Chl = Chloramphenicol; Cip = Ciprofloxacin; Ery = Erythromycin; Gen = Gentamicin; Mep = Meropenem; Oxa = Oxacillin; Pen = Penicillin; Van = Vancomycin

Table 3. Relationship between antibiotic regimen and final outcome in the neonates studied in Jos University Teaching Hospital

Antibiotic regimen	Total no. n=218 freq. (100%)	Discharged N=173 freq. (%)	DAMA N=15 freq. (%)	Died N=30 freq. (%)	X^2	df	P value
Ampicillin + Cloaxacillin							
Administered	133	104(78.2)	7(5.3)	22(16.5)	2.271	2	.20
Not Administered	85	69(81.2)	8(9.4)	8(9.4)			
Ampicillin + Cloxacillin and Ceftazidime							
Administered	57	49(86.0)	2(3.5)	6(10.5)	2.287	2	.32
Not Administered	161	124(77.0)	13(18.)	24(14.9)			
Ampicillin and Cefotaxime							
Administered	35	28 (80.0)	1 (2.9)	6 (17.1)	1.330	2	.51
Not Administered	183	145 (79.2)	14(7.7)	24 (13.1)			
Ciprofloxacin and Gentamicin							
Administered	42	32 (76.2)	5 (11.9)	5 (11.9)	2.104	2	.35
Not Administered	176	141 (80.1)	10 (5.7)	25 (14.2)			
Ceftriaxone and Gentamicin							
Administered	30	21 (70.0)	2 (6.7)	7 (23.7)	2.702	2	.26
Not Administered	188	152 (80.9)	13 (6.9)	23(12.2)			

DAMA = Discharged Against Medical Advice; DF = Degree of Freedom

Table 4. Relationship between antibiotic regimen and final outcome in the neonates with positive culture results in Jos University Teaching Hospital

Antibiotic regimen	Total no n=75 freq. (100%)	Discharged n=50 freq. (%)	DAMA n=7 freq. (%)	Died n=18 freq. (%)	X^2	df	P value
Administered	54	33 (61.1)	5 (9.3)	16(29.6)	3.441	2	.18
Not Administered	21	17 (81.0)	2 (9.5)	2 (9.5)			
Ampicillin+Cloxacillin and Ceftazidime							
Administered	19	13 (68.4)	3 (15.8)	3 (15.8)	1.809*	2	.41
Not Administered	56	37 (66.1)	4 (7.1)	15 (26.8)			
Ampicillin and Cefotaxime							
Administered	17	12 (70.6)	1 (5.9)	4 (23.5)	0.364*	2	.83
Not Administered	58	38 (65.6)	6 (10.3)	14 (24.1)			
Ciprofloxacin and Gentamicin							
Administered	36	29 (80.6)	2 (5.6)	5 (13.9)	6.177*	2	.046
Not Administered	39	21 (53.8)	5 (12.8)	13 (33.3)			
Ceftriaxone and Gentamicin							
Administered	17	10 (58.8)	3 (17.6)	4 (23.5)	1.612*	2	.45
Not Administered	58	40 (69.0)	4 (6.9)	14 (24.1)			

*DAMA = Discharged Against Medical Advice; DF = Degree of Freedom; * Fishers exact*

The finding of *K. pneumoniae* as the commonest isolate in this study is in contrast to that of a previous study in this center [8] is only proof that organisms causing neonatal sepsis in this environment are changing and therefore therapy protocols should change as the organisms evolve. This further confirms the varying nature of bacterial isolates in neonatal sepsis within the same country or same geographical region. The distribution of neonatal sepsis in our environment as it relates to the socioeconomic and demographic features indentified in this group of neonates have been discussed previously [38].

Neonatal sepsis is a life threatening emergency and any delay in treatment may result in death [39,40]. Most of the Gram negative isolates in this study showed multiple antibiotic resistant profiles which imply that drugs used for empirical therapy were mostly ineffective in treating sepsis in these neonates. This amounts to a technical delay in treatment with appropriate drugs. The multidrug resistant nature of *K. pneumoniae* especially to the cephalosporins in this study is in keeping with findings in other studies carried out in north central [33] and south western Nigeria [14] as well as in Jordan [26] and India [22,23,27,36]. *K. pneumoniae* had a sensitivity of 83% to carbapenems in this study, though this was not in agreement with the finding in the Abuja study where 100% sensitivity to carbapenems was reported [33]. The reasons for the higher sensitivity to carbapenem in such studies may be related to stringent antibiotic stewardship policies and compliance to hospital infection control practices both of which are important factors in the control of spread of antibiotic resistant phenotypes. This indicates that there may be selective pressure since physicians have started prescribing the carbapenems without laboratory evidence and pharmacies are also dispensing them without approval from our clinical microbiology departments. However, the *Enterobacter* species and *E. coli* isolates were 100% sensitive to meropenem in this study. Suffice to note that the numbers of the *Pseudomonas aeruginosa*, *Salmonella* species, *Enterobacter* species, *Citrobacter* species and Proteus mirabilis were too small to warrant any reasonable conclusions. In addition, we did not observe any *H. influenza* isolate in this study despite the lack of routine *H. influenza* type b (Hib) immunization in Nigeria.

Amongst the gram positive isolates, *S. aureus* had excellent sensitivity to the cephalosporins at a percentage greater than 70% which concurs with the findings in similar studies in North Central [33] and South Western, Nigeria [32]. However, *S. aureus* sensitivity to ceftazidime was about 60% in this study. A much less sensitivity of *S. aureus* to ceftazidime was also seen in a study in Osogbo, Nigeria [14] and in another study in Uganda [9]. The *S. aureus* isolates were most sensitive to cefotaxime (94% sensitive). Ironically, *S. aureus* sensitivity to cefotaxime in a previous study in the same unit was much lower [8]. The higher sensitivity in the current study is not surprising as cefotaxime is not commonly used in this center. It is well known that microorganisms tend to become resistant to commonly used antibiotics while becoming more sensitive to the rarely used ones. The overly high resistance of *S. aureus* to ampicillin (61%) questions the rationale behind the use of an ampicillin formulation (ampicillin+cloxacillin) as an antibiotic used for empirical treatment in the management of neonatal sepsis in our environment. *S. aureus* has also been shown to have marked resistance to cloxacillin, the other component of the formulation in several studies [8,9,32]. Moreover, oxacillin which is in the same class as cloxacillin was also highly resistant in this study. Selective pressure on ampicillin+cloxacillin formulations as it is widely used as an over the counter medication for almost every ailment where an infection is suspected and as prophylaxis in surgical and dental procedures may be responsible for the high level of resistance exhibited by most of the bacterial isolates. These factors may also have contributed to the recent trends in resistance patterns of CoNS, methicillin resistant staphylococcus aureus (MRSA), vancomycin-intermediate sensitive *Staphylococcus aureus* (VISA) and Vancomycin resistant *Staphylococcus aureus* (VRSA). Many studies have reported that prevalence of these organisms is increasing even in developing countries [21-27]. Antimicrobials such as vancomycin, linezolid, teicoplanin and combinations such as piperacillin-tazobactam may have to be included in subsequent studies and indeed tested for routinely due to these multidrug resistance patterns.

Furthermore, 68% of all the isolates tested in this study were sensitive to gentamicin which was similar to the sensitivity to gentamicin (67%) in a previous study conducted in this center several years ago [8] but still markedly lower than the sensitivity in a much earlier study where gentamicin sensitivity was 98% [41]. In the absence of amikacin which may be a better alternative, gentamicin is recommended as part

of the empirical therapy for neonatal sepsis in this environment. Gentamicin in combination with cefotaxime seems fairly appropriate as first line empirical therapy for neonatal sepsis based on the findings in this study. Ciprofloxacin was the most sensitive antibiotic for all isolates tested in this study which is similar to the findings in most other studies where a flouroquinolone had the best antibiotic sensitivity profile [14,32,34]. Unfortunately, flouroquinolones may not be used as first line empirical therapy in neonates as they are given with caution because of the possibility of adverse effects. The flouroquinolones are also a major arsenal against multidrug resistant tuberculosis (MDR-TB) hence the advocacy to still keep it as a reserve drug. However, its high sensitivity suggests that ciprofloxacin may be used as a second line antibiotic of choice in neonatal sepsis. This assertion is even more pertinent as the antibiotic regimen that contained ciprofloxacin had the best clinical outcome in this study.

The discontinuation of use of ampicillin plus cloxacillin as a drug for empirical therapy in neonatal sepsis as currently practiced in many neonatal units including that of JUTH is recommended based on the findings in this study. Cefotaxime and gentamicin are recommended as first line antibiotics for empirical therapy and ciprofloxacin as second line antibiotic. These recommendations are in line with international guidelines for empirical therapy of serious bacterial infections in the neonate where a cephalosporin in combination with an aminoglycoside is recommended [25-28]. Carbapenems like meropenem should still be kept as reserve drug used only when laboratory evidence suggests its use and in consultation with the medical microbiology department. Indiscriminate prescription and use of most antibiotics especially the carbapenems should be discouraged. It is noteworthy to state that organisms such as Chlamydiae and viruses which could have been the cause of sepsis in some of the neonates studied were not found due to our limitations of lacking molecular diagnostic facilities.

5. CONCLUSION

Antibiotic resistance is a major contributory factor to the morbidity and mortality of neonatal sepsis everywhere in the world. As the isolates in this study showed variable antibiogram with complicated patterns of resistance, culture and sensitivity tests should be performed in all cases of neonatal sepsis. However, cefotaxime in combination with gentamicin is recommended as first line empirical therapy due to the concerns with the use of ciprofloxacin and the excellent susceptibility of the gram positive isolates to cefotaxime. Developing hospital antibiotic policies and commitment to good antibiotic stewardship will go a long way in reducing the morbidity and mortality of neonatal sepsis in this environment.

CONSENT

All authors declare that written informed consent was obtained from the parents or guardians of the neonates.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX I

Clinical criteria used in screening patients into the study

IMCI criteria for severe bacterial infections and WHO young infant study group criteria

Convulsions
Respiratory rate > 60 breaths/minute
Severe chest indrawing
Nasal flaring
Grunting
Bulging fontanelle
Pus draining from the ear
Redness around umbilicus extending to the skin
Temperature > 37.7 0C OR < 35.5 0C
Lethargic or unconscious (not aroused by minimal stimulus)
Reduced movements (change in activity)
Not able to feed (not able to sustain suck)
Not attaching to the breast
No suckling at all
Crepitations
Cyanosis
Reduced digital capillary refill time

***Any of the signs listed implies high suspicion of serious bacterial infection.**

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