Multi-drug resistance in early and late onset neonatal sepsis in a tertiary hospital in Nigeria

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Abstract

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, and accompanied by bacteremia in the first month of life and is responsible for 30-50% of total neonatal deaths, each year in developing countries. This study investigated multi-drug resistant organisms associated with early and late onset neonatal sepsis in the University of Ilorin Teaching Hospital (UITH). It was a descriptive cross-sectional study. One hundred and sixty-two blood samples from neonates admitted into the neonatal intensive care unit of UITH with clinical diagnosis of sepsis were obtained. One milliliter of blood was taken per neonate and cultured aerobically in brain heart infusion broth and sub cultured onto blood and MacConkey agar plates. Identification of the isolates was carried out by colonial morphology, Gram stain microscopy and several biochemical tests. Antibiotic susceptibility test was done using the modified Kirby-Bauer method, screening for methicillin resistance staphylococcus aureus (MRSA) and extended spectrum beta lactamase (ESBL) was done by the cefoxitin-based methods and double disc synergy test respectively. Data analysis was carried out using Microsoft excel version 2007 and Epi-info version 2012. Sepsis was confirmed bacteriologically in 22.2% of the samples. The prevalence of multidrug resistant isolate was 29.0%. The prevalence of MRSA was found to be 37% while that of ESBL producing Enterobacteria was 44.4% with ESBL producing Klebsiella pneumoniae and Escherichia coli prevalence of 50% and 25% respectively. This study shows a high prevalence of Methicillin Resistant Staphylococcus Aureus and Extended Spectrum Beta Lactamase producing Klebsiella pneumoniae and Escherichia coli causing neonatal sepsis in UITH Ilorin.

Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, and accompanied by bacteremia in the first month of life. Sepsis is the most common cause of neonatal mortality, and is responsible for 30-50% of total neonatal deaths, each year in developing countries.1

Neonatal sepsis may be classified according to the time of onset of the disease: early onset (EOS) and late onset (LOS). The distinction has clinical relevance, as EOS is mainly due to bacteria acquired before and during delivery, and LOS is due to bacteria acquired after delivery (nosocomial or community sources). In the literature, however, there is little consensus as to what age limits apply.2 Etiological causes of neonatal sepsis could be bacterial, viral, fungal or even toxin mediated. Both Gram negative and positive bacteria have been isolated from blood, and predominance of one type over the other varies from place to place and even in the same
place over time. Bacteria commonly isolated in the samples included Klebsiella pneumoniae, Escherichia coli, Enterobacter species, Pseudomonas aeruginosa and Staphylococcus aureus. Also, many multi-drug resistant (MDR) organisms have also been isolated such as Methicillin Resistant Staphylococcus aureus (MRSA) and Extended Spectrum beta lactamases (ESBLs).

Methicillin resistance Staphylococcus aureus (MRSA) are strains of Staphylococcus aureus that are resistant to the isoxazoyl penicillins such as methicillin, oxacillin and flucloxacillin, the mechanism of resistance is as a result of an alteration in the target of the antibiotics. They were once confined largely to hospitals, other health care environments, and patients frequenting these facilities. Since the mid-1990s, however, there has been an explosion in the number of MRSA infections reported for populations lacking risk factors for exposure to the health care. This increase has been associated with the recognition of new MRSA strains, often called community-associated MRSA (CA-MRSA) strains, that have been responsible for a large proportion of the increased disease burden observed in the last decade.

MRSA in Neonatal Intensive Care Units (NICU) has been reported and is commonly associated with episodic outbreaks from a single clone. Epidemics of MRSA infection have been associated with understaffing, overcrowding, improper cleaning of equipment and hands. Due to extensive use of beta-lactam antibiotics over the last several decades in clinical practice, various beta-lactamases have emerged. Extended-spectrum beta-lactamases (ESBLs) which were first reported in 1983, are mutant plasmid-mediated beta-lactamases derived from older, broad-spectrum beta-lactamases (e.g., TEM-1, TEM-2, SHV-1). These enzymes are most commonly produced by Klebsiella spp and Escherichia coli but may also occur in other Gram-negative bacteria, including Enterobacter, Salmonella, Proteus, Citrobacter spp, Morganella morganii, Serratia marcescens, Shigella dysenteriae, and Pseudomonas aeruginosa. They are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. The cephamycins i.e. cefoxitin and cefotetan are resistant to the hydrolytic effect of these enzymes because of their methoxy group. The carbapenems i.e imipenem and meropenem are also not affected.

The emergence of these superbugs (MRSA and ESBLs) poses a serious antibiotic management problem because of their resistance to multiple drugs and also these genes are easily transferred from one organism to the other via plasmids. This study therefore assesses the prevalence of MRSA and ESBLs among aerobic bacterial isolates of neonatal sepsis in University of Ilorin Teaching Hospital (UITH) Ilorin Kwara State Nigeria.

Materials and Methods

This descriptive cross-sectional study was conducted at the Neonatal Intensive Care Unit of the UITH. The Neonatal Intensive Care Unit of the hospital admits an average of 2000-2500 babies per annum, this comprises babies born within the hospital and those referred to the unit. The study population included all neonate’s new-born aged 0-28 days (Table 1) with clinical diagnosis of sepsis admitted into the Neonatal Intensive Care Unit of the Hospital from October 2013-February 2014. Presence of any or a combination of the following signs and symptoms were used by the attending physician to establish a clinical diagnosis of neonatal sepsis: temperature instability, lethargy, irritability, mottling, pallor, petechiae, feeding intolerance, vomiting, diarrhea, abdominal distention, or respiratory distress and neonates with risk factors for sepsis such as new-born of mothers with prolonged rupture of membrane (PROM) [rupture of membranes for more than 18 hours prior to delivery], maternal fever and or Vulval urinary or lower gastrointestinal infection before or during labor, foul smell of amniotic fluid and meconium stained fluid.

One milliliter of venous blood from a peripheral vein, was taken by resident doctors in the neonatal intensive care unit (NICU) under aseptic conditions, and before commencement of antibiotics, from 162 neonates. The blood was then transferred, using a new sterile needle through the rubber liner of the bottle cap, into the blood culture bottle containing 9 mL Brain Heart Infusion broth. A fresh ethanol-ether swab was used to wipe the top of each culture bottle and the tape was replaced. Each bottle was clearly labelled with the reference ID number of the neonate, and the date and time of collection. Samples were transferred to the microbiology laboratory of the University of Ilorin, Teaching Hospital within an hour of collection for further processing.

Samples were processed using the inoculated Brain Heart Infusion medium which was incubated at 37°C for 7 days and were examined daily for visible signs of bacterial growth. Growth was usually indicated by hemolysis of the red blood cells, gas bubbles in the medium, turbidity, or the appearance of small aggregates of bacterial growth in the broth, on the surface of the sedimented red cell (cotton balls) or occasionally along the walls of the bottle. Subcultures from blood cultures suspected of being positive were made on 5% sheep blood agar and MacConkey agar. Strict aseptic technique was ensured to avoid contaminating the culture.

The blood and MacConkey agars were prepared according to the manufacturer’s standard and one plate per batch of each of the agars was examined for sterility by incubating at 37°C for 24-48 hours. The plates were then stored in the refrigerator, at a temperature of between 2°C to 10°C and used within seven days of production. The Blood agar and MacConkey agar plates were incubated at 37°C aerobically overnight. Bottles without evidence of growth were followed up by examining the broth daily, a final subculture was done at the end of 7 days or at appearance of turbidity, whichever occurred earlier.

Identification of isolates was carried out using standard laboratory procedures which includes colonial morphology, Gram stain microscopy and several biochemical tests as hemolytic activity on blood agar plates, catalase, coagulase (free and bound), DNase production, and growth on mannitol salt agar for Gram-positive isolates, and triple sugar iron (TSI), motility, indole, citrate utilization, urease, oxidase and hydrogen sulphide production and Voges-Proskauer (VP) test for Gram-negative bacilli as described by Cheesebrough and Forbes. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using the modified Kirby-bauer method (CLSI 2012). For this study the following antibiotics were tested against the isolates following the procedures stated above: Gentamicin (10 µg), Ceftriaxone (30 µg), Augmentin (30 µg), Ceftazidime (30 µg), Unasyn (10/10 µg), Imipenem (10 µg) and Vancomycin (30 µg) while Staphylococcus aureus ATCC

Table 1. Age and sex distribution of neonates.

| Age (hours)       | Female | Male | Total (%) |
|-------------------|--------|------|-----------|
| Early onset sepsis (<72) | 58     | 67   | 125 (77.2) |
| Late onset sepsis (≥72) | 12     | 25   | 37 (22.8)  |
| Total (%)         | 70 (43.2) | 92 (56.8) | 162 (100.0) |
25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

### Meticillin resistance Staphylococcus Aureus

The MRSA was detected by the Cefoxitin-based methods recommended in the guidelines of the Clinical Laboratory Standards Institute. (CLSI, 2012)\(^{15}\) All strains of *Staphylococcus aureus* were tested with 30 µg cefoxitin discs (Mast, UK) on Mueller–Hinton agar plates. For each strain, the bacterial suspension was adjusted to 0.5 McFarlands standard. MRSA ATCC 3391 (Oxoid) and locally identified Meticillin sensitive *Staphylococcus aureus* (MSSA) were used as positive and negative controls respectively. The zone of inhibition was determined after 24 hours of incubation at 35°C. Zone size was interpreted according to CLSI (2012) criteria: susceptible, ≥22 mm; resistant, ≤21 mm.

### Extended Spectrum Beta Lactamases

According to CLSI guidelines, strains showing zone of inhibition of ≤22 mm for ceftazidime, ≤27 mm for cefotaxime, and ≤25 mm for ceftriaxone were selected for conformational tests for ESBL. The Double Disc Synergy Test (DSST) was used. The isolated colonies were inoculated in peptone water at 37°C for 2–6 hour. The turbidity was adjusted to 0.5 McFarlands standard and lawn culture was made on Mueller-Hinton agar using sterile swab, an Augmentin disc (20/10 µg) was placed in the center of plate. On both sides of Augmentin disc, a disc of cefotaxime (30µg) and ceftazidime (30µg), was placed with center to center distance of 15 mm to the centrally placed disc (amoxicillin-clavulanic acid disc). The plate was incubated at 37°C for 16–18 hours. ESBL production was interpreted as the 3rd-generation cephalosporin disc inhibition increases towards the Augmentin disc or if neither disc were inhibitory alone but bacterial growth was inhibited where the two antibiotics diffuses together.\(^{16}\)

Data was entered into a computer and analyzed using Microsoft Excel version 2007 and Epi-info version 2012 software. Associations of categorical variables were tested using Chi square while statistical significance was set at P<0.05. Results were presented in tables and charts.

### Results

Majority (77.2%) of the neonates recruited for the study were less than 72 hours old which is EOS and this was not significant (χ\(^2\)=3.75, P=0.053). Gram negative organisms occurred more commonly as a cause of EOS (15 of 25 isolates) than Gram positive organisms (10 of 25 isolates), but this was not statistically significant (χ\(^2\)=0.22 and P=0.63).

Eleven out of the thirty-eight isolates were multi-drug resistant thus giving a prevalence rate of 29.0% (Figure 1). Three out of the eight *Staphylococcus aureus* were Meticillin resistant giving MRSA prevalence rate of 37.5% (Figure 2). Out of the eighteen (18) *Escherichia coli* and *Klebsiella pneumoniae* isolated, eight (8) were ESBL producers, giving a prevalence rate of 44.4% (Figure 3). Half (50%) of the *Klebsiella pneumoniae* isolated were ESBL producers.

### Discussion

Neonatal sepsis remains an important cause of morbidity and mortality especially in developing countries,\(^{17}\) despite the increasing awareness of hospital infection control practices and introduction of new and more potent antimicrobial agents.

Our result showed a much higher occurrence of EOS compared to LOS. This is similar with report from Zhiling et al.,\(^{18}\) where EOS is higher (66) than LOS (49). Consistently, early onset sepsis has been found to occur more frequently than late onset sepsis in neonates, and with a higher morbidity and mortality.\(^{18}\)

![Figure 1. Prevalence of multi-drug resistant isolates.](image)

### Table 2. Distribution of isolates by age.

| Isolates                  | Early onset sepsis ≤72 hrs (%) | Late onset sepsis >72 hrs (%) | Total (%) |
|---------------------------|-------------------------------|-----------------------------|----------|
| **Gram negative**         |                               |                             |          |
| *Escherichia coli*        | 3 (75.0)                      | 1 (25.0)                    | 4 (100.0) |
| *Klebsiella pneumoniae*   | 9 (64.3)                      | 5 (35.7)                    | 14 (100.0) |
| *Pseudomonas aeruginosa*  | 5 (100.0)                     | 0 (0.0)                     | 3 (100.0) |
| **Gram positive**         |                               |                             |          |
| *Staphylococcus aureus*   | 3 (37.5)                      | 5 (62.5)                    | 8 (100.0) |
| *Staphylococcus saprophyticus* | 6 (75.0)                       | 2 (25.0)                    | 8 (100.0) |
| *Enterococcus faecalis*   | 1 (100.0)                     | 0 (0.0)                     | 1 (100.0) |
| **Total**                 | 25 (65.8)                     | 13 (34.2)                   | 38 (100.0) |
Out of the 36 patients with positive blood culture, 65.8% had early onset sepsis (EOS) while 34.2% had late onset sepsis (LOS). This however contrasts with an earlier study by Mokuolu et al.4 where two-third of the early onset infections were due to Gram negative organisms with Klebsiella pneumoniae accounting for 57%.

In the late onset category, Gram positive organisms predominated, accounting for 54% with Staphylococcus aureus constituting 71.4% and Staphylococcus saprophyticus 28.6%. This is also similar with the findings of Mokuolu et al.4 where Gram positive organisms also predominated in the late onset category and Staphylococcus aureus accounted for 38.8%. These similarities may be due to the fact that the two studies were carried out in the same area hence, same prevailing strains.

The prevalence of MRSA as shown in Figure 2 is 37%; this is similar to the findings of Ghandi et al.,19 who reported MRSA prevalence of 31.25%. It is however, higher than the findings of Prabhu et al.,20 and Bhat et al.,5 where MRSA prevalence of 29% and 23.07% were reported. This is also lower than MRSA prevalence of 50.94%, 66% and 66.7% reported by Singh et al.,21 Karthikeyan et al.,22 and Hannan et al.23 The difference in prevalence may be due to the different prevailing strains, antibiotic practices in these locations and also the screening method used; for this study the cefoxitin based method was used while some of the previous studies used the oxacillin method. Cefoxitin induces mecA gene better and gives a more accurate and reproducible results.

In this study extended spectrum beta-lactamase (ESBLs) prevalence of 44.4% was recorded among Klebsiella pneumoniae and Escherichia coli isolated (Figure 3). This is similar to the findings of Prabhu et al.,20 and Bhat et al.,5 where the prevalence of ESBL producing Gram negative bacilli were found to be 32% and 35% respectively. This is far lower than the reports of Shalini et al.,6 and Dengre-Munday et al.,24 which reported ESBL prevalence of 72.3%, and 95% among Gram negative bacilli. This may be due to different prevailing strains and antibiotic practices as the methodology is quite similar.

The prevalence of ESBL producers among Klebsiella pneumoniae was found to be 50%, while 25% of the Escherichia coli isolated were ESBL producers. This is lower than the 80% and 63.6% prevalence of ESBL producing Klebsiella pneumoniae and Escherichia coli reported by Shalini et al.6 It is also lower than the 58% prevalence of ESBL producing Klebsiella pneumoniae reported by Jain et al.,25 and 52.9% of ESBL producing Escherichia coli reported by Ghandi et al.19

Conclusions

This study shows a high prevalence of Methicillin Resistant Staphylococcus Aureus and Extended Spectrum Beta Lacitamase producing Klebsiella pneumoniae and Escherichia coli causing neonatal sepsis in UITH Ilorin. MRSA and ESBL pose a challenge in the management of neonatal sepsis, they lead to an increase in the duration of hospital stay thereby increasing the cost of treatment. Also, by being multidrug resistant they increase mortality rate. We therefore recommend an effective hospital infection control program and periodic review of the hospital antibiotic policy.

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