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Incidence and Antimicrobial Sensitivity Assay of Gram Positive Cocci Isolated from Neonates with Suspected Septicaemia in Tertiary Hospital in Zaria

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Gram positive coccal bacteria were isolated from 16 of the 65 samples representing 24.62% of the organisms responsible for neonatal septicaemia. Of this number (16), ten (62.5%) were *Staphylococcus* species, with a prevalence of 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. β-haemolytic *Streptococcus sp* had a prevalence of 9.23% and accounted for 37.5% of the isolates, being the predominant organism responsible for neonatal septicaemia in the study area. 60% of the *Staphylococcus aureus* isolates were sensitive to chloramphenicol, augmentin and amoxicillin, 40% to cotrimazole, erythromycin and tetracycline and 20% were sensitive to gentamicin and cloxacillin. Strains AB-010 and AB-061 exhibited multiple resistance to most of the antibiotics. The mean zone of inhibition ranges from 26mm for gentamicin and cloxacillin to 34.3 mm for augmentin. All *Streptococcus* strains were sensitive to gentamicin while 83.33% were sensitive to chloramplenicol. The least sensitivity was to tetracycline which was 50%. The mean zone of inhibition ranges from 29.4mm for gentamicin to 36.5 mm recorded for cotrimazole The CONS isolates were 100% sensitive to Chloramphenicol, augmentim and amoxicillin. 80% were sensitive to gentamicin, cotrimazole and cloxacillin while 40% were sensitive to erythromycin and tetracycline. The mean zone of inhibition ranges from 30mm for tetracycline to 35.5 mm recorded for cloxacillin

Key words: Antibacterials, Antibiotics, Neonates, Septicaemia, Zaria.

Neonatal sepsis may be categorized as early or late onset (Ali *et al*., 2004). Eighty-five percent of newborns with early-onset infection present within 24 hours, 5% present at 24-48 hours, and a smaller percentage of patients present between 48 hours and 6 days of life. Onset is most rapid in premature neonates. Early-onset sepsis syndrome is associated with acquisition of micro-organisms from the mother by Trans-placental infection or an ascending infection from the cervix may be caused by organisms that colonize in the mother's genitourinary tract (Chiesa, *et al,* 2004). The infant may acquire the microbe by passage through a colonized birth canal at delivery. The microorganisms most commonly associated with early-onset infection include group B *Streptococcus* (GBS), *Escherichia coli, Haemophilus influenzae,* and *Listeria monocytogenes.* The risk is greater in males than females with a ratio of 2:1 (Mokuolu *et al*, 2002) and in newborns with congenital malformations, particularly of the GI tract

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Neonates are deficient in humoral and cellular immunity; they produce immunoglobulins at a lower rate than adults (Wilson, 1986) making them dependent on maternal antibodies for their first months of life. Transplacentally acquired maternal antibodies majorly mediate humoral immunity primarily; hence very low birthweight (VLBW) premature infants are less likely to receive as many immunoglobulins as term infants. T–cell function is also less efficient in neonates (Schelonka and Infante, 1998). Complement function and phagocytic function inclusive of phagocytosis, phagocyte migration and toxin production are also deficient (Berger, 1990).

Gram positive bacteria acquired from the birth canal constitute a leading cause of invasive bacterial infections in newborns (Lukacs *et al*, 2004). Commonly isolated gram positive bacteria in neonatal sepsis include Group B *Streptococcus* (GBS) (Lukacs *et al*, 2004, Puopolo, and Madoff, 2007)). *Staphylococcus aureus* and coagulase negative *Staphylococcus* (Roy *et al*, 2002).

Rapid physiologic changes during the neonatal period significantly affect the pharmacokinetic and toxicologic properties of antibiotics, necessitating complex dosage calculations. Empiric treatment, usually a combination of ampicillin and an aminoglycoside, or, when appropriate, ampicillin and a broad-spectrum cephalosporin with good CSF penetration, is often started pending results of cultures and antimicrobial susceptibility tests. Prevalence data on antibiotic-resistant organisms in a nursery would be helpful in choosing the treatment.

This study aims at identification and characterization of gram positive organisms associated with neonatal sepsis from neonates with suspected cases of septicaemias well as to establish the antimicrobial sensitivity pattern of the isolates to common antimicrobials.

Study Period

The study was conducted in a tertiary hospital within the months of June to December, 2006 covering a period of seven months (both months inclusive). The period under study, only neonates (ages 0-28) admitted into the hospital with clinical symptoms, of sepsis was included. Older babies as well as those with ailments other than sepsis as diagnosed by the physicians were excluded. A total of 65 neonates were sampled in the period under study. Ethical permit was granted for the study by the authorities of the Hospital.

MATERIAL AND METHODS

A total of one millilitre of venous blood was collected aseptically into bottles containing Castaneda modified blood culture medium (Baker *et al*., 2001) and transported to the Laboratory. The culture bottles were incubated for 48 hours and then sub-cultured for the initial isolation on blood agar plates. Growths were sub-cultured on fresh sterile medium for purification and transferred to slants for identification. The tests carried out to identify the organisms include Grams reaction and Microscopic morphology, Cagulase, Catalase, Hemolysis on blood agar, Sugar fermentation tests: sucrose, glucose, fructose, sorbose, sorbitol, raffinose, ramnose, inositol, arabinose, maltose, mannitol, xylose, lactose, Optocin sensitivity test, DNAse activity and Bile solubility test. The method used was that described by Cheesbrough, (2001).

Antibiotic sensitivity testing

The Kirby Bauer method was used for the sensitivity assay. This was done using the multi-disc diffusion method. Broad spectrum antibiotic included in the preparation include Gentamicin, Cotrimazole, Chloramphenicol, Augmentin, Amoxycillin, Erythromycin, Tetracycline and Chloxacillin (Abtek Labs. London).

The surface of sterile Mueller Hinton agar were seeded with 0.1ml of the organism suspension (McFarland No 1), spread evenly with a sterile glass rod and allowed to stand for 10 minutes. Commercially prepared paper discs, incorporated with broad spectrum Gram positive antibiotics (Abtek Labs. London) were then aseptically placed on the surfaces and the plates incubated for 24 hours at 37°C. The results were read and sensitivity determined.

RESULTS

Gram positive coccal bacteria were isolated from 16 of the 65 samples representing 24.62% of the organisms responsible for neonatal septicaemia. Of this number (16), ten (62.5%)

Table 1. Biochemical Characteristics of Isolates

Isolate code	Hemolysis reaction	Coagulase reaction	Mannitol fermentation		Bile	Catalse solubiilty	DNAse	6.5%	Optochin sensitivity test	Inference
			O	F						
AB – 002	-ve	-ve	-ve	-ve	-ve	-ve	Partial Positive	+		*Staph sp*
AB – 010	α Hemolysis	+	+	+	-	+	-	+		*S. aureus*
AB – 016	β haemolysis	-ve	-ve	-ve	+	+	-	+	-ve	*Streps*
AB – 017	α- hemolysis	+	++	++	-	+	+	+		*S.aureus*
AB – 022	α Hemolysis	+	+	+	-	+	-	+		*S. aureus*
AB – 023	-ve	-ve	-ve	-ve	±ve	-ve	+	+		*Staph sp*
AB – 024	-ve	-ve	+	+	-	-ve	-	+		*Staph sp*
AB – 028	-ve	-ve	+	-ve	-ve	+	-	+		*Staph sp*
AB – 031	α hemolysis	+	+	+	-	+	+	+		*S. aureus*
AB – 032	β hemolysis	-ve	-ve	-ve	-ve	+	-	+	-ve	*Streps*
AB – 033	-ve	-ve	-ve	ve	-ve	-ve	Partial Positive	+		*Staph sp*
AB – 035	β hemolysis	-ve	-ve	-ve	+	+	-	+	-ve	*Streps*
AB – 039	β haemolysis	-ve	-ve	-ve	+	+	-	+	-ve	*Streps*
AB – 049	β haemolysis	-ve	-ve	-ve	+	+	-	+	-ve	*Streps*
AB – 057	β Hemolysis	-ve	-ve	-ve	+	+	-	+	-ve	*Streps*
AB – 061	α Hemolysis	+	+	-ve	-	+	+	+		*S. aureus*

were *Staphylococcus* species, with a prevalence of 15.38%. *S. aureus* with a positive DNAse reaction was isolated from five (5), representing 31.25%, with a prevalence of 7.69%. Coagulase negative *Staphylococcus sp* accounted for the remaining five (5) (7.69%) of the isolates. B-haemolytic *Streptococcus sp* had a prevalence of 9.23% and accounted for 37.5% of the isolates, being the predominant organism responsible for neonatal septicaemia in the study area. These results are presented below in Table 1 and Fig.1. This result showed that group B-*Streptococcus* remains the predominant gram positive organism associated with neonatal sepsis.

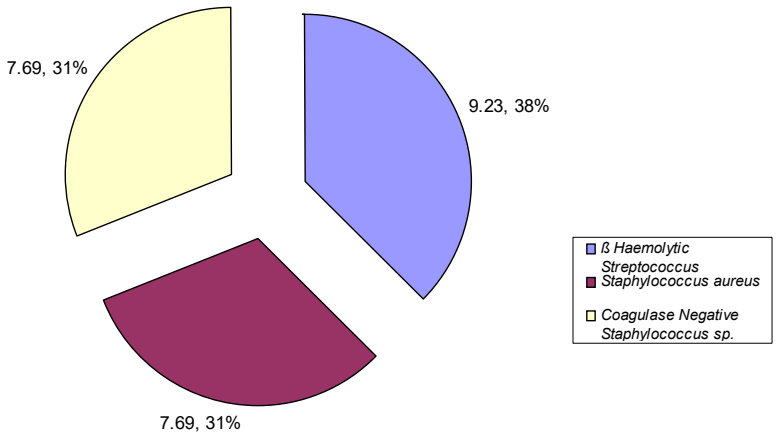

Fig. 1. Distribution of isolatea in percentage

Sensitivity pattern of the isolates

Sixty percent of the *Staphylococcus aureus* isolates were sensitive to chloramphenicol, augmentin and amoxicillin**.** Only forty percent were sensitive to cotrimazole, erythromycin and tetracycline while only twenty percent were sensitive to gentamicin and cloxacillin. Strains AB-010 and AB-061 exhibited multiple resistance to most of the antibiotics. The mean zone of inhibition ranges from 26mm for gentamicin and cloxacillin to 34.3 mm for augmentin (Table 2a, Fig.2).

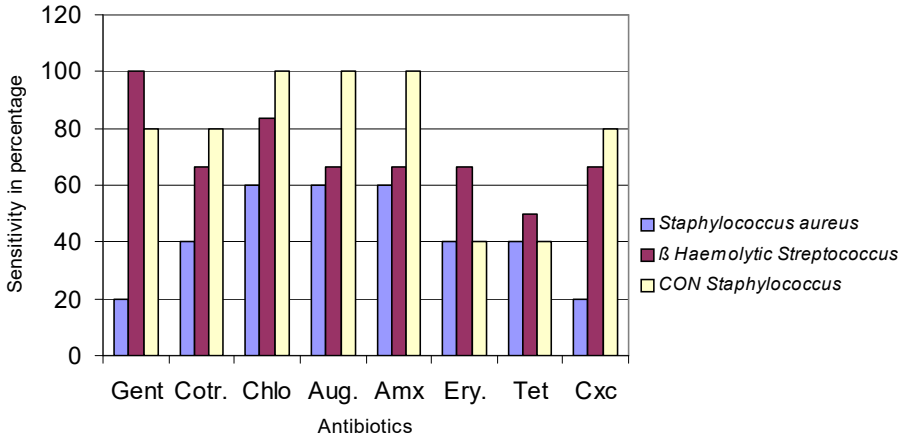

Fig. 2. Percentage sensitivity of organisms to antibiotics used

All *Streptococcus* strains were sensitive to gentamicin while 83.33% were sensitive to chloramplenicol. The least sensitivity was to tetracycline which was 50% (Table 2b, Fig.2). The mean zone of inhibition ranges from 29.4mm for gentamicin to 36.5 mm recorded for cotrimazole.

The coagulase negative *Staphylococcus* isolates were 100% sensitive to Cloramplenicol, augmentim and amoxicillin. 80% were sensitive to gentamicin, cotrimazole and cloxacillin while 40% were sensitive to erythromycin and tetracycline (Table 2c, Fig.2). The mean zone of inhibition ranges from 30mm for tetracycline to 35.5 mm recorded for cloxacillin

Table 2a. Sensitivity pattern of *S. aureus* isolates

Isolate code	Inference	Gent	Cotr.	Chlo	Aug.	Amx	Ery.	Tet	Cxc
AB – 010	*S. aureus*	16	12	26	12	Re	Re	Re	Re
AB – 017	*S.aureus*	15	Re	25	30	29	30	Re	Re
AB – 022	*S. aureus*	26	40	42	42	38	30	2.8	2.6
AB – 031	*S. aureus*	21	32	26	31	30	Re	26	25
AB – 061	*S. aureus*	Re	Re	Re	12	Re	Re	Re	Re
% sensitivity		20	40	60	60	60	40	40	20
Mean Zone of Inhibition (mm)		26	36	31.3	34.3	32.3	30	27	26

Table 2b. Sensitivity pattern of *Streptococcus* isolates

Isolate code	Inference	Gent	Cotr.	Chlo	Aug.	Amx	Ery.	Tet	Cxc
AB – 016	*Streps*	26	32	36	9	9	Re	11	Re
AB – 032	*Streps*	30	42	32	40	32	38	32	32
AB – 035	*Streps*	30	Re	27	30	35	35	30	30
AB – 039	*Streps*	34	32	25	35	30	19	21	28
AB – 049	*Streps*	30	40	32	40	35	38	32	36
AB – 057	*Streps*	27	Re	30	18	16	26	20	9
% sensitivity		100	66.67	83.33	66.67	66.67	66.67	50	66.67
Mean Zone of Inhibition (mm)		29.5	36.5	31.4	36.3	33	34	31.3	31.4

Table 2c. Sensitivity pattern of CON *Staphylococcus* isolates

Isolate code	Inference	Gent	Cotr.	Chlo	Aug.	Amx	Ery.	Tet	Cxc
AB – 002	*Staph sp*	26	20	32	38	32	25	Re	41
AB – 023	*Staph sp*	26	32	30	30	31	22	20	30
AB – 024	*Staph sp*	40	41	42	38	36	34	30	35
AB – 028	*Staph*	25	31	35	30	35	21	9	Re
AB – 033	*Staph sp*	41	34	38	38	36	34	30	36
% sensitivity		80	80	100	100	100	40	40	80
Mean Zone of Inhibition (mm)		33	34.5	35.4	34.8	34	34	30	35.5

DISCUSSION

The finding that group B *Streptococcus* was the prominent organism with a prevalence of 9.23% showed that this organism is common in the environment and could also be an indication of acquired maternal infection. Colonization of the neonate by maternal infection usually occurs after rupture of the maternal membranes (Klein, 2001). In most cases, the infant is colonized with the microflora of the birth canal during delivery. Vaginal bacteria may ascend and in some cases produce inflammation of the fetal membranes, umbilical cord, and placenta (Bernirschke, 1960). The organisms most commonly isolated from infected amniotic fluid are anaerobic bacteria, group B streptococci, *Escherichia coli*, and genital mycoplasmas (Gibbs and Duff, 1991). Infection of the mother at the time of birth, particularly genital infection, is the principal pathway of maternal transmission (Prober, 1997) and can play an important role in the development of infection in the neonate (Chiesa, *et al*, 2004). Clinical trials have demonstrated the effectiveness of intrapartum antibiotics to reduce early-onset GBS disease in neonates (Allen *et al*, 1993).

The predominance of group B *Streptococcus* in neonatal sepsis have been documented by various workers (Vesikari, *et al*, 1985, Lyytikäinen *et al*., 2003, Lukacs *et al,* 2004) This result differ from that of Mokuolu *et al* (2002) who established that *Staphylococcus* was the predominant organism in their finding in a research based Ilorin in which no group B *Streptococcus sp* was isolated. Roy, *et a*l (2002) also reported in their work that coagulase negative staphylococci (CONS) were more frequently isolated (16.5 percent) than *Staphylococcus aureus* (14 percent). In Trotman and Bell (2006) also reported the incidence of *Staphylococcus aureus*, coagulase negative *Staphylococcus*, Group B *Streptococcus* as well as Group D *Streptococcus* from neonates in Kingston the capital city of Jamaica. In their findings, Group B *Streptococcus* was the predominant organism isolated. The prevalence of gram positive cocci bacteria in this study was 24.62%. This was lower than that found in a retrospective study was conducted at the newborn unit in Kenyatta National Hospital in Nairobi, Kenya where gram positive organisms accounted for 33.4% of the organisms associated with neonatal sepsis (Simiyu, 2005).

Streptococcus sp. accounted for the prevalent gram positive cocci isolated from neonates in the period of the study with a prevalence of 14.5%. *Staphylococcus* was in next with a prevalence of about 11% though pathogenic *Staph* accounted for only 4.8% and the rest by *Staph epidermidis.*

Resistance was quite low for the penicillin giving the hope that these organism are still sensitive to cheap and easily available drugs. This study found that 60% of the *Staphylococcus aureus* isolates were sensitive to Chloramphenicol, augmentin and amoxicillin. These are broad spectrum antibiotics commonly employed in clinical services. Sensitivity to the sulphonamides was 40% while most isolates were resistant to gentamicin, a member of the aminoglycoside. *Staphylococcus aureus* isolate AB-010 was resistant to all the antibiotic except chloramphenicol in which the zone of inhibition was only 26mm. AB-061 was resistant to all of the antibiotics. CONS were more sensitive to the antibiotics used, with 100% sensitivity to Chloramphenicol, augmentin and amoxicillin. 80% of the CONS were sensitive to gentamicin, cotrimazole and cloxacillin while only 40% were sensitive to erythromycin and tetracycline. The *Streptococcus* species were sensitive to most of the antibiotics with a sensitivity of over 60% except for tetracycline to which 50% were sensitive. All the isolates were sensitive to gentamicin while 83.33% were sensitive to chloramphenicol.

A report based in Northern India indicated a resistance of *Staphylococcus aureus* to most of the antibiotics ranging from 42.8% for tetracycline to 95.9% for penicillin. In the same report, resistance of CONS to the same antibiotics varied from 43.1% for erythromycin to 89.6% for penicillin, showing a high resistance to the B-lactams (Roy *et al*, 2002). In this report, however, only 40% of the *Staphylococcus aureus* isolates were resistant to amoxicillin, a member of the B- lactamases to which all the CONS were sensitive. Mokuolu *et al* (2002) reported that

Gram positive organisms in their findings had 100% sensitivity to Azythromcin, 85% to ampicillin-sulbactam, 63% to ceftazidime and 62.5% to gentamicin. Though our findings agree with the sensitivity to amoxicillin with a resistance of 40%, the sensitivity to gentamicin was lower than that reported by them among the *Staphylococcus aureus* isolates while the CONS were 100% sensitive to gentamicin. However all the *Streptococcus* species isolated were sensitive to gentamicin. Another report based in Ethiopia showed a high resistance to the penicillins among the gram positive organisms (Gebreselassie, 2002). According to Gebreselassie (2002), the resistance patterns of *S. aureus*, CONS, *S. pneumoniae* and enterococci to penicillin was 91.5%, 94.4%, 7.7% and 100% respectively. Penicillin, ampicillin and cloxacillin showed low effects (< 60%) in their findings on both *S. aureus* and CONS. Multi-drug resistance was observed in all the gram-positive isolates, especially higher in staphylococcus species. All isolates of *S. aureus* (100%) were susceptible to vancomycin, clindamycin and gentamicin.

This result showed that multi-drug resistance may not be as high as often reported in neonatal septicaemia tough most cases of multi-drug resistance were reported in isolates from adults that could be as a result of abuse of antibiotics (Gebreselassie 2002). The sensitivity profile revealed that most of the isolates were sensitive to the common antimicrobials in use.

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