Research Article

High Rates of Bacteria Isolates of Neonatal Sepsis with Multidrug Resistance Patterns in Jos, Nigeria

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Abstract

The era of multiple antibiotic resistances in bacteria causing infections have led to increased morbidity and mortality and has posed huge therapeutic challenges to clinicians. Consequently, we determined the presence of these resistance patterns in the common bacterial isolates of neonatal sepsis in a tertiary care hospital.

A total of 75 non duplicate bacteria isolates from 218 neonates with suspected sepsis were analyzed by standard methods. Antibiotic susceptibility testing was carried out by Kirby Bauer's disc diffusion method and specific multidrug resistance patterns identified.

The common bacterial isolates were *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. Fifteen (62.5%) of the *K. pneumoniae* and five (62.5%) *E. coli* isolates produced Extended Spectrum Beta Lactamases (ESBLs). Amp C genes were identified in eight (33.3%) *K. pneumoniae* and three (37.5%) *E. coli* isolates. Two (66.7%) *Citrobacter* isolates and one (50.0%) *Enterobacter species* produced both ESBLs and Amp C properties. *Klebsiella pneumoniae* Carbapenemases (KPC) production was seen in four (16.7%) *K. pneumoniae* isolates. Methicillin resistance was identified in six (26.1%) and three (60.0%) of the *S. aureus* and Coagulase Negative Staphylococci (CoNS) isolates respectively. This study has shown high rates of multiple antibiotic resistance patterns especially amongst the gram negative isolates which invariably reduce therapeutic options. We recommend culture, sensitivity and resistance testing in all cases of neonatal sepsis. Further studies evaluating institutional surveillance mechanisms for these resistant organisms and the infection control practices in our hospitals are required.

INTRODUCTION

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 [1]. The mortality rate in neonatal sepsis may be as high as 50% for untreated infants [2]. Infection is a major cause of fatality during the neonatal period [2]. Neonatal sepsis is very prevalent in sub-saharan Africa and contributes up to 69% to neonatal mortality in Nigeria and other parts of Africa [2-10]. In preterm infants, profound morbidity is associated with neonatal sepsis which contributes to significant neurological damage in the neonate, undermines the quality of life of the neonate, traumatizes mothers and care givers with eventual loss of productive hours as neonates are admitted for many days or

weeks [10].

In recent times, changing patterns of resistance and changes in the prevalent causative organisms in neonatal sepsis have been widely documented [2-11]. Moreover, reports of multidrug resistant bacteria causing neonatal sepsis in developing countries are increasing, particularly in intensive care units [11,12]. *Klebsiella* and *Enterobacter* species are often reported in this context [11]. Spread of resistant organisms in hospitals is a recognized problem, although babies admitted from the community may also carry resistant pathogens. The wide availability of over the counter antibiotics in Nigeria and the inappropriate use of broad spectrum antibiotics in the community 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 predominant resistance phenotypes amongst the common bacteria isolates of neonatal sepsis in our environment.

MATERIALS AND METHODS

This study was a prospective cross sectional study.

Study area

The study was designed for and carried out in the Special Care Baby Unit (SCBU) of the Jos University Teaching Hospital (JUTH), a 600 bed capacity tertiary health care institution in North Central, Nigeria. The SCBU has a capacity of 30 beds where neonates in need of intensive and special care are managed.

Ethical consideration

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 was given and parent or guardian agreed to sign or thumbprint in the consent form before recruitment and collection of samples.

Recruitment of participants

Specimens were collected from all neonates in the SCBU of the hospital who had a clinical diagnosis or suspicion of neonatal sepsis and whose parents or guardians consented to participate in the study. The WHO criteria for neonatal sepsis screening were used in conjunction with the Integrated Management of Childhood Illnesses (IMCI) criteria to select subjects for the study [13]. All neonates admitted into the SCBU were evaluated by a specialist senior registrar in the neonatology unit of the hospital. Recruitment into the study was based on a suspicion of neonatal sepsis in any neonate who presented with at least one of the signs in the WHO/IMCI tool (Chart 1) [13].

Sample Collection

Samples collected include blood, cerebrospinal fluid (CSF), urine, aspirates and swabs from discharging sites. Standard aseptic techniques were observed in both collection and processing of samples. Blood for culture was obtained by venipuncture of any two peripheral veins after adequate aseptic preparations. They were inoculated immediately at the site of collection into brain heart infusion broth at a ratio of 1:10 (Blood: Broth). Cerebrospinal fluid samples were collected by lumbar puncture between the 4th and 5th spinal arachnoid space after adequate aseptic preparation. Urine samples were obtained by suprapubic aspiration after ensuring that the neonate had not voided in the preceding 30 minutes to one hour. The CSF and urine samples were collected in sterile universal bottles. All samples were transported to the microbiology laboratory immediately.

Laboratory methods

Samples collected from the neonates were processed in the microbiology laboratory by standard methods [14, 15]. The samples collected from each neonate were inoculated on Blood agar, chocolate agar (Oxoid, Basingstoke, UK) and MacConkey agar (Fluka medica) plates using a sterile platinum wire loop. MacConkey and blood agar plates were incubated aerobically at a temperature of 35-37°C for 24-48 hours, while chocolate agar plates were incubated in a candle extinction jar (to provide 5% CO_2) to facilitate growth of fastidious organisms.

Blood cultures were incubated at a temperature of 35-37 °C 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 MacConkey, 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 [14,15]. Antibiotic susceptibility and resistance testing was carried out by the modified Kirby-Bauer disc diffusion method. Resistance testing was by standard protocols [16,17]. Results were determined based on the Clinical Laboratory Standards Institute (CLSI) protocols [16].Control strains were used for quality control [15-17]. EPI Info version 3.5.3 statistical package was used for statistical analysis.

RESULTS AND DISCUSSION

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%).

Of the 218 neonates recruited into the study, 75 (34.4%) had culture positive sepsis with 75 non duplicate bacterial isolates. The Gram positive isolates were 31 (41.3%) while the Gram negative isolates were 44 (58.7%). Twenty four (54.5%) of the Gram negative isolates were *K. pneumoniae* making them the commonest Gram negative isolate and the most prevalent isolate in the study. Similarly, 23 (74.2%) of the Gram positive isolates were *S. aureus* while five (16.1%) were Coagulase Negative Staphylococci (CoNS). Eight (18.2%) of the Gram negative isolates were *E. coli*. Other less common isolates were *Enterococcus species, Salmonella species, Proteus mirabilis, Citrobacter species, Enterobacter species* and *Streptococcus pneumoniae*.

Extended Spectrum Beta Lactamase (ESBL) production and

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presence of Amp C characteristics were observed in 24 (54.5%) and 14 (31.8%) of the gram negative isolates respectively. *K. pneumoniae* Carbapenemases (KPC) production were seen in four (16.7%) of the 24 *K. pneumoniae* isolates while methicillin resistance was identified in six (26.1%) and three (60.0%) of the *S. aureus* and CoNS isolates respectively. About 62.5% of *K. pneumoniae* and *E. coli* isolates produced ESBL, while Amp C properties were identified in 33.3% and 36.5% of *K. pneumoniae* and *E. coli* respectively. Two (66.7%) of the *Citrobacter* isolates and one (50.0%) of the *Enterobacter species* produced both ESBLs and Amp C properties. There were no multidrug resistance patterns identified in the *Enterococcus species, Salmonella species, Proteus mirabilis,* and *Streptococcus pneumonia* (Table 1 and Figure 1).

Multidrug resistance amongst organisms causing neonatal sepsis has posed a major problem to neonatologists [11]. The capacity for ESBL production in an organism confers resistance to all cephalosporins and all penicillins including the extended spectrum penicillins except clavulanic acid. Moreover, in the presence of Amp C resistance gene, the organism in addition to the resistance conferred by ESBL production also becomes resistant to clavulanic acid and the cephamycins such as cefoxitin or cefotetan. These resistance phenotypes are increasingly being seen in gram negative bacteria that cause a large proportion of neonatal sepsis in the developing world including Nigeria [3-7]. This study has shown a high rate of isolation of these gram negative organisms which posses both ESBL and Amp C

resistance phenotypes. Thus greatly reducing the arsenal the neonatologist can use in treating infections with such pathogens. The drug of choice in this case becomes a carbapenem such as imipenem, meropenem, doripenem or ertapenem. These drugs are expensive and often times not readily available in many hospitals in Nigeria.

However, in this study, it was found that some of the *K. pneumoniae* also produced carbapenemases (KPCs). The future outlook for treating cases with KPCs becomes even gloomier when we are faced with almost no other alternative antibiotic therapy. Colistin has been suggested in some centers but there is paucity of data to support the effectiveness of this.

It is noteworthy to state that the KPCs found in this study were not in tandem with the finding in a related study in Nigeria where 100% sensitivity to carbapenems was reported [7]. The reasons for the absence of KPCs in such studies may be related to stringent antibiotic stewardship policies and compliance to hospital infection control practices such as hand hygiene both of which are important factors in the control of spread of antibiotic resistant phenotypes [12]. This indicates that there may be selective pressure in the emergence of these resistance phenotypes since physicians have started prescribing the carbapenems without laboratory evidence and pharmacies are also dispensing them without approval from clinical microbiologist in our environment. However, the *Enterobacter species* and *E. coli* isolates were 100% sensitive to meropenem in this study.

Table 1: Specific resistance properties in the isolates from the neonates studied in the University Teaching Hospital.

Resistance Properties	Present		Absent	
	Frequency	Percentage	Frequency	Percentage
ESBL production in Gram negative bacilli (n = 44)	24	54.5	20	45.5
Amp C Gene in Gram negative bacilli (n = 44)	14	31.8	30	68.2
KPC Production in Klebsiella pneumoniae (n = 24)	4	16.7	20	83.3
Methicillin resistance in Staphylococcus aureus (n = 23)	6	26.1	17	73.9
Methicillin resistance in Coagulase Negative Staphylococci (n = 5)	3	60	2	40

ESBL = Extended Spectrum Beta Lactamase,
KPC = *Klebsiella pneumoniae* Carbapenemases
Chart 1
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 °C OR < 35.5 °C
Lethargic or unconscious (not aroused by minimal stimulus)
Reduced movements (change in activity)
Not able to feed (not able to sustain suck)
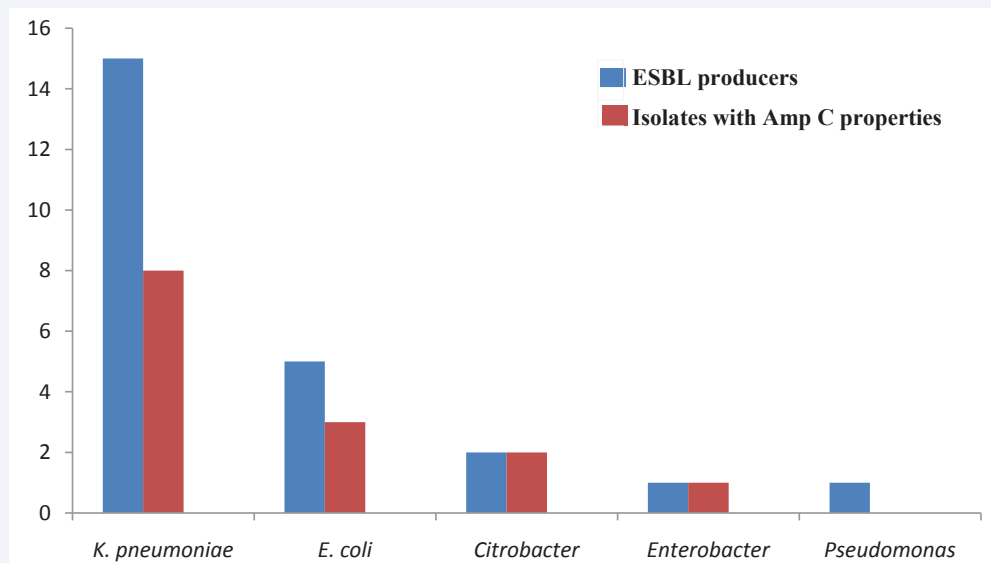Not attaching to the breast
No suckling at all
Crepitations
Cyanosis
Reduced digital capillary refill time
*Any of the signs listed implies high suspicion of serious bacterial infection.

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Figure 1 Distribution of ESBL producing and Amp C exhibiting Gram negative isolates from the neonates studied in Jos University Teaching Hospital.
Figure Legend 2: x axis = Bacterial isolates, y axis = Number of isolates

The high level of ESBL production and the multidrug resistant nature of the gram negative organisms were in keeping with findings in studies carried out in several centers in Nigeria [7-10] and in other parts of the world [18-25]. A related South African study [9] reported AmpC de-repressed mutants only amongst the *Enterobacter* species while in this study, they were observed amongst other Gram negative isolates. This may represent the plasmid transfers of AmpC genes from *Enterobacter* species to other Gram negative isolates. Interventions such as hand hygiene may help in curbing transfer of these resistant phenotypes in our hospital [12]. We are now advocating for proper hand hygiene practices in our hospital with the aim of reducing the spread of such multidrug resistant organisms in the hospital. A subsequent study evaluating the impact of this intervention may be necessary.

Our finding of MRSA and MRCoNS is not surprising as other researchers in Nigeria [6,10] and South Africa [9] also found Staphylococci with such resistance phenotypes in hospitals. Methicillin resistance implies that the Staphylococci isolate is intrinsically resistant to all penicillins and cephalosporins. In such cases, the laboratory would report all penicillins and cephalosporins as resistant irrespective of what the antibiotic susceptibility testing shows. The drug of choice in this case is vancomycin. We were not able to identify those that also possessed vancomycin resistance genes. Although, vancomycin is a toxic drug that requires close monitoring of peak and trough levels. The facilities for such monitoring may not be available in most centers in developing countries. In our center, we are unable to monitor the peak and trough levels of this drug. Moreover, vancomycin and indeed many other antibiotics required for multidrug resistant organisms are not readily available in hospitals in many developing countries including Nigeria[26].

It is therefore not surprising that there are an increasing number of neonates with poor clinical response to existing therapy protocols [3-7, 11]. It is likely that resistance to drugs used for empirical treatment of neonatal sepsis contribute significantly to the risk of mortality since it may amount to technical delay in the commencement of appropriate and effective antibiotics therapy [11]. Well-controlled studies are required to examine the contribution of multiple antibiotic resistances in treatment of newborn sepsis to poor outcome.

In addition, every neonate recruited into this study received broad spectrum antibiotics, many of them with culture negative results. This adds to the unnecessary use of antibiotics in a huge proportion of neonates who eventually may not have needed these antibiotics. It must be noted that a culture negative result does not completely rule out bacteria sepsis in the neonate as certain organisms such as the Rickettsia and Chlamydia group are not easily culturable on artificial media [14]. There were no facilities for molecular identification of these organisms in this study. Collection of samples after commencement of antibiotic therapy might have affected recovery of organism causing sepsis in the neonate [14]. However, this was controlled for as samples were collected from all neonates enrolled into the study before commencement of empirical therapy. The empirical use of these broad spectrum antibiotics in neonates suspected of an infection may have contributed to the rate of antibiotic resistance in our setting. Subsequently, further studies may be required to modify the screening guidelines for neonatal sepsis and arrive at a guideline that will reduce the excessive use of antibiotics in this group of neonates in our environment.

The high rate of isolation of organisms possessing resistance phenotypes such as ESBLs, AmpC, KPC or MRSA warrant that they be included in all studies on sepsis and possibly tested for routinely in the laboratory to stem transmission and give early warning signs. However, the cost of this surveillance and the access to the required antibiotic if multidrug resistance exists may not be feasible in our setting at the moment. Many patients and care givers cannot afford to purchase these antibiotics even when they are available as bills are paid out of pocket. Only a small percentage of the populace has health insurance policies in

Nigeria as is experienced in many developing countries. It is an unfortunate situation when faced with such financial dilemma in healthcare delivery. We hope that our governments in this part of the world will dedicate more funding to healthcare and ensure universal health coverage for its people.

Selective pressure on broad spectrum antibiotics which are widely used as an over-the-counter medication in our environment for almost every ailment where an infection is suspected and as prophylaxis in surgical and dental procedures may have contributed to the high level of these resistance phenotypes.

We recommend that hospital policy in Nigeria and in settings such as ours stipulates that carbapenems like meropenem are kept as reserve drug and used only when laboratory evidence suggests its use and in consultation with the medical microbiologists. Indiscriminate prescription and use of most antibiotics especially the carbapenems should be discouraged. We also recommend further studies to evaluate the infection control practices and the institutional policies for surveillance of these multidrug resistant organisms in our setting.

Another impediment that may arise will be the cost of implementing these surveillance and infection control recommendations as many of our hospitals are poorly funded. A cost benefit analysis of the price of resources needed to implement this versus the potential cost saving could also be another area of further research.

CONCLUSION

This study has shown high rates of multiple antibiotic resistance patterns especially amongst the gram negative isolates which invariably reduce therapeutic options for neonatal sepsis. We recommend culture, sensitivity and resistance testing in all cases of neonatal sepsis. Control of antibiotic purchase and use in our communities as well as adherence to proper hand hygiene practices in our hospital may stem transmission.

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