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High Rate of Antibiotic Resistance in a Neonatal Intensive Care Unit of a University Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSE designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ADE performed the statistical analyses and managed literature searches. Author UBOU managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Management of infections in new-born remain a major problem globally due to their delicate nature. Bacteremia in new born has resulted in high mortality. Determining the prevalence and antibiotic susceptibility pattern of *Escherichia coli, Klebsiella pneumoniae and Staphylocccus aureus* which dominates in sepsis is important.
Study Design: During a 4 month period in 2015, 98 blood samples were collected from new-born admitted to a university hospital in Delta State, Nigeria.
Methodology: Isolation of organisms were based on growth patterns, morphological appearance and biochemical analysis. Antimicrobial susceptibility were determined following Kirby-Bauer disc diffusion methods, using 11 different antibiotics which include Gentamicin (10 µg), Ofloxacin, (5 µg) Ciprofloxacin, (5 µg) Amoxicillin-clavulanic acid (30 µg), Ceftazidime (30 µg), Cefuroxime, (30 µg) Trimethoprim-sulphamethoxazole (25 µg), Nitrofurantoin (300 µg), Cefixime (5 µg), Cloxacillin (10 µg) and Erythromycin (10 µg).
Results: A total of 30 (30.61%) *Escherichia coli*, 20 (20.41%) *Klebsiella pneumoniaè* and 18 (18.37%) *Staphylococcus aureus* were isolated. Susceptibility results indicate that all isolates were

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highly resistant to Gentamicin and to the two lower generation cephalosporins tested; Ceftazidime and Cefuroxime. In addition, all isolates were multidrug resistant.
Conclusion: Our data has revealed that a serious problem of antimicrobial resistance exist among bloodstream isolates of new-born in our hospital.

Keywords: New-born; sepsis; resistance; blood.

1. INTRODUCTION

Neonatal sepsis is a major contributor of morbidity and mortality in developing countries than in developed countries [1]. Health care usually is not a priority in most developing countries due to economic reasons. In some cases, there are shortages in disposables used in invasive procedures [2] there are inefficient prenatal and postnatal cares. The new born is highly susceptible to infections due to their impaired immune system [3]. WHO estimates that there are about 5 million neonatal deaths yearly, with 98% occurring in developing countries [4]. Neonatal sepsis remain a major cause of morbidity and mortality in neonates The incidence of neonatal sepsis in Nigeria varies from 30%-50% [5]. This may be linked to the level of poverty in Nigeria. Though Nigeria is blessed with enormous oil wealth, over 70% of her populace live on less than 1 US dollar per day [6]. Affordability of healthcare is impaired because care for sick newborn is expensive. A major health issue affecting neonatal care in Nigeria is that competent neonatal care is exclusively available in tertiary and a few extremely expensive private hospitals. This is why the majority of births occur in unorthodox heath facilities, where health care is cheap but the level of hygiene is poor and certain harmful practices are carried out. New born are usually brought to the hospitals when complications that are irreparable occur, thereby increasing the chances of deaths. More than a quarter of under-five deaths in Nigeria occur in the neonatal period. The prevalence of neonatal sepsis varies in Nigeria. [7] observed while working on childhood mortality at University College Hospital, Ibadan, Nigeria that septicaemia was the most common cause of death. [8] in Calabar observed 16% while [9] in Abuja reported 22%.

Ethiologic causes of bacterial infection in children in Nigeria differs depending on the type of infection. The most common bacteria are usually those associated with respiratory diseases and enteric organisms [10].

The spectrum of organisms implicated in neonatal sepsis changes from time to time and varies from one geographical location to another, even in the same locations [11]. Neonatal sepsis is caused by Gram-negative and Gram-positive bacteria. However a predominance of *Klebsiella peumoniae* followed by *Staphylococcus aureus* and *Escherichia coli* have been reported in many parts of the country [12,13].

The increasing cases of septicaemia in developing countries is further compounded by the development of antimicrobial resistance in organisms. Increasing antimicrobial resistance in neonates has been reported by some researchers in Nigeria [5,13] and elsewhere in the world [14,15,1].

Constant assessment of the etiological agents responsible for neonatal sepsis and their antimicrobial resistance pattern is important in order to guide empiric therapy. Empiric therapy in blood infection is important because the pathogens and their toxins can be carried to other organs, in the body if treatment is not commenced early. Therefore this study was undertaken to evaluate the common pathogens responsible for neonatal sepsis and their resistance patterns.

2. MATERIALS AND METHODS

For each neonate, 2 ml of venous blood was collected and aseptically introduced into two culture bottles, each containing 5ml of brain hearth infusion. The broth cultures were transported and processed in the Department of Microbiology Laboratory, Faculty of Science, Delta State University, Abraka. The inoculated blood cultures were sub cultured every day for 7 days on Macconkey agar, blood agar and chocolate agar plate (Oxoid, UK) and incubated at 37ºC for 24 hours. Blood cultures were considered negative if after 7 days no growth was observed. Organisms were considered pathogens if the same organism was obtained in the 2 broth culture bottles and contaminants if either the growth was obtained in only one culture bottle or a mixed growth obtained.

Bacteria isolated were first identified by gram staining reaction. Their characteristic

appearances on their respective media was evaluated. Their identity was further confirmed by the following biochemical tests; indole production, H_2S production, citrate utilization, mobility test, urease test, oxidase, carbohydrate utilization tests, coagulase and catalase tests. Procedures was according to the Clinical Laboratory Standard Institute [16].

2.1 Susceptibility Testing

Antimicrobial sensitivity was determined on Muller Hinton agar (Oxoid, UK) by Kirby-Bauer disc diffusion methods and interpreted according to national committee for clinical laboratory standards recommendations (NCCLS, 2004). Antimicrobial susceptibility tests was carried out on both gram negative and gram positive isolates. Gentamicin (10 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), Amoxicillin-clavulanic acid (30 µg), Trimetroprim-sulfametroxazole (25 µg), Nitrofurantion (300 µg) and Cefixime (5 µg) were used to determine susceptibility in Gram negative isolates. Cloxacillin (10 µg) and Erythromycin (10 µg) were used only on Gram positive isolates. Interpretation of susceptibility was based on Clinical and laboratory standards Institute [16].

2.2 Statistical Analysis

Statistical analysis was carried out using SPSS Version 20.

2.3 Ethical Approval

Ethical approval for the study was obtained form the Ethics and Research Committee of Delsu Teaching Hospital, Delta State Nigeria.

3. RESULTS

For 4 months, in 2015, a total of 98 blood samples of neonate were evaluated for the presence of 3 important pathogens, *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* causing blood stream infections. *Escherichia coli* (30.61%) was the most frequently isolated of the 3 pathogens. This was followed by *Klebsiella pneumoniae* (29.41%) and *Staphylococcus aureus* (18.37%). The information is presented in Table 1.

Susceptibility result revealed varying levels of resistance amongst the isolates. The gram negative organisms were highly resistant to Gentamicin and to the two lower generation cephalosporins; Cefuroxime and Ceftazidime used in the study. However low level of resistance to a higher (4^{th}) generation cephalosporin, cefixime used in the study was observed. All *Klebsiella pneumonia*e isolate were 0% resistant to cefixime. *E. coli* was 20% resistant to cefixime. The least in resistance of the antimicrobial agents tested on *Staphylococcus aureus*, the only gram positive organism studied was the fluoroquinolones. Ofloxacin and Ciprofloxacin were 44.4% resistant in *Staphylococcus aureus*. All other antimicrobial agent tested on *staphylococcus aureus* were over 60% resistant. Detailed information on the resistance pattern of gram negative and gram positive isolate are shown in Table 2.

Table 1. Prevalence of *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* in blood of new born

Isolate	Prevalence (%)
Escherichia coli	30 (30.61)
Klebsiella pneumoniae	20 (20.41)
Staphylococcus aureus	18 (18.37)
Total	68 (69.39)

Multi-drug resistance (resistance to 3 or more antimicrobial agents) was also observed in the study. The multidrug resistance pattern of the isolates are shown in Tables 3, 4 and 5.

4. DISCUSSION

Neonatal sepsis are an important cause of mortality and morbidity worldwide. The infection has potential life threatening consequences that may lead to death. Death from neonatal sepsis can be minimized by constant periodic evaluation of organisms responsible for sepsis and their sensitivity patterns.

Neonatal sepsis varies from region to region and changes over time, even in the same place [17]. Out of 98 blood samples obtained from neonates in intensive care unit of a tertiary hospital in Delta State, 68 (69.39%) cultures were found positive [18,19,20]. Have all reported high incidence of positive blood cultures. The incidence of the 3 isolates investigated was 30.61% for *Escherichia coli*, which was the most prevalent followed by *Klebsiella pneumoniae* (20.41%) and *Staphylococcus aureus* (18.37%). The predominant gram negative organism varied from *Escherichia coli* to *Klebsiella pneumoniae* in

different reports. [21] and [22] reported *E. coli* as the most common organisms causing neonatal sepsis. [21,18,1,23,24] all reported *Klebsiella spp* as the most common organism isolated. In this study, the gram negative organisms encountered in 50 (50.02%) cases were mainly responsible for neonatal sepsis. [17] reported 67.85% incidence of gram negatives in their study.

Table 2. Antimicrobial resistance patterns of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*

Antibiotic group	Antibiotics (μg/disc)	Resistance in *Escherichia coli* N = 30 (%)	Resistance in *Klebsiella pneumonia* N = 20	Resistance in *Staphylococcus aureus* N= 18
Aminoghycosides	Gentamicin (10)	30 (100.00)	12 (60.00)	12 (66.67)
Cephalosporins	Cefixime (30)	6 (20.00)	0.(0.00)	-
	Ceffazidime (30)	20 (66.67)	12.(60.00)	18 (100.00)
	Cefuroxime (30)	20 (66.67)	16 (80.00)	18 (100.00)
β- lactams	Amoxicillin –Clavulanic acid (30)	23 (76.67)	12 (80.00)	16 (88.89)
	Cloxaxillin (10)	-	-	12 (66.67)
Macrolides	Erythromycin (10)	-	-	14 (77.78)
Fluoroguirolones	Ofloxaclin (5)	16 (53.33)	14 (70.00)	8 (44.44)
Sulforiamides	Ciprofloxacine (5)	8 (26.67)	12 (60.00)	8 (44.44)
	Trimethroprin-Sulfametrixazole (25)	10 (33.33)	16 (80.00)	14 (77.88)
Synthetic drug	Nitrofurantrin (300)	26 (86.67)	12 (60.00)	-

- *Not tested*

Table 3. Resistance pattern of *Escherichia coli*

Resistance pattern	No of antibiotic	No of isolates
GEN, OF.L, AMX-CLA, NTT, CPR, CAZ, CR x CXM SXT	9	2
GEN, AMX-CLA, NIT, CPR, CAZ, CRX, SXT	7	3
GEN OFLA, AMX-CLA, NIT, CAZ, CRX	6	5
GEN, OFL, AMX-CLA, NIT, CAZ, CRX	5	8
GEN, OFL, AMX-CLA, CAZ, CRX	5	2
GEN OFL, NIT, CAZ, CXM	5	8
GEN, CAZ CRX	3	1
GEN OFL AMX	3	1

Table 4. Resistance pattern of *Klebsiella pneumoniae* resistance pattern

Resistance pattern	No of antibiotics	No of isolates
GEN, OFL, AUG, NIT, CPR, CAZ, CRX, SXT	8	2
GEN OFL AMX-CLA, NIT, CPR, SXT	6	8
GEN, OFL, AMX-CLA, CAZ, CRX SXT	6	4
GEN, OFL, NIT, CPR, CRX, SXT	6	2
GEN OFL CPR, CAZ, CRX	5	4

Table 5. Resistance pattern of *S. aureus*

Resistance pattern	No of antibiotic	No of isolates
GEN, AMX-CLA, NIT, CAZ CRX CRY, OFL, CPX, CXC	9	6
GEN, AMX-CLA, NIT, CAZ, OFL CPX CRX	6	6
CXC, CAZ, SXT, CRX	4	5
CXC, AMX-CLA, CAZ, CRX	4	1

Among the gram-positive organisms, *S. aureus* has been consistently reported as the most prominent gram positive organism causing sepsis in children [24]. In Port-Harcourt, showed a predominance of *Klebsiella pneumoniae* followed by *S. aureus*, then *E. coli*. In Jos, [25] reported *E. coli* as the most predominant followed by *S. aureus* and *Klebsiella pneumonia*. Analysis of variance (ANOVA) indicates the differences in values of the organism were significant at ($p<0.05$).

An overall increase in the level of resistance was observed in all the isolates in this study. High level of resistance to gentamicin was observed. This maybe because of the abuse in the use of both gentamicin antibiotics and ointment to threat neonates. It was also observed that all *Staphylococcus aureus* were resistant to ceftazidime and cefuroxime. Amongst the gram negative isolates, over 60% resistance was observed in Ceftazidime and Cefuroxime (Table 2). The high rate of resistance observed in the cephalosporins could be as a result of the expression of β-lactamase enzymes such as extended spectrum beta lactamases (ESBLs). ESBLs are mostly plasmid associated, as such can spread among bacteria. High resistance to the cephalosporins has been reported [26,27]. The production of ESBLs confer resistance to β-lactam drugs particularly the cephalosporins and to other classes of drugs. Production of β-lactamases has complicated treatment of gram negative pathogens. Previous studies in India has reported ESBL production among gram negative isolates from neonatal septicaemia [19]. However, an important observation in this study is that the gram negative pathogens were still susceptible to the fourth–generation Cephalosporins, Cefixime. Cefixime is therefore a good choice for emperic therapy in our environment.

5. CONCLUSION

Antibiotic resistance in neonate sepsis in this study was high. This is a delicate matter and a life threatening emergency; therefore we stress the need for constant periodic evaluation of isolates causing neonatal sepsis and their resistance pattern.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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