Bacteriology and Sensitivity Profile Bacterial Agents Responsible for Neonatal Septicaemia in a Tertiary Hospital of Kaduna Metropolis

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A total of 50 samples and patients analyzed and out of these, 13 had evidence of bacterial growth, accounting for 26% Gram positive bacteria were isolated from 5 patients representing 10% of the total samples (n = 50) and 38.46% of the isolates (n = 13). Group B-Streptococcus was isolated from one patient, (7.69%), Staphylococcus aureus was also isolated from one patient (7.69%) while the remaining three gram positive isolates were coagulase negative Staphylococcus sp. (23.08%) while the remaining 8 isolates were gram negative organisms representing a prevalence of 16% and 61.54% of the total bacterial isolates. Enterobacter sp had the highest incidence accounting for 23% Klebsiella sp and Escherichia coli were 15.38%, Citrobacter flexneri 7.69%. The incidence of septicaemia was higher in males than in females with the ratio being 3:1 80% was sensitive to Cloxacillin, 60% of the gram positive bacteria were sensitive to chloramphenicol, augmentin and amoxicillin while 40% were sensitive to gentamicin and cotrimazole. All the isolates were resistant to tetracycline and only one isolate (20%) was sensitive to erythromycin. On the other hand there was a remarked resistance to the antibiotics among the gram negative bacteria with 100% to ampicillin, cotrimazole, nalizidic acid and colistin. The sensitivity to gentamicin, nitrofurantoin, streptomycin and tetracycline was only 12.5%.

Key words: Neonatal Septicaemia, Bacterial, strains, Mortality and Morbidity.

Neonatal bacterial sepsis (NBS) remains as an important cause of mortality and morbidity among infants especially in developing countries. Its incidence varies with geographical area and may change in the same area with time (Movahedian *et al*, 2006). In most of the developing countries, gram-negative bacilli remain the major cause of neonatal sepsis (Haque, 2001, Bhutta *et al*, 1991). Reported incidence rates vary from place to place and range from 1 to 8 per 1000 live births. The highest rates are for low birth weight preterm infants. Bacterial sepsis contributes to prolonged hospitalization, additional hospital costs, and increased neonatal mortality. Reported sepsis case

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fatality rates range from <under 10% to >50% for neonates and infants; the highest rates are for neonates with early-onset disease (i.e., illness onset during the first week of life) (Stoll *et al*, 1998). In a report based in Ilorin, *Staphylococcus aureus* was the commonest pathogen, accounting for 18 (29.5%) of the total isolates while coagulase negative *Saphylococcus albus* 15 (24.6%), Klebsiella spp 10 (16.4%) and unclassified Coliforms 9 (14.8%) (Mokuolu *et al*, 2002).

In a research finding based in Zaria, an incidence of 22.5% of the bacterial organisms responsible for neonatal septicaemia was reported for gram negative organisms (Ella et al., 2007). The incidence of 24.62% was indicated in another report by Ella et al (2008) for gram positive organisms. In that same report (Ella et al 2008), the prevalence of Staphylococcus species was 15.38%. S. aureus was isolated from five (5), representing 31.25%, with a prevalence of 7.69%. CONS accounted for the remaining five (5) (7.69%) of the isolates while β -haemolytic Streptococcus sp had a prevalence of 9.23%. Reports of high resistance to antibiotics have been documented by many workers (Roy et al, 2002). This is however not a general trend as there are reports of sensitivity in some findings necessitating the need for sensitivity assay for all isolates

This research is a hospital based in Kaduna, a cosmopolitan capital city in Northern part of Nigeria. This present study was undertaken to survey the microorganisms causing sepsis in a tertiary hospital in a selected tertiary hospital in Kaduna as well as review their sensitivity pattern to locally available antibiotics. The patients included for the study were in-patient neonates diagnosed as having sepsis by the pediatrician. Approval for this study was granted by the ethical board of the state Ministry of Health Kaduna and informed consent of the patients was obtained.

MATERIAL AND METHODS

The study period covered one year from August 2006 to July 2007 and within the period, a total of 50 neonates were sampled. The inclusion criterion was those neonates diagnosed by physician as having sepsis. Samples were collected before the administration of antibiotics.

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Neonates admitted in relation to illnesses other than sepsis were excluded.

One to two millilitre of blood was collected from each patient by medical personnel using proper aseptic precautions and inoculated immediately into 5 mL of Castenada medium, a modification of Mueller Hinton agar overlaid with Mueller Hinton Broth (Baker et al, 2001), and incubated for 48-72 hours. The broths were observed daily for evidence of growth. The broths showing growth were sub-cultured on chocolate agar, MacConkey agar, Mannitol salt agar, Eosin methylene blue agar and Blood agar. A negative result (the bottles without evidence of growth) was followed up by examining the broth daily and doing a final subculture at the end of 7 days or at appearance of turbidity, whichever was earlier. Growth was identified by Grams reaction, colonial characteristics and standard biochemical tests as described by Cheesbrough, (2001).

RESULTS

A total of 50 samples and patients analyzed and out of these, only 13 had evidence of bacterial growth, accounting for 26% Gram positive bacteria were isolated from 5 patients representing 10% of the total samples (n = 50) and 38.46% of the isolates (n = 13). Group B- *Streptococcus* was isolated from one patient, (7.69%), *Staphylococcus aureus* was also isolated from one patient (7.69%) while the remaining three gram positive isolates were coagulase negative *Staphylococcus sp.* (23.08%) (Fig 1), while the remaining 8 isolates were gram negative organisms representing a prevalence of 16% and

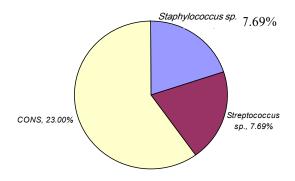


Fig. 1. Gram positive bacteria isolated

61.54% of the total bacterial isolates. The biochemical characterization of the gram negative organisms were isolated from 8 of the patients representing (61.54%, n = 13). Preliminary identification showed that *Enterobacter sp* had the highest incidence accounting for 23% while that of *Klebsiella sp* and *Escherichia coli* were 15.38% respectively. *Citrobacter flexneri* was the least accounting for 7.69% of the total isolates (Fig 2). In relation to sex distribution, the incidence of septicaemia was higher in males than in females with the ratio being 3:1(Fig 3).

There was a general resistance to most

of the antibiotics. 80% was sensitive to Cloxacillin, 60% of the gram positive bacteria were sensitive to chloramphenicol, augmentin and amoxicillin while 40% were sensitive to gentamicin and cotrimazole. All the isolates were resistant to tetracycline and only one isolate (20%) was sensitive to erythromycin (Table 1). On the other hand there was a remarked resistance to the antibiotics among the gram negative bacteria with 100% to ampicillin, cotrimazole, nalizidic acid and colistin. The sensitivity to gentamicin, nitrofurantoin, streptomycin and tetracycline was only 12.5% (Table 2).

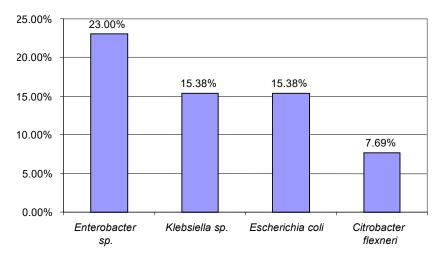


Fig. 2. Gram negative bacteria isolates

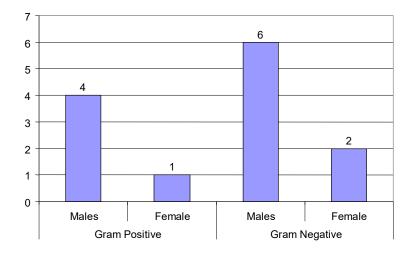


Fig. 2. Isolation in relation to sex

Sample no	Inference	Gen	Cot	Chlo	Aug	Amx	Ery	Tet	Cxc
BD - 004	Staph sp	28	30	32	31	32	28	16	32
BD - 009	Staph sp	23	24	12	31	25	23	Re	31
BD - 030	Streps sp	12	Re	32	28	21	24	12	26
BD - 040	Staph sp	13	Re	20	20	Re	Re	21	Re
BD-043	S.aureus	27	29	30	21	30	Re	19	28
% sensitivity		40	40	60	60	60	20	0	80
Mean Zone of Inhibition (mm)		27.5	29.5	31.33	30	29	28	0	29.25

Table 1. Sensitivity test result of Gram Positive Bacterial isolates

Table 2. Sensitivity test result of Gram Negative Bacterial isolates

Sample no	Inference	Amp	Cot	Gent	Nal	Nit	Col	Stre	Tet
BD – 019	Enterobacter	Re	Re	9	21	11	8	13	Re
BD-021	Klebsiella sp	Re	Re	26	Re	Re	10	32	Re
BD - 023	Citrobacter	23	Re	16	Re	Re	Re	24	26
BD - 025	Enterobacter	Re	Re	6	18	12	8	16	21
BD - 026	Escherichia coli	Re	Re	6	Re	25	Re	13	Re
BD - 036	Escherichia coli	Re	Re	Re	20	10	7	15	Re
BD - 038	Klebsiella	Re	Re	Re	18	14	10	Re	Re
BD - 039	Enterobacter	Re	Re	Re	18	16	7	Re	6
% sensitivity		0	0	12.5	0	12.5	0	12.5	12.5
Mean Zone of Inhibition (mm)		0	0	26	0	25	0	32	26

DISCUSSION

A total of 50 samples and patients analyzed and out of these, only 13 had evidence of bacterial growth, representing 26% with positive blood isolation. A breakdown of the bacterial analysis revealed that CONS and *Enterobacter species* were the most prominent isolates, accounting 23% each. *Klebsiella sp* and *Escherichia coli* had incidences of 15.38% each, while surprisingly, the incidence of *S. aureus* was 7.69% alongside *Streptococcus sp* and *Citrobacter flexneri*.

In a similar study in Ilorin by Mukuolu *et al.*, (2002), of the 198 neonates screened for sepsis, there were 61 (30.8%) positive blood cultures. In another report based in Ilesha In western Nigeria by Owa and Olusanya (1988), a prevalence of 35% was reported for neonatal septicaemia. A low prevalence of 30.8% was the

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outcome of the findings of another research work in Ilorin in central Nigeria by Mokuolu *et al*, (2002). The finding in this study in however, within the range reported is other part of the country though that reported by the latter workers was low compared to our findings. The finding contrasts that reported in Ile-Ife, a town in the western part of Nigeria by Ako - Nai *et al* (1990) in which they reported a prevalence of 55% and the findings of Haque *et al* (1990) in Riyadh, Saudi Arabia that had a prevalence of 15%. In a report based in Iran in which blood culture reports of 1266 clinically suspected neonatal septicemia cases were reviewed, a culture positivity rate of 24.88% was observed (Mathur *et al*, 1994)

The result revealed that Gram-ve organisms had the highest incidence of 61.54% while Gram+ve incidence was 38.46% which is an indication that most of the sepsis cases were caused by gram negative organisms in the study population. In most of the subjects, Sepsis was diagnosed within the first week of life. Mokuolu et al (2002), reported similarly that the predominant organisms in the first 48 hours were Gram negative bacilli; accounting for (70%) of their isolates. This is in agreement with our findings, in which gram negative bacilli accounted for 61.54%. Mathur et al, (1994) and Movahedian et al, (2006) reported that Gram negative bacilli constituted 87.1% and 72.1% of the total isolates respectively in their works based in Iran. The dominance of Gram negative organisms (n=60, 66.6%), as compared with Gram positive organisms (30; 33.4%) in the findings of Simiyu (2005) was in agreement with our findings that Gram positive accounted for 38.46%. The incidence of septicaemia was higher in males than in females with the ratio being 3:1in our finding. A ratio of 2: 1 was reported for males and females respectively by Mokuolu et al, (2002).

There was a general resistance to most of the antibiotics. The gram negative bacteria exhibited multiple resistances as compared with the gram positive bacteria. Multiple antibiotic resistance has been reported by many workers. This property is seen common in most gram negative bacteria. In our findings, there was 100% resistance to ampicillin, cotrimazole, nalizidic acid and colistin. The sensitivity to gentamicin, nitrofurantoin, streptomycin and tetracycline was only 12.5% meaning that 87.5% were resistant. The gram positive organisms were fairly sensitive to the antibiotics with 80% sensitive to Cloxacillin, 60% sensitive to chloramphenicol, augmentin and amoxicillin while 40% were sensitive to gentamicin and cotrimazole. All the isolates were resistant to tetracycline and only one isolate (20%) was sensitive to erythromycin. Reports based in Ethiopia by Asrat and Amanuel (2001) indicated that susceptibility for gram positive range from 12% to 76%, and for gram negatives range from 8% to 46% a similar range was obtained in our findings. Multiple resistance by gram negative bacteria was also reported by Mathur et al, (1994), Roy et al, (2002) and Movahedian et al, (2006). Resistant strains have been known to be associated with bacteria isolates obtained from intensive care units. This could account for the multiple resistances seen in the sensitivity pattern. This finding was also reported

by Biedenbach *et al*, (2004). The findings differ from our earlier report in which sensitivity was high among neonatal pathogen in another children intensive care unit (Ella, *et al*, 2008). This shows that antibiotic resistances differ from regions and geographical areas. This variation was also observed by Biedenbach *et al*, (2004). There are reports that antimicrobial sensitivity pattern differs in different studies as well as at different times in the same hospital. This is because of emergence of resistant strains as a result of indiscriminate use of antibiotics (Mathur *et al*, 1994).

CONCLUSION

The bacterial profiles vary little in our neonatal ICU. However the emergence of resistant strains poses serious concern to the health of our neonates. There is need therefore to review the bacterial profile associated with neonatal sepsis as the pattern as well as sensitivity to antibiotics in use do vary with respect to geographical location and time.

REFERENCES

- Ako-Nai K A, Taiwo O, and Adeniran M O. Bacterial Isolates involved in cases of septicaemia in a Nigerian hospital. *East African Medical Journal*. 1990; 67: 407–412
- Asrat D, and Amanuel Y. W., Prevalence and antibiotic susceptibility pattern of bacterial isolates from blood culture in Tikur Anbassa Hospital, Addis Ababa, Ethiopia. *Ethiop Med* J. 2001; 39(2):97-104
- Baker, F.J., Silverton, R.E. and Pallister, C.J. Introduction to Medical Laboratory Technology. (7eds). Bounty Press, Ltd. London. 2001; 348-419
- Bhutta Z. A, Naqvi S. H, Muzaffar T, and Farooqui B.J. Neonatal sepsis in Pakistan: Presentation and pathogens. *Acta Paediatr Scand*, 1991; 80(6-7): 596- 601.
- Biedenbach D. J, Moet G. J, and Jones R. N., Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002) *Diagn Microbiol Infect Dis.* 2004; 50(1): 59-69
- Cheesbrough, M. Medical Laboratory Manual for Tropical Countries. (2eds). Butterworths-Heinemann. Great Britain. 2001; 540

J. Pure & Appl. Micro., 2(1), April 2008.

- Ella, E. E., Ahmad, A. A., Ogala, W. N., Umoh, V.J. and Balogun T. B. Characterization and Antimicrobial Sensitivity Assay of Gram Negative rods isolated from Neonates with septicaemia in Zaria. *Journal of Pure* and Applied Microbiology 2007; 1(2): 191-196.
- 8. Ella, E. E., Ahmad, A. A., Ogala, W. N., Umoh, V.J. and Balogun, T.B., Incidence and Antimicrobial Sensitivity Assay of Gram Positive Cocci isolated from Neonates with suspected septicaemia in tertiary hospital in Zaria Journal of Pure and Applied Microbiology 2008; **2**(1).
- Haque K.N. Update on management of neonatal sepsis. Proceedings of 10th National Annual Paediatrics Conference Bhurban/Murree, Lahore. Pak Paediatr Assoc: 2001; 19
- Haque K N, Chagia A H, and Shaheed M. M.Half a decade of neonatal sepsis, Riyadh, Saudi Arabia. *Journal of Tropical Pediatrics*. 1990; **36**: 20-23
- 11. Mathur M, Shah H, Dixit K, Khambadkone S,

Chakrapani A, and Irani S., Bacteriological profile of neonatal septicemia cases (for the year 1990-91). *J Postgrad Med* 1994; **40**:18-20

- 12. Mokuolu AO, Jiya N, Adesiyun OO. Neonatal septicaemia in Ilorin: bacterial pathogens and antibiotic sensitivity pattern. *Afr J Med Med Sci.* 2002; **31**(2): 127-30
- Movahedian, A.H, Moniri, R and Mosayebi, Z Bacterial Culture of Neonatal Sepsis Iranian J Publ Health, 2006; 35(4): 84-89
- Owa J A and Olusanya O. Neonatal bacteraemia in Wesley Guild Hospital, Ilasha, Nigeria. Annals of Tropical Paediatrics. 1988; 8: 80-84
- Roy, I, Jain, A., Kumar, M. and Agarwal S. K. Bacteriology of Neonatal Septicaemia in a Tertiary Care hospital of Northern India *Indian Journal of Medical Microbiology*,2002; 20 (3):156-159
- Simiyu, D. E., Neonatal septicaemia in low birth weight infants at Kenyatta National Hospital, Nairobi. *East African Medical Journal* 2005; 82(3): 148-152.

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