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An epidemiological survey of neonatal sepsis in a hospital in western Nigeria

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The study examined occurrence of bacterial pathogens associated with neonatal sepsis in a hospital setting. A prospective cross-sectional study was carried out on neonates with sepsis who were admitted to the neonatal ward of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria over a six-month period. One hundred samples for microbiological evaluation were collected from the neonates excluding those on prior antibiotic therapy. Microbiological examination and analysis of a variety of samples, processed according to standard procedures for isolation and identification of pathogenic bacteria and antibiotic sensitivity testing, was carried out on bacterial isolates. Among the 100 samples collected and processed, *Staphylococcus aureus* accounted for 28%, *Klebsiella* and *Pseudomonas* species 13% each, *Proteus* species 10%, other *Enterobacteriaciae* 9%, *Neisseria gonorrhoea* 8%, beta- haemolytic *Streptococcus* 5 and 14% showed no bacterial growth. Antibiotic sensitivity patterns of the bacterial isolates showed that most were sensitive to oxfloxacin. However, a significant number of them showed resistance to commonly used antibiotics such as ampicillin and penicillin. Bacterial pathogens in neonates with sepsis vary from Gram-positive bacteria, mostly *S. aureus* to Gram- negative bacilli, mainly K *lebsiella* and *Pseudomonas* species. Improvement in hygienic practices in both the wards and nurseries is required to reduce mortality rate in the hospital.

Key words: Neonatal sepsis, samples, infants, bacteria, antibiotic, Nigeria.

INTRODUCTION

Bacterial sepsis is considered to be an important cause of neonatal mortality (deaths in the first 28 days of life) (WHO, 1999; Dawodu et al., 2002; Rubin *et* al., 2002; Motara et al., 2005; Movahedian et al., 2006) . The World Health Organization estimated that there are approximately five million neonatal deaths per year of which 98% occur in developing countries (WHO, 1996). These neonatal deaths are attributed principally to infection, birth asphyxia and consequences of premature birth and low birth weight. It is known that risk factors related to neonatal bacterial sepsis are complex and include interaction of maternal-foetal colonization, transplacental immunity and physical and cellular defence mechanisms of the neonate (Jumah and Hassan, 2007). The incidence of neonatal bacterial sepsis depends on geographic area and may vary from country to country as well as within the same country. In developing countries, neonatal mortality resulting from all causes of neonatal sepsis is about 34 per 1000 live births, occurring mainly in the first week of life, whilst it is 5 per 1000 live births in developed countries (Costello et al., 2001). Neonatal mortality is about 34 per 1000 live births in Asia, 42 per 1000 live births in Africa and 17 per 1000 live births in Latin America and the Caribbean (Vergnano et al., 2005). Furthermore, neonatal mortality for different African countries ranges from 68 per 1000 live births in Liberia to 11 per 1000 live births in South Africa (Costello et al., 2001).

Bacterial organisms causing neonatal sepsis may differ among countries, however, in most developing countries, Gram-negative bacteria remain the major source of infection (Dawodu et al., 2002). In addition, bacterial organisms causing neonatal sepsis have developed increased drug resistance to commonly used antibiotics, making its management a challenge for both the public and private health sectors (Motara et al., 2005). Knowledge of local

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Table 1. Distribution, nature of samples collected in the neonatal ward and bacterial isolates from neonates with suspected sepsis

Source of sample	Nature of sample	Total sample	No of bacterial Isolates
Blood	Blood	27	21 (78%)
Wound	Swab	21	21 (100%)
Eye	Swab	26	24 (92%)
Umbilical cord	Swab	14	14 (100%)
CSF	CSF	12	06 (50%)
	Total	100	86 (86%)

and regional health problems is a prerequisite for establishing an effective health care delivery system and epidemiological and statistical information regarding neonatal sepsis is the basis for establishing a sound programme for early detection of infection or an outbreak (WHO, 1996; Ako-Nai et al., 1999; Anah et al., 2008). This information also has a significant influence on treatment assessment and subsequent outcome. The epidemiology of neonatal sepsis and antibiotic resistance patterns of pathogens may be used to develop guidelines for management of neonatal sepsis in hospital including choice of empiric antibiotic therapy. Therefore, the study was designed to examine the occurrence of aerobic bacterial organisms causing neonatal sepsis at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife and to report the antibiotic sensitivity patterns of the bacterial pathogens that were isolated.

MATERIALS AND METHODS

This was a prospective cross-sectional study and all samples for microbiological assessment were collected from neonates that were diagnosed as having neonatal sepsis in the neonatal ward of Obafemi Awolowo University Teaching Hospital. The samples and bacterial isolates were treated in the Medical Microbiology Laboratory of the Hospital according to standard procedures (Oguntibeju and Fabode, 2002; Oguntibeju and Nwobu, 2003). Since this study was mainly laboratory-based, clinical information about neonates were not considered as essential part of the study, thus clinical information about the neonates were not obtained and hence were not utilized in the analysis and interpretation of the results of this study.

For blood cultures, two samples of I ml of blood each were collected under aseptic conditions, inoculated into glucose and thioglycollate broths and incubated at 37^{U}C for a period of 1-7 days. The cultures were examined daily for evidence of bacterial growth and macroscopically for gas formation, turbidity and clot formation. The presence of gas, turbidity or clot formation may indicate bacterial growth and in such cases the blood sample was subsequently sub-cultured onto chocolate and blood agar plates and incubated at 37^{U}C for 24 to 72 h. For all pathogens, infection was confirmed by presence of the organisms in even a single blood, chocolate and MacConkey agar culture plates.

Smears were made of swabs obtained from the eye, wounds, umbilical cords and cerebrospinal fluid (CSF) of neonates and stained by Gram's staining technique. These samples were inoculated on MacConkey agar, chocolate agar and blood agar plates and incubated aerobically at 37^{U}C for 24-48 h. Colonial appearance and morphological characteristics of isolated bacteria were noted and the colonies subjected to appropriate biochemical tests for identification and classification.

Bacterial isolates were subjected to relevant biochemical tests such as carbohydrate fermentation, indole production, citrate utilization and ability to produce urease using standard procedures. *Pseudomonas* species were subjected to oxidase and citrate tests. Catalase and coagulase tests were carried out on all *Staphylococcus* species. Bacitracin sensitivity was performed on suspected colonies of group B-haemolytic *Streptococcus* species (Movahedian et al., 2006).

Sensitivity of the bacterial isolates to different antibiotics was determined using the Kirby-Bauer disc diffusion method. Antibiotic sensitivity was tested for ampicillin (AMP), penicillin (PEN), cloxacillin (CLX), tetracycline (TET), gentamicin (GEN), cotrimoxazole (COT), chloramphenicol (CHL), carbenicillin (CAR), streptomycin (STR), erythromycin (ERY), ofloxacin (OFX), cefuroxime (CXM), ceftazidime (CAZ) and colistin (COL) and interpreted according to National Committee for Clinical Laboratory Standards recommendations.

RESULTS

One hundred samples were collected from the neonatal ward of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife. Of these, 86 samples yielded bacterial growth of which *Staphylococcus aureus* accounted for 28%, *Klebsiella* and *Pseudomonas* species 13% each, *Proteus* species 10%, other *Enterobacteriaciae* 9%, *Neisseria gonorrhoea* 8%, beta-haemolytic *Streptococcus* 5% (Figure 1) and 14% showed no bacterial growth.

Twenty–seven (27%) samples were from blood, 26 (26%) eye swabs, 21 (21%) wound swabs, 14 (14%) umbilical cord swabs and 12 (12%) from CSF. The highest rates of isolation (100%) were from wound and umbilical cord swabs, followed by eye swab (92%), blood (78%) and CSF (50%) (Table 1) . Sixty-five (65%) samples were from males and 35 (35%) from females.

Fifty-four (54%) samples were collected from neonates aged between 1-14 days while 46 (46%) were from those aged between 15-28 days.

The general antibiotic sensitivity patterns showed that, most bacterial pathogens were sensitive to ofloxacin. However, the majority of bacterial pathogens were resistant or less sensitive to the commonly used antibiotics such as ampicillin and penicillin. Distribution of samples and bacterial isolates and distribution of bacterial isolates according to age are presented in Tables 2.

Almost all *S. aureus* isolates were highly sensitive to ofloxacin, carbenicillin, erythromycin and gentamicin but less sensitive to ampicillin, and tetracycline. *Pseudomo-*

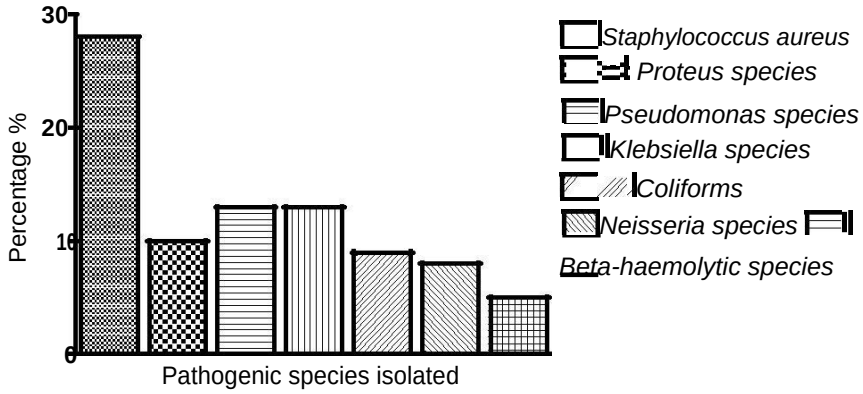

Figure 1. Percentage distribution of bacteria isolated from different samples.

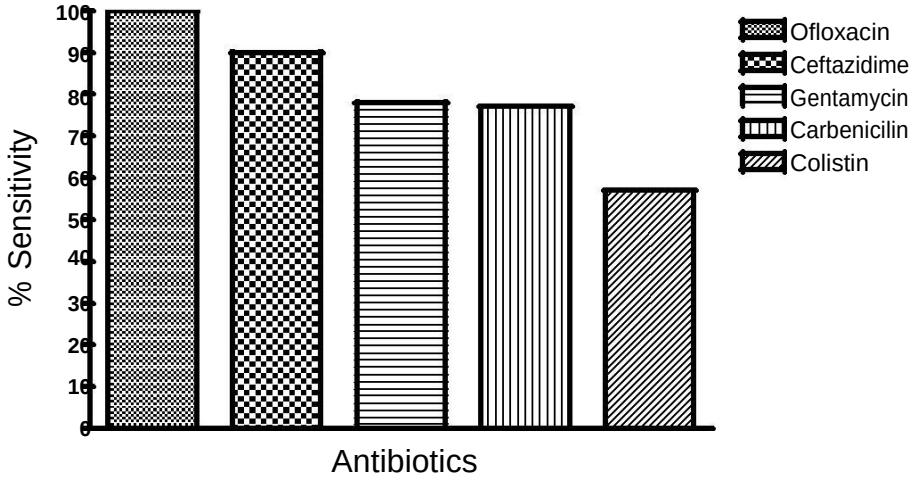

Figure 2. Graphical representation of *Pseudomonas species* susceptibility testing.

Table 2. Distribution of bacterial isolates according to age of patient

Age(Days)	No Examined	No with bacterial growth
1-9	41	36 (88%)
10-18	34	28 (82%)
19-28	25	22 (88%)
Total	100	86 (86%)

nas species were found to be highly sensitive to carbenicillin and gentamicin but less sensitive to colistin. Figures 2-4 show antibiotic sensitivity testing of selected bacterial isolates. Of the 41 samples collected from 1-9 day old neonates 36 (88%) showed bacterial growth. Twenty-eight (82%) of 34 samples from neonates aged 10-18 days indicated bacterial growth and of the 25 samples from neonates aged from 19- 28 days, 22 (88%) were culture positive.

DISCUSSION

Infants are generally more susceptible to infections than adults (Anah et al., 2008). This is due to a number of fac-tors including an inadequately developed immune system making sepsis a risk to the newborn especially under poor hygienic conditions. Despite improvements in diag-nosis and management of neonatal sepsis in recent years, it is still a major cause of neonatal morbidity and mortality especially in developing countries. In the pre-antibiotic years, mortality from neonatal sepsis exceeded 90% but now with the availability of antibiotics, the mortality rate has been reduced to between 10 and 50% (WHO, 1999; Rubin et al., 2002; Gheibi et al., 2005; Yalaz et al., 2006). Karlowicz et al. (2000) reported that Gram-positive organisms caused 73% of bacterial sepsis but Gram-negative organisms were responsible for the highest mortality rate. As bacterial pathogens responsible for neonatal sepsis tend to undergo mutation over time and certain bacteria continue to develop increased antibiotic resistance, our study was carried out to determine

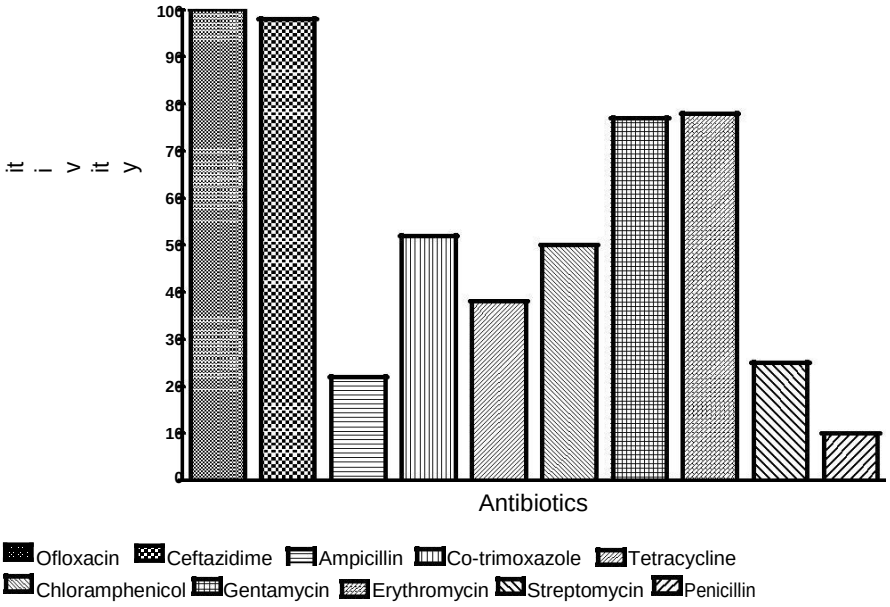

Figure 3. Graphical representation of *Staphylococcus aureus* susceptibility testing.

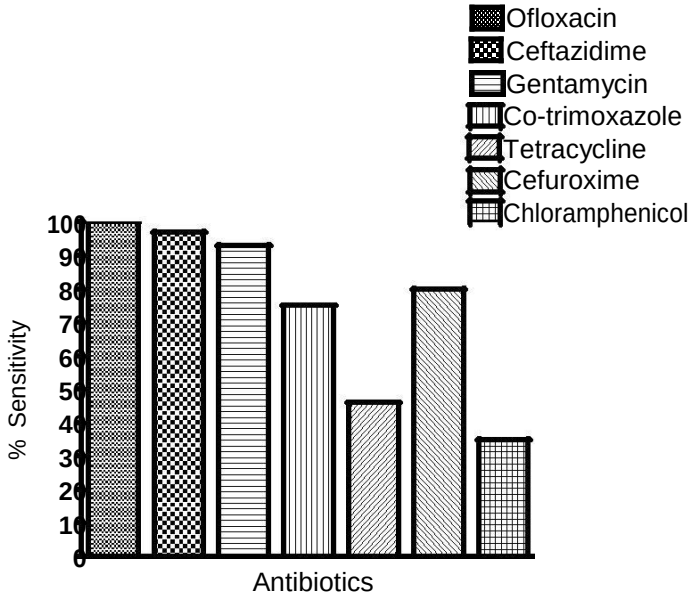

Figure 4. Graphical representation of *Proteus* species susceptibility testing.

(2008) found that *S. aureus* was the dominant bacterial isolate implicated in neonatal sepsis. The high percentage of *S. aureus* in our study may be related to contamination of the instruments used during clinical procedures at birth; hence the organism may be a hospital acquired organism (nosocomial infection). Prevalence of other causative organisms such as *Pseudomonas* and *Klebsiella* species (13% each) reported in our study is lower than previously reported in India (Orrett and Shurland, 2001; Roy et al., 2002; Ali, 2004; Ghotaslou et al., 2007) and Nigeria (Ako-Nai et al., 1999). This difference may be due to geographic location and distribution of bacterial organisms, exposure to antibiotics, time of collection and processing as well as methods of isolation.

Prevalence of bacterial infection was higher in samples obtained from male (65%) neonates than from females (35%) . The majority of the patients studied were aged be-tween 1-9 days but the result did not differ significantly from other age group. In general, our prevalence rate is the frequency and patterns of antimicrobial sensitivity/resistance of bacteria associated with sepsis among neonates at the Obafemi Awolowo Teaching Hospital Com-

plex, Ile-ife in the Western part of Nigeria.

S. aureus, a Gram-positive organism was the commonest major bacterial isolate (28%) associated with neonatal sepsis. The predominance of *S. aureus* corroborates the findings of others (Ghadamli, 1998; Behjati, 1998; Basher and Gharebaghi, 2001; Huda et al., 2001; Gheibi et al., 2005; Mozaffari et al., 2005; Bindayna et al., 2006; Yalaz et al., 2006). In a previous study, Ako-Nai et al. (1999) reported a prevalence rate of 33.8% for *S. aureus* among bacteria cultured from cases of neonatal sepsis in Nigeria. In a three-year survey of neonatal sepsis at Calabar Teaching Hospital, Anah et al. higher than those reported in developed countries such as the USA (Gessner et al., 2005).

Almost all the bacterial isolates were sensitive to oxfloxacin while most were found to be resistant to commonly used antibiotics such as ampicillin and penicillin. Antimicrobial sensitivity patterns differ in studies and at different times. This is due to emergence of resistant strains as a result of indiscriminate use of antibiotics (Basher and Gharebaghi, 2001; Oguntibeju and Nwobu, 2003; Dawodu et al., 2002; Motara et al., 2005). The high resistance rates in our study may be associated with frequent use of antibiotics for both prophylaxis and treatment of neonates in hospital. In view of this, we suggest that strategies of antibiotic usage in neonates be reviewed periodically.

It is important to improve hygienic practices of various wards and ensure that all surgical equipment used during clinical procedures is properly sterilized. Infected pregnant women should be followed up and treated. Pregnant women with wound infections should be adequately treated to reduce the risks of exposing their newborn to bacterial infection.

Furthermore, we advise that health education be given to the public on the dangers of the indiscriminate use of antibiotics which is currently considered to be a menace in Nigerian society and which has been responsible for the ineffectiveness of most commonly used antibiotics such as penicillin and ampicillin as observed in our study.

Conclusion

This study showed that *S. aureus* is the most common Gram-positive bacteria while *Psuedomonas species* is the commonest Gram-positive bacilli associated with neonatal sepsis in a hospital setting in the western part of Nigeria. We believe that the outcomes of the study will contribute to preventing and treating neonatal sepsis in the hospital.

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