JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2007. Vol. **1**(2), p. 191-196

Characterization and Antimicrobial Sensitivity Assay of Gram Negative rods isolated from Neonates with Septicaemia in Zaria

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(Received: 18 August 2007; accepted: 27 September 2007)

A total of sixty five neonates suspected to be septicaemia were sampled. Nine Gram negative organisms were isolated out of the 40 samples with positive blood culture accounting for 22.5% of the bacterial organisms responsible for neonatal septicaemia within the period of study. Of this number, the most prevalent was Enterobacter cloacae, *Enterobacter cloacae* accounting for 4 out of a total of 40 patients (10%) and 44.44% of the total Gram negative organisms. *Klebsiella pneumoniae* was next with an incidence of 7.5% which represents 33.33% of the gram negative organisms. *Citrobacter freundii* and *Escherichia coli* had incidence of 2.5% each constituting 11.11% of the total gram negative organism respectively. Incidence was higher in males 55.56% while in females it was 44.44%. The overall sensitivity percentage to ampicillin and gentamicin was 77.78 %, followed by nitrofurantoin , streptomycin and tetracycline which was 66.67%. the percentage sensitivity to colistin was 55.56% while sensitivity to cotrimazole and nalixidic acid was 44.44% respectively. All the *Enterobacter* isolates were sensitive to ampicillin but one isolate showed multiple resistance to the other antibiotic. the E coli strain also showed evidence of multiple resistance, being resistant to the ampicillin and gentamicin and streptomycin. It was however sensitive to tetracycline and nitrofurantoin.

Keywords: Antimicrogram, Antibiotics, Sensitivity, Septicaemia.

The health need of the newborn has come to limelight as they lack the ability to produce their own immunity, often needing the protection from their mother.

Throughout pregnancy and until the membranes rupture, the fetus is relatively protected from the microbial flora of the mother by the chorioamniotic membranes, the placenta, and poorly understood antibacterial factors in amniotic fluid (Chiesa*, et al,* 2003). Initial colonization of the neonate usually takes place after rupture of the maternal membranes (Chiesa*, et al,* 2003).). In most cases, the infant is colonized with the microflora of the birth canal during delivery. However, particularly if the rupture of membranes lasts longer than 24 h, vaginal bacteria may ascend and in some cases produce inflammation of the fetal membranes, umbilical cord, and placenta (Chiesa*, et al,* 2003).

In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, septicaemia continues to be a major cause of mortality and morbidity among neonates around the world (Roy *et al*., 2002). Bacterial sepsis remains the leading

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cause of neonatal mortality in the United States, affecting up to 32,000 live births annually (Lukacs, *et al,* 2004).

Neonatal bacterial sepsis (NBS) remains as an important cause of mortality and morbidity among infants. Its incidence varies with geographical area and may change in the same area with time. NBS has been classified as either early onset (0-7 d of age) or late onset (7-28 d of age) (Ali, 2004). The incidence of NBS varies from 0.3 to 3 / 1000 live birth (LB) in Europe (Vesikari, *et al*, 1985), 1 to 4/1000 LB in North America (Fisher *et al*, 1983), 1.4 /1000 LB in a hospital study in Jamaica (MacFarlane, 1987), 8.9 /1000 LB in Guadeloupe (Robillard *et al*, 1993) and 10/ 1000 LB at the San Fernando general hospital in South Trinidad (Orrett and Shurland, 2001).

In most of developing countries, gram-negative bacilli remain the major cause of neonatal sepsis (Haque, (2001). An incidence of 7.04/1000 for in-born patients was recorded in Ilorin, Nigeria (Mokuolu, *et al*, 2002). .These organisms have developed increased drug resistance over the last two decades (Haque, 2001, Joshi, *et al,* 2000, Movahedian, *et al,* 2006) and management of patients is becoming a major problem.

This present study was undertaken to survey the gram negative organisms causing sepsis in a tertiary hospital in Zaria as well as the sensitivity pattern to locally available antibiotics. The patients included for the study were those diagnosed as having sepsis by the pediatrician.

MATERIAL AND METHODS

The study cover neonates (age 0 – 28 days) admitted into the intensive care baby unit of the hospital with suspected case of septicaemia. Ethical permit was obtained from the hospital ethical board as well as consent from the patients. One ml of blood was obtained by venous puncture from the neonates aseptically using a 2ml needle with a 22 gauge needle and dispensed immediately into a sterile medical bottle containing 20mls of Brain heart infusion broth overlaid on nutrient agar slant according to modified system of Castaneda (Baker *et al.,* 2001). These were incubated at 37^0C for 1-4 days and monitored for growth daily as evidenced by growth on the surface of the nutrient agar slant as well as turbidity of the broth. Bottles with evidence of growth were sub-cultured unto plates of Blood agar, purified by further sub-cultured. Gram stain was employed to determine the Gram's reaction and the target organisms (i.e. Gram negative rods) were transferred to slants for further identification

Bacterial identification

A series of biochemical test was employed to identify the organisms. These include IMVIC (Indole, Methyl Red, Voges Praskauer, and Citrate), Urease, Tryptophan deaminase, Phenylalanine deaminase, Motility, H_2S production and a battery of sugar fermentation as well as reaction on KIA slants (Cheesbrough, 1987)..

Antibiotic sensitivity testing

The Kirby Bauer method was used for the sensitivity assay**.** This was done using the multi-disc diffusion method (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 prepared for gram negative organisms, incorporated with the antibiotics were then aseptically placed on the surfaces and the plates incubated for 24 hours at 37^0C. The results were read and sensitivity determined. The antibiotics used include Ampicillin, Cotrimazole, Gentamicin, Nalicidic Acid, Nitrofurantoin, Colistin, Streptomycin and Tetracycline

RESULTS

A total of 65 patients were sampled for this study. Out of this number, 40 (61.54%) had evidence of bacterial growth while 25 (38.46%) yielded no growth by plate isolation. The incidence of Gram negative organisms isolated from neonates with suspected septicaemia was 22.5% (9 out of 40) within the period of study. The biochemical reactions of the isolates as well as their fermentative abilities on a number of sugars are presented in tables 1 and 2. These reactions formed the basis for their identification. The remaining organisms, i.e. 31 were Gram positive cocci or bacilli and excluded in further study. Of the Gram negative organisms, the organism with the highest incidence was *Enterobacter cloacae* accounting for 4 out of a total of 40 patients (10%) and 44.44% of the total Gram negative organisms. *Klebsiella*

Table 1. Biochemical test on isolated organisms

Sample No	Indole	MR	VP	Citrate	Motility	Urease	Try	Phe	S/F	Inference
AB – 014	-		-	+	+	+	+	-	-	K/A *Enterobacter*
AB – 011	-	-	+	+	+	+	-	-	K/A	*Enterobacter*
AB – 041	-	-	+	+	+	-	-	-+	K/A	*Enterobacter*
AB – 006	-	+	-	+	-	-	-	-	K/A	*Klebsiella*
AB – 059	-	-	+	+	+	+	-	-	K/A	*Enterobacter*
AB – 027	-	-	+	+	-	+	-	-	K/A	*Klebsiella*
AB – 037	-	+	-	+	+	+	-	+	-	K/AGH_2S *Citrobacter*
AB – 062	-	-	-	+	+	-	-+	-	K/A	*Klebsiella*
AB – 030	+	+	-	-	+	-	+	-	K/A G	*Escherichia coli*

MR: Methyl red VP: Voges prausker Try: Tryptophan Phe: Phenylalanine S/F: Sugar fermentation

Table 2. Sugar Oxidation and Fermentation Reaction of Isolates

Sample No.	Glu OF	Suc OF	Fru OF	Sor OF	Lac OF	Sob OF	Mal OF	Mann OF	Xyl OF	Ram OF	Ara OF	Ino OF	Raf OF
AB – 014	+ -	+ +	- -	- -	+ +	- -	+ +	- -	+ +	+ +	- -	+ +	+ -
AB – 011	- -	- -	- -	- -	+ +	- -	+ +	- -	- -	- -	- -	+ +	+ +
AB – 041	+ +	- -	- -	+ -	+ +	- -	+ +	- -	- -	- -	- -	- -	+ -
AB – 005	+ +	+ +	+ +	+ +	- +	+ +	- -	+ +	+ +	+ +	+ +	+ +	+ +
AB – 059	- -	- -	- -	- -	+ +	- -	+ +	- -	- -	- -	- -	- -	- -
AB – 027	- -	- -	- -	- -	+ +	- -	+ +	- -	+ -	+ -	+ -	+ -	+ -
AB – 037	+ +	+ -	- +	+ -	+ +	- -	- -	- -+	- -	+ +	+ +	+ +	+ +
AB – 062	- -	- -	- -	- -	+ +	- -	+ +	- -	+ -	- -	- -	- -	- -
AB – 030	- -	- -	- -	- -	+ +	- -	+ +	- -	+ -	+ -	-+ -	- -	-+ -

Glu –Glucose Suc. Sucrose Fru. Fructose Sor. Sorbose Lac. Lactose Sob. Sobitol Mal. Maltose Mann. Mannitol Xyl. Xylose Ram. Ramnose Ara. Arabinose Ino. Inositol Raf. Raffinose O. Oxidation F. Fermentation

pneumoniae was next with an incidence of 7.5% which represents 33.33% of the gram negative organisms. *Citrobacter freundii* and *E. coli* had incidence of 2.5% each constituting 11.11% of the total gram negative organism respectively. This is presented in Table 3.

In relation to sexes, the male patients were more, 55.56% and the females, 44.44%. This is presented in Table 4. Gram negative organisms present themselves from day four (4) upwards. No case was isolated from neonates younger than four days old.

The antibiotic sensitivity pattern showed that all the *Enterobacter cloacae* strains were sensitive to most of the antibiotics. However, most were resistant to Nalizidic acid except strain AB – 011. *Klebsiella* strain AB – 027 exhibited multiple resistance to most of the antibiotics while the other two strains were sensitive to most of the antibiotics. The *E. coli* strain AB – 030 as well as the *Citrobacter freundii* strain AB – 037 exhibited multiple resistances to most of the antibiotics used. The result is presented in Table 5. The overall sensitivity percentage to ampicillin and gentamicin was 77.78 %, followed by ntrofurantoin , streptomycin and tetracycline which was 66.67%. the percentage sensitivity to colistin was 55.56% while sensitivity to cotrimazole and nalixidic acid was 44.44% respectively. This result are presented in Table 6.

Table 3. Distribution of isolates according to sex and age

Isolate code	Organisms Isolated	Sex	Age
AB – 014	*Enterobacter cloacae*	Female	4
AB – 011	*Enterobacter cloacae*	Male	4
AB – 041	*Enterobacter cloacae*	Female	4
AB – 006	*Klebsiella pneumoniae*	Male	7
AB – 059	*Enterobacter cloacae s*	Female	14
AB – 027	*Klebsiella pneumoniae*	Male	8
AB – 037	*Citrobacter freundii*	Male	6
AB – 062	*Klebsiella pneumoniae*	Female	6
AB – 030	*Escherichia coli*	Male	5

Table 4. Summary of Isolates

Oganism	No of isolates	% of total positive isolates	% of Gram negative samples
Enterobacter cloacae	4	10	44.44
Klebsiella pneumoniae	3	7.5	33.33
Citrobacter freundii	1	2.5	11.11
Escherichia coli	1	2.5	11.11

Table 5. Sensitivity results with zones in millimeters

Sample No	Amp	Cot	Gen	Nal	Nit	Col	Strep	Tet
Enterobacter cloacae	36	RE	26	RE	30	18	35	23
Enterobacter cloacae	37	34	28	30	27	13	26	24
Enterobacter cloacae	20	RE	RE	RE	RE	15	RE	RE
Klebsiella pneumoniae	12	22	18	26	18	RE	16	20
Enterobacter cloacae s	30	32	21	RE	RE	RE	10	19
Klebsiella pneumoniae	10	RE	11	RE	RE	RE	RE	RE
Citrobacter freundii	RE	RE	20	20	11	RE	15	RE
Klebsiella pneumoniae	30	32	28	24	24	15	28	30
Escherichia coli	RE	RE	RE	RE	24	15	RE	29

Table 6. Percentage Sensitivity to various antibiotics

Sample No	Amp	Cot	Gen	Nal	Nit	Col	Strep	Tet
No Sensitive	7	4	7	4	6	5	6	6
Percentage	77.78	44.44	77.78	44.44	66.67	55.56	66.67	66.67
No Resistant	2	5	2	5	3	4	3	3
Percentage	22.22	55.56	22.22	55.56	77.77	44.44	33.33	33.33

Amp. Ampicillin Cot. Cotrimazole Gen. Gentamicin Nal. Nalicidic Acid Nit. Nitrofurantoin
Col. Colistin Strep. Streptomycin Tet. Tetracycline

DISCUSSIONS

Bacterial sepsis contributes to prolonged hospitalization, additional hospital costs, and increased neonatal mortality (Stoll *et al*., 1996). Reported sepsis case fatality rates range from <under 10% to >50% for neonates and infants in the United States; the highest rates are for neonates with early-onset disease (i.e., illness onset during the first week of life) (Klien and Marcy, 1995) The survival of smaller, more immature infants has resulted in a larger cohort of preterm neonates and infants who are at greatest risk for bacterial sepsis. Gram negative organisms was isolated from nine (9) of the forty samples that yielded positive results by blood culture representing 22.5 % of organisms causing sepsis in neonates. The causative organisms in our study were similar to findings from India, though the incidences and percentages differ slightly, where Gram negative organisms were responsible for 67.2% of their cases. *Pseudomonas aeruginosa* was the most common organism (38.3%), followed by *Klebsiella* spp. (30.4%) and *Escherichia coli* (15.6%) (Joshi, *et al*., 2000). The result showed that *Enterobacter cloacae* had the highest occurrence accounting for 4 of the nine organisms isolated representing 44.44% of the total Gram negative organisms. *Enterobacter spp* was next with percentage isolation of 3.08%. Most of the isolates belong to the family enterobacteriaceae, often responsible for the enteric fever. The incidence of *Enterobacter spp* in neonatal sepsis has been documented by many scholars. Ray *et al.,* (2002) in their work, found that most frequent offender in neonatal sepsis were *Klebsiella spp*. (24.5 percent) and *Enterobacter spp* (22.8 percent). In this present study, *Enterobacter spp* was prominent with an incidence rate of 10% while *Klebsiella* was 7.5% lower than figures reported by Ray *et al*., (2002). Sharma *et al* (2002) also reported the incidence of bacteraemia to be 33.9% in neonates and Gram negative organisms (88.8%) isolating *Klebsiella, Salmonella* and *Pseudomonas.*

Mahapatra *et al.,* (2002) reported the isolation of *Enterobacter cloacae* was in 39.5% followed by *Klebsiella pneumoniae* in 23.2%, *E. coli* in 11.6%, *Citrobacter freundii* 4.6%, and *P.mirabilis* 2.3%. The findings in this study established 2.5% for both *Citrobacter freundii* and *Escherichia coli* showing that *Citrobacter freundii* and *E. coli* both have low incidence in neonatal sepsis. These organisms are colon organisms often transmitted via the use of contaminated water and feeding utensils.

The antibiotic sensitivity pattern revealed varying degree of sensitivity to the conventional antibiotics with prominent zones of inhibition. The percentage sensitivity to the various antibiotics showed that of the Gram negative organisms assayed, percentage sensitivity to ampicillin and gentamicin was 77.78%. These findings were not compatible with other studies (Joshi *et al*., 2000, Koksal *et al*., 2001). On the contrary studies from Sydney Neonatal Infection Surveillance (Levine *et al*., 1999) have mentioned that all the Gram negative organisms were susceptible to gentamicin and third generation cephalosporins. In another study in Ilorin, Mokuolu *et al.*, (2002) showed that 94% of the organisms were sensitive to azythromicin, 77.8% to streptomycin, 73.3% to gentamicin and 69.2% to ampicillin-sulbactam.

All the *Enterobacter* isolates were sensitive to ampicillin but one isolate showed multiple resistance to the other antibiotics. *Klebsiella spp* were moderately sensitive to ampicillin and gentamicin. One of the strains exhibited multiple resistance. The *E. coli* showed multiple resistances being resistant to the ampicillin and gentamicin and streptomycin. It was however sensitive to tetracycline and nitrofurantoin.

This study revealed that the conventional drug are still very effective and explored in place of more costly ones which should be reserved for chronic cases Positive blood culture remains the gold standard in the diagnosis of neonatal sepsis. However, in the absence of proof of sepsis many clinicians and neonatologists felt obliged to continue antibiotics for a full course (Bhutta, *et al.,* 1991). This is usually done in good faith to save the life of the patient. In most cases, the laboratory result takes two to three days before a conclusive result can be issued. Saving the life of the child becomes necessary and therapy is often started. If, however, the infant is not infected he or she is being subjected to unnecessary treatment. There is also danger of removing useful or susceptible organisms and encouraging resistant

ones. If this occurs we shall reach a stage to go on to use more expensive antibiotics, or we have no more chance to use new drug combinations.

ACKNOWLEDGEMENTS

I wish to acknowledge the assistance and support by the ethical committee of the Ahmadu Bello University Teaching Hospital Zaria and the Staff of the Special care Baby Unit. Similarly I acknowledge the lecturers, technologist of the Dept. of Microbiology, Ahmadu Bello University Zaria.

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