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Research Article

Antibiogram and ESBL Production Among Neonatal Blood Stream Infections in Intensive Care Units of Selected Hospitals in Delta State, Nigeria

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Abstract

Antimicrobial resistance in neonatal bloodstream infections (BSI) is a threat to the health system and a major contributor to morbidity and mortality within neonatal intensive care units. This study was carried out to determine the prevalence of BSI, antimicrobial resistance, and Extended Spectrum Beta Lactamase (ESBL) production. We carried out a cross-sectional study of newborns admitted to three hospitals in Delta State, Nigeria. Blood samples were collected aseptically, cultured on MacConkey, Blood and Chocolate agar respectively. Isolated bacteria were identified based on morphology, Gram stain and standard biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Phenotypic testing for ESBL production was carried out using the double-disc diffusion method according to CLSI guidelines. A plasmid curing test was performed on ESBL-producing isolates using 10% sodium dodecyl sulphate. A total of 70 bacterial isolates were detected in 180 blood samples, of which 50 (27.8%) were Gram-negative and 20 (11.1%) were Gram-positive. The most frequently isolated pathogen was Escherichia coli (19; 27.1%). A total of 19 (10.6%) isolates were from early-onset BSI, while 51 (28.3%) were from late-onset infection. A high rate of resistance was observed with Gentamicin and fluoroquinolone resistance being over 50% in *E. coli* and *Klebsiella pneumoniae*. All *Staphylococcus aureus* were resistant to erythromycin and trimethoprim-sulfamethoxazole. Twenty of the 50 Gram-negative isolates (40%) were ESBL producers with E. coli being 26.0% (13), and all harboured plasmids. Regular monitoring of pathogen spectrum and antimicrobial resistance patterns will help clinicians use drugs rationally in clinical management.

Keywords: Neonates; Intensive Care Units; Bloodstream Infection; Gram Positive Bacteria; Gram Negative Bacteria; ESBL Producers

Introduction

Although neonatal mortality has decreased to between 11–19% in high-income countries [1], significant burden still occurs in low-income countries, with Asia and Africa being among the continents with the highest burden of neonatal BSIs [2]. In Africa, mortality rates are highest in sub-Saharan Africa [3,4]. Insufficient infection control and surveillance, abuse of antibiotics and increase of antimicrobial resistance may contribute to this situation [5,6]. Monitoring resistance in disease-causing pathogens is of particular importance for neonatal BSIs in Africa, where most empirical treatments follow prescribed guidelines without recourse to local resistance data. There are two major definitions of BSIs in neonates; early-onset sepsis is bacteraemia in the first 72 hours of life, and late-onset sepsis occurs after 3 days of birth [7]. Neonates admitted to intensive care units are at high risk for infection due to factors such as an immature immune system, gestational age, prolonged hospitalization, and frequent use of single or multiple invasive devices.

The occurrence of antimicrobial resistance is one of the greatest challenges in the treatment of neonatal bloodstream infections. In sub-Saharan Africa, 66% of neonatal sepsis cases are caused by

antibiotic-resistant bacteria [4]. Drug-resistant bacteria, especially extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-PE), in hospital settings represent a major threat [4]. Although they are mostly associated with urinary tract infections, the incidence of BSIs caused by ESBL-PE has been reported and is increasing worldwide [8]. Neonatal bloodstream infections (NBSIs) with ESBL-PE are associated with longer hospital stays and consequently higher hospital expenses, and worse outcomes [8,9]. The global public health antibiotic resistance threat of ESBL-producing *Enterobacteriaceae* stems from their ability to acquire genes conferring drug resistance through mobile genetic elements such as plasmids and transfer them between the same or different species [10,11]. These plasmids are often large and able to harbour genes conferring resistance to other antimicrobial classes such as aminoglycosides, fluoroquinolones, and trimethoprim/sulfamethoxazole, thus resulting in multidrug-resistant isolates [12]. and difficult-to-treat infections [8].

The spectrum of bloodstream pathogens encountered among admitted neonates include Gram-positive and Gram-negative bacteria, and sometimes fungi. Bacteria causing neonatal sepsis are constantly changing and may be due to local patterns of antibiotic use. The aetiology varies with the time of onset of illness, local ecology, and bacterial niche. In some developing countries, Gram-negative bacteria such as *Klebsiella pneumoniae are* far more prevalent as neonatal pathogens [13-15,17-19]. *In others, it is Escherichia coli [15] and* this is usually with a higher incidence of antimicrobial resistance. In developed countries, Gram-positive organisms such as *Streptococcus agalactiae* have been identified as the most common cause of sepsis in neonates [16]. Similarly, there is considerable variation in antibiotic resistance patterns in different countries. Antimicrobial susceptibility patterns and ESBL production in NBSIs have been reported in some countries in America, Europe, Asia, and Africa.

Nevertheless, published data on antimicrobial resistance patterns of neonatal sepsis pathogens are limited, possibly due to a lack of prioritization, the absence of routine surveillance, and the tendency to only report outbreaks. Therefore, this study was conducted to determine the prevalence of BSI, antimicrobial resistance and BSI due to ESBL.

Material and Methods

Ethical approval

Prior to the commencement of the study, ethical approval was obtained from the ethics and research committee of the Faculty of Science and Research Committee of Delta State University, Abraka while permission to collect samples were sought from the ethical committees of the different hospitals sampled.

Study population

This was a hospital-based, cohort study carried out from February to June 2019. Neonates aged 0-28 days admitted to the intensive care units (ICU) of General Hospital Warri, Delta State University Teaching Hospital and Central Hospital Eku due to sepsis or other bacterial infections were included in this study. Neonates included in the early-onset cohort were neonates considered at risk for early-onset bacteraemia by admission into the ICU in the first 72 hours of birth. Neonates included in the late-onset cohort were considered at risk for late-onset bacteraemia by admission into the ICU during the risk period of 3 days of birth or later.

Sample collection and identification of bacteria

From sepsis-suspected neonates, 1ml of blood sample in 5 ml of brain heart infusion broth was collected and cultured as described in our previously published work [17]. Samples were immediately transported to the microbiology laboratory and incubated at 37°C. The culture bottles were examined for evidence of bacteria growth such as turbidity and gas production. The samples were then subcultured onto Blood agar, MacConkey agar (aerobic, 37°C), and chocolate agar (anaerobic) and incubated for 7 days. After the incubation period, the blood cultures were reported as positive or negative. Gram staining was performed on pure colonies of isolated bacteria and characterized using standard biochemical tests [18], followed by antimicrobial susceptibility testing [19].

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standard Institute (19) guidelines using the Kirby-Bauer disk diffusion method. Direct colony suspension in sterile physiological saline, equivalent to 0.5 McFarland standard, was prepared and inoculated on Muller-Hinton agar (MHA) using a sterile cotton swab. Multiple antibiotic disks (Abtek Ltd, UK) were

placed on the agar surface and incubated at 35–37 °C for 18–24 h. The zone of growth inhibition around each disk was measured and interpreted as resistant or susceptible based on the CLSI guidelines.

Fifteen (15) antibiotics, including trimethoprim-sulfamethoxazole (30µg), streptomycin (30µg), erythromycin (30µg), ceftriaxone (30µg), gentamicin (30µg), amoxicillin (30µg), ciprofloxacin (30µg), chloramphenicol (30µg), sparfloxacin (30µg), amoxicillin-clavulanic acid (30µg), ofloxacin (30µg), pefloxacin (30µg), cefuroxime (30µg), ceftazidime (30µg) and cefotaxime (30µg), were used.

Detection of ESBL production in isolated organisms

Phenotypic testing for ESBL production was carried out using the double-disc diffusion method according to CLSI guidelines [19]. Disks containing ceftazidime and cefotaxime (30 µg each) were placed on Mueller Hinton agar at 20m center to center around a disk containing amoxicillin (20 µg) plus clavulanic acid (10 µg). After incubation for 24 hours at 37°C, ESBL production was determined. ESBL phenotype was considered positive if there was a difference of ≥5 mm in the inhibition zone diameter around the ceftazidime or cefotaxime disks in combination with amoxicillin-clavulanic acid compared to the zones around the disks containing only ceftazidime or cefotaxime [19].

Plasmid curing

Plasmid curing was performed on ESBL-producing isolates as previously described [20] using a sub-inhibitory concentration of 10% sodium dodecyl sulphate (SDS). Briefly, an overnight broth culture of the ESBL isolate was used to inoculate 4.5 ml of nutrient broth. Then 0.5 ml SDS (10% concentration) was added, and the mixture was incubated at 37°C for 48 h. After incubation, 0.5 ml of the broth was added to a freshly made 4.5 ml nutrient broth and incubated at 37°C for an additional 24 h. After curing, susceptibility testing was repeated as earlier described. Cured isolates were identified by their failure to grow in the presence of antibiotics, indicating that the resistance genes were carried on the plasmids eliminated by curing.

Results

Microbial aetiology of neonatal bloodstream infections

A total of 70 (38.9%) isolates were obtained from the 180 blood samples collected from neonates. A total of 19 (10.6%) isolates were from early-onset infection while 51 (28.3%) were from late-onset infection (Table 1). Gram-negative organisms predominated (n = 50; 27.8%) compared to the Gram-positives (n = 20; 11.1%). *Escherichia coli* (n = 19; 27.1%), *Staphylococcus aureus* (n = 15; 21.4%), and *Klebsiella pneumoniae* (10; 10.0%%), were the three most prevalent organisms (Table 2). Other isolates in decreasing order of prevalence include *Alcaligenes* (8.6%), *Providencia* sp (8.6%), *Citrobacter* sp (7.1%), and *Staphylococcus epidermidis* (7.1%).

Table 1: Prevalence of early- and late-onset BSI in neonates.

Organism	B Number of isolates (%)	Early Onset	Late Onset
Gram-positive	20 (11.1)	6 (3.3)	14 (7.7)
Gram-negative	50 (27.7)	13 (7.2)	37 (20.6)
Total	70 (38.9)	19 (10.6)	51 (28.3)

Table 2: Prevalence of Organisms Isolated from Neonatal Blood Culture.

Bacterial Isolates	Onset of Infection		Total Isolates
	Early Onset	Late Onset	
Staphylococcus aureus	6 (8.6)	9 (12.9)	15 (21.4)
Staphylococcus albus	0 (0.0)	5 (7.1)	5 (7.1)
Escherichia coli	8 (11.4)	11 (15.7)	19 (27.1)
Klebsiella pneumoniae	5 (7.1)	2 (2.9)	7 (10)
Pseudomonas aeruginosa	0 (0.0)	7 (10)	7 (10)
Providencia species	0 (0.0)	6 (8.6)	6 (8.6)
Citrobacter species	0 (0.0)	5 (7.1)	5 (7.1)
Alcaligenes species	0 (0.0)	6 (8.6)	6 (8.6)
Total	19 (27.1)	51 (72.8)	70 (38.9)

Table 3: Antibiotic susceptibility Patterns of Gram-negative isolates obtained from neonatal BSI.

Number of bacterial isolates (%)							
Class of antimicrobial agent	**Antimicrobial agent**	***E. Coli.* (%) n = 19**	***K. pneumonia* (%) n = 7**	***Pseudomonas aeruginosa* (%)n = 7**	***Providencia sp.* (%) n = 6**	***Acaligen sp.* (%) n = 6**	***Citrobacter sp.* (%) n = 5**
Penicillin	Amoxicillin	11 (57.9)	2 (28.6)	7 (100.0)	6 (100.0)	0 (0.0)	0 (0.0)
Cephalosporin	Cefotaxime	10 (53)	2 (28.6)	4 (57.1)	5 (83.3)	5 (83.3)	3 (60.0)
	Ceftazidime	6 (31.6)	2 (28.6)	3 (43.0)	6 (100.0)	5 (83.3)	3 (60.0)
B-lactamase inhibitor	Amoxicillin-clavulanic acid	15 (79)	4 (57.1)	3 (43.0)	6 (100.0)	5 (83.3)	4 (80.0)
Aminoglycoside	Gentamycin	14 (73.7)	4 (57.1)	7 (100.0)	6 (100.0)	4 (66.7)	5 (100.0)
Aminoglycoside	Streptomycin	11 (57.9)	4 (57.1)	7 (100.0)	6 (100.0)	4 (66.7)	2 (40.0)
Phenicols	Chloramphenicol	11 (57.9)	5 (71.4)	7 (100.0)	2 (33.3)	1 (16.7)	2 (40.0)
Quinolones	Sparfloxacin	14 (73.7)	2 (28.6)	7 (100.0)	6 (100.0)	4 (66.7)	5 (100.0)
Fluoroquinolone	Pefloxacin	15 (78.7)	4 (57.1)	7 (100.0)	6 (100.0)	6 (100)	5 (100.0)
	Ofloxacin	13 (68.4)	4 (57.1)	7 (100)	6 (100.0)	6 (100)	5 (100.0)
	Ciprofloxacin	19 (100)	7 (100)	7 (100.0)	6 (100.0)	6 (100)	5 (100)
Sulfonamide	Trimethoprim-sulfamethoxazole	11 (57.9)	4 (57.1)	2 (28.0)	2 (23.3)	0 (0.0)	0 (0.0)

Table 4: Antibiotic susceptibility Patterns of Gram-positive isolates obtained from neonatal BSI.

		Number of bacterial isolates (%)	
Class of antimicrobial agent	**Antimicrobial agent**	***S. aureus* (%) n = 5**	***S. epidermidis* (%) n = 5**
Penicillin	Amoxicillin	12 (80.0)	5 (100.0)
	Ampicillin	12 (80.0)	3 (60.0)
Cephalosporin	Ceftriaxone	12 (80.0)	5 (100.0)
	Cefuroxime	4 (26.7)	5 (100.0)
B-lactamase inhibitor	Amoxicillin-clavulanic acid	6 (40.0)	3 (60.0)
Aminoglycoside	Gentamycin	14 (93.3)	3 (60.0)
Aminoglycoside	Streptomycin	12 (80.0)	5 (100.0)
Fluoroquinolone	Pefloxacin	15 (100.0)	3 (60.0)
	Ciprofloxacin	15 (100.0)	5 (100.0)
Macrolide	Erythromycin	15 (100.0)	3 (60.0)
Sulfonamide	Trimethoprim-sulfamethoxazole	15 (100.0)	3 (60.0)

The prevalence of antimicrobial resistance was assessed among the isolates, focusing on seven antibiotics classes: beta-lactams, beta-lactam inhibitors, cephalosporins, fluoroquinolone, aminoglycosides, macrolides, phenicols, and sulfonamides. Resistance to cephalosporins, aminoglycosides and fluoroquinolone was most frequently detected among the Gram-negative isolates. Gentamicin and fluoroquinolone resistance of over 50% was detected in *E. coli* and *K. pneumoniae*. However, *K. pneumoniae* showed low resistance (28.6%) to sparfloxacin. Low resistance rates to cephalosporins of between 0 to 28% were observed in *K. pneumoniae*.

Amongst the cephalosporins, *E. coli* exhibited high rates of resistance (> 50%) to ceftriaxone, cefuroxime, and cefotaxime, but

a low resistance rate to ceftazidime (31.6%). All isolates (100%) were resistant to ciprofloxacin in this study. *Staphylococcus aureus* and *Staphylococcus epidermidis* showed high rates of resistance to the antibiotics tested. However, *S. epidermidis* exhibited an overall higher rate of resistance than *S. aureus* to most of the antibiotics. Although for erythromycin and trimethoprim-sulfamethoxazole, *S. aureus* was found to show a higher resistance of 100%.

ESBL prevalence among Gram-negative isolates

The double-disk method revealed that 20 of the 50 Gram-negative isolates (40%) showed synergy between amoxicillin-clavulanic acid and at least one of the cefotaxime or ceftazidime β-lactams. As shown in Table 4, the most common ESBL-producing strain was *E. coli* (13; 26.0%), followed by *Klebsiella pneumoniae* (4; 8.0%), *Pseudomonas* (2; 4.0%), and *Citrobacter* sp. (1; 2.0%).

Prevalence of ESBL-producing isolates

Isolates	No of isolates (%)	ESBL positive (%)
E. coli	38 (27.1)	13 (26)
Klebsiella pneumoniae	14 (10.0)	4 (8.0)
Pseudomonas aeruginosa	14 (10.0)	2 (4.0)
Citrobacter sp.	10 (7.1)	1 (2.0)
Providencia sp.	12 (8.6)	0 (0.0)
Alcaligenes sp.	12 (8.6)	0 (0.0)
Total	100 (55.6)	20 (40.0)

Table 5

Discussion

Bloodstream infections with ESBL-producing bacteria have been reported in Africa, particularly in vulnerable populations of neonatal intensive care units [15,20,21]. A high rate (38.9%) of bloodstream infections among neonates was observed in this study, which is comparable with studies of Olorukooba., *et al*. 2020 and Edmond., *et al*. [16,18]. In their studies, *E. coli* was the most prevalent isolate, with a high prevalence of 37.6% and 30.61%, respectively. Most authors have also observed a high prevalence of neonatal sepsis in Nigeria, with rates ranging from 25% to 55% [15,22]. A late onset of 28.3% was observed in this study. This suggests nosocomial infections as most pathogens were likely acquired after delivery in the hospital. Late-onset neonatal sepsis is caused by organisms from the environment and may be nosocomial in origin [15]. It is important to note that most newborns admitted to the ICU have more serious underlying diseases, may undergo more invasive procedures, and have a significantly increased risk of infection. It is therefore necessary to observe preventive measures in other to avoid nosocomial infections. Gram-negative rods such as *Klebsiella* species*, Escherichia coli, Staphylococcus aureus,* and Group B Streptococci (GBS) are responsible for 60 to 70% of blood culture-positive infections in neonates [23,24] and predominate in early-onset neonatal sepsis [23]. An early-onset BSI prevalence rate of 10.6% was observed. *E. coli* and *K. pneumonia*e were the most predominant isolates in this study, albeit these were mostly recovered from late-onset BSI (28.3%). *E. coli* and *K. pneumoniae* have also been demonstrated as the most common Gram-negative bacterial pathogen for neonatal sepsis [25,26] which is consistent with studies conducted in other developing countries [27]. Similar research reports of neonatal sepsis from Nigeria showed that the infections were mainly caused by Gram-negative bacteria [15]. However, studies from other countries [26,28] reported mainly Gram-positive bacteria. These differences in the distribution of common pathogens suggest that predominant pathogens of NBSI may vary based on differences in research time, research locations, or research objects. *S. aureus* has been reported in between 8–22% of the bloodstream isolates in different regions of the world [29]. Gram-positive bacteria gain access to the bloodstream through invasive procedures and various neonatal care equipment like intravenous lines and mechanical ventilators.

Both vertical and horizontal colonization have been implicated in neonatal bloodstream infection in most African countries [30,31]. Mshana., *et al*. [32] reported patient-to-patient or mother-to-child transmission, contaminated equipment, and healthcare workers as possible sources of colonization.

The antibiotic resistance results showed that the drug resistance rates of the Gram-positive isolates, *S. aureus* and *S. epidermidis* to erythromycin and the penicillins were high (Table 3). The resistance rates of *S. aureus* and *S. epidermidis* to erythromycin were 100% and 60%, respectively. Resistance of 91.11% and 50% to erythromycin has been reported in *S. agalactiae and S. aureus* respectively [28]. An increasing resistance to erythromycin has occurred in recent times mainly because macrolides have been widely used in neonatal and perinatal diseases in recent years [32]. The rise in the prevalence of multidrug-resistant bacteria (particularly

multidrug-resistant Gram-negative pathogens) has major implications for neonatal care in resource-limited settings where antibiotic availability is limited [5]. In this study, *E. coli* exhibited high rates of resistance (> 50%) to most of the drugs tested, especially fluoroquinolones, gentamicin, and cefotaxime. This may be due to the abuse of empiric treatment in this vulnerable group as prompt treatment is usually done bearing in mind that delays in treatment initiation may increase mortality rates. The high resistance rate of *E. coli* to cefotaxime, a third-generation cephalosporin, has been shown to be associated with usage. In most African countries, Nigeria inclusive, cefotaxime is one of the extensively used antibiotics in pediatric wards and pediatric intensive care units [33]. Cefotaxime has received wide acceptance as a first-line antibiotic for many neonatal infections [34]. Resistance of *E. coli* (and other members of the Enterobacteriaceae) to cefotaxime has been recognised as a critical threat to public health by the World Health Organization [35]. Resistance to cefotaxime and other third-generation cephalosporins is often due to the production of enzymes such as ESBLs. The occurrence of ESBL-producing *E. coli* and concomitant treatment failure of cephalosporin therapy has become an important epidemiological event [36]. The prevalence of ESBL production among E. *coli* in this study was 26.0%. Other ESBL producers in decreasing order of prevalence were *K. pneumoniae* (8.0%), *Pseudomonas aeruginosa* (4.0%) and *Citrobacter* spp. (2.0%).

Genes encoding ESBLs are typically carried on large plasmids that also carry other antibiotic resistance genes, making ESBL-producing strains multidrug-resistant [37]. Our results indicate that all ESBL-producing *E. coli* in this study harboured plasmids. This explains the high levels of resistance observed in most antibiotics tested. All *E. coli* isolates were resistant to fluoroquinolone, and 73.7% were resistant to gentamicin. Some authors have demonstrated an increased level of ESBL-producing *E. coli* isolates to these 2 frontline antibiotics [38,39]. The high rate of resistance to gentamicin specifically is likely influenced by its inclusion as a first-line antibiotic for neonatal sepsis in the WHO's guidelines [40], thereby promoting widespread use in hospitals.

On the global level, the increasing level of antimicrobial resistance of ESBL-producing *E. coli* to frontline antibiotics is a critical threat. It threatens health systems by limiting the therapeutic choices used for treating BSI and highlights a bigger threat of the emergence of pan-drugs resistance in ESBL-producing *E. coli* [40]. Active local antibiotic surveillance will enable modifying empiric antibiotic use, rather than relying entirely on empirical treatment based on Nigeria's standard treatment guidelines. It is most likely that the sampled hospitals frequently initiate empiric treatment using fluoroquinolones and gentamicin without recourse to local empiric studies (though lacking).

Plasmids carrying ESBL genes have been shown to be transferable by conjugation. Person-to-person spread, and hospital surfaces-person spread are likely possibilities in this study due to the observed poor cleaning of potential environmental sources. Clonal outbreaks are therefore underscored in this study. Cohorting or contact isolation and special cleaning of potential environmental sources should be carried out to limit the occurrence of resistance.

Conclusion

This study has provided insight into bloodstream infections, the pathogens implicated, the pattern of drug resistance, and the production of ESBLs by isolates. Gram-negative bacteria were the main cause of BSI in this study. Regular monitoring of pathogen spectrum and antimicrobial resistance patterns will help clinicians use drugs rationally in neonates, adjust medications when necessary, and better prevent and control the occurrence of neonatal bloodstream infections. However, there were some limitations in this study. Genotypic detection of ESBL and plasmid DNA extraction were lacking due to funds. There was also a lack of exploration of factors that increase the risk of infection or colonization with ESBL-producing Enterobacteriaceae.

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Conflict of Interest

The authors have no conflict of interest.

Authors Contribution

Conception and design of the study was carried out by Olivia Sochi Egbule, acquisition of data, analyses, and interpretation was by Obaro Levinson Oyubu and Mary Oghenenyerhovwo Okotie drafting the article was Patricia Konye Omenogor while critical revising of the manuscript was by Bernard Onyekweli Ejechi.

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Bibliography

1. Fleischmann-Struzek C. "The global burden of paediatric and neonatal sepsis: a systematic review". *Lancet Respiratory Medicine* 6 (2018): 223-230.

2. Naghavi M., *et al*. "Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016". *The Lancet* 390 (2017): 1151-210.

3. Lawn JE., *et al*. "4 million neonatal deaths: when? where? why?" *The Lancet* 365 (2005): 891-900.

4. Okomo U., *et al*. "Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: A systematic review and meta-analysis in line with the STROBE-NI reporting guidelines". *Lancet Infectious Disease* 19 (2019): 1219-1234.

5. Chaurasia S., *et al*. "Neonatal sepsis in South Asia: huge burden and spiralling antimicrobial resistance". *BMJ* 364 (2019): k5314.

6. Folgori L., *et al*. "Tackling antimicrobial resistance in neonatal sepsis". *Lancet Global Health* 5 (2017): e1066-8.

7. Simonsen KA. "Early-onset neonatal sepsis". *Clinical Microbiology Review* 27 (2014): 21-47.

8. Zaoutis TE., *et al*. "Risk factors for and outcomes of bloodstream infection caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species in children". *Pediatrics* 115 (2005): 942-949.

9. Ndir A., *et al*. "Epidemiology and Burden of Bloodstream Infections Caused by Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in a Pediatric Hospital in Senegal". *PLoS ONE* (2016): e0143729.

10. Bitew A and Tsige E. "High Prevalence of Multidrug-Resistant and Extended-Spectrum β Lactamase-Pro-ducing Enterobacteriaceae: A Cross-Sectional Study at Arsho Advanced Medical Laboratory, AddisAbaba, Ethiopia". *Journal of Tropical Medicine* (2020): 6167234.

11. Shilpakar A., *et al*. "Prevalence of multidrug- resistant and extended-spectrum beta-lactamase producing Gram- negative isolates from clinical samples in a tertiary care hospital of Nepal". *Tropical Medical Health* 49 (2021): 23.

12. Egbule OS. "Occurrence of extended spectrum beta-lactamases and sul 1 in multi-drug resistant *Escherichia coli* and *Salmonella* isolated from poultry feeds". *Sci African* (2022): 18: e01362

13. Adler A., *et al*. "The continuing plague of extended-spectrum β-lactamase producing enterbacterales infections: an update". *Infectious Disease Clinics of North America* 34 (2020) :677-708.

14. Jyothi P., *et al*. "Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates". *Journal of Natural Science, Biology, and Medicine* 4 (2013): 306-309.

15. Omoregie R., *et al*. "Microbiology of neonatal septicemia in a tertiary hospital in Benin City, Nigeria". *Biomark Genomic Medicine* 5 (2013): 142-144.

16. Olorukooba AA., *et al*. "Prevalence and Factors Associated with Neonatal Sepsis in a Tertiary Hospital, North West Nigeria". *Nigerian Medical Journal* 61 (2020): 60-66.

17. Egbule OS., *et al*. "High Rate of Antibiotic Resistance in a Neonatal Intensive Care Unit of a University Hospital". *BMRJ* 15.1 (2016): 1-6.

18. Edmond KM., *et al*. "Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis". *Lancet* 379 (2012): 547-556.

19. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Wayne, Clinical and laboratory Standards Institute (2018).

20. Sijhary TJ., *et al*. "Experiment with Gene Fusions". 5th Edition, Cold Spring Harbor Laboratory Press, New York, (1984).

21. Ballot DE., *et al*. "Bacterial bloodstream infections in neonates in a developing country". *ISRN Pediatrics* (2012): 2012508512.

22. Maharath A and Ahmed MS. Bacterial Etiology of Bloodstream Infections and Antimicrobial Resistance Patterns from a Tertiary Care Hospital in Malé, Maldives". *International Journal of Microbiology* 18 (2021): 3088202.

23. Garba B., *et al*. "A study of neonatal mortality in a specialist hospital in Gusau, Zamfara, North-Western Nigeria". *International Journal of Tropical Disease and Health* 28 (2017): 1-6.

24. Obiero CW., *et al*. "Empiric treatment of neonatal sepsis in developing countries". *The Pediatric Infectious Disease Journal* 34 (2015): 659-661.

25. Khan AM., *et al*. "Neonatal and perinatal infections". *Pediatric Clinics of North America* 64 (2017): 785-798.

26. Shane AL., *et al*. "Neonatal sepsis". *Lancet* 390.10104 (2017): 1770-1780.

27. Jing Z and Big L. "Analysis on the applicability of neonatal blood flow infection and antimicrobial regimen in a third class hospital". *Chin Med Rec* 20 (2019): 96-101.

28. Dat VQ., *et al*. "Bacterial bloodstream infections in a tertiary infectious diseases hospital in Northern Vietnam: aetiology, drug resistance, and treatment outcome". *BMC Infectious Diseases* 17 (2017): 493.

29. Zhang X., *et al*. " Epidemiology and Drug Resistance of Neonatal Bloodstream Infection Pathogens in East China Children's Medical Center from 2016 to 2020". *Frontiers in Microbiology 13* (2022): 820577.

30. Tong SY., *et al*. "*Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management". *Clinical Microbiology Review* 28 (2015): 603-661.

31. Guyot K., *et al*. "Raman spectroscopic analysis of the clonal and horizontal spread of CTX-M-15-producing Klebsiella pneumoniae in a neonatal intensive care unit". *European Journal of Clinical Microbiology and Infectious Diseases* 31 (2012): 2827-2834.

32. Mshana SE., *et al*. "Predominance of Klebsiella pneumoniae ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania". *BMC Infectious Disease* 13 (2013): 466.

33. Wang HM. "Analysis of pathogen distribution and drug resistance of neonatal infectious pneumonia from 2010 to 2013". *Chinese Journal of Infection Control* 13 (2015): 411-412.

34. Koopmans LR., *et al*. "Paediatric antimicrobial use at a South African hospital". *The International Journal of Infectious Disease* 74 (2018): 16-23.

35. World Health Organization. WHO priority pathogens list for R&D of new antibiotics. WHO (2017).

36. Wu PC., *et al*. " Prevalence and risk factors for colonization by extendedspectrum β-lactamase-producing or ST 131 Escherichia coli among asymptomatic adults in community settings in Southern Taiwan". *Infectious Drug Resistance* 12 (2019): 1063-1071.

37. Bush KGA Jacoby. "Updated functional classification of beta-lactamases". *Antimicrobe Agents Chemotherapy* 54 (2010): 969-976.

38. Mshana SE., *et al*. "Multiple ST clonal complexes, with a predominance of ST131, of escherichia coli harbouring blaCTX-M-15 in a tertiary hospital in Tanzania". *Clinical Microbiology and Infection* 17.8 (2011): 1279-1282.

39. Rogers BA., *et al*. "*Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain". *Journal of Antimicrobe Chemotherapy* 66 (2011): 1-14.

40. Egbule, O. S. Antimicrobial Resistance and β-Lactamase Production among Hospital Dumpsite Isolates. Journal of Environmental protection 7.07 (2016): 1057-1063.

41. Majumder MAA., *et al*. "Antimicrobial Stewardship: Fighting Antimicrobial Resistance and Protecting Global Public Health". *Infectious Drug Resistance* 29 (2020): 4713-4738.