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Research Article

Horizontal Transmission of Multidrug Resistant Gram Negative Blood Borne Infections in A Tertiary Hospital Obstetric Wards in South West Nigeria

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Abstract: The thrust of this study is on newborn health with the objective of determining the percentage horizontal transmission of blood borne Gram negative infections to neonates within the first 7 days of postnatal life, in the obstetric wards of the hospital. It is with a view to assessing the degree of compliance with the prevailing infection control protocols. This was a prospective study, carried out among pregnant women in labor and their newborn babies suspected of sepsis within 7 days of delivery. HVS were collected from the women in first stage of labor and cultured. Isolates of *E.coli* and *K. pneumoniae* were used as surrogates for vagina colonizing Gram negative species. Identification to species was by *Microbac*t identification kits. Antibiogram of blood isolates of babies suspected of sepsis was determined using Kirby Bauer disk diffusion method. The plasmid DNA Profiles of both maternal vaginal and newborn blood isolates were determined by PCR. Mother and newborn plasmid DNA profiles were compared to identify horizontally transmitted early onset neonatal sepsis. Fifty seven (16.3%, n=350) babies were suspected of sepsis. Twelve (21.0%, n=57) were culture positive for Gram negative bacilli. *Klebsiella pneumoniae* 4 (7.0%) and E.coli 4 (7.0%) were the predominant isolates. The antibiogram was characterized by widespread resistance. *Klebsiella pneumoniae* 4(7.0%) was the dominant ESBL producer. The percentage horizontal transmission of multidrug resistant Gram negative neonatal sepsis was 75%. There is need for re-appraisal of infection control protocols in the wards and enforcement of compliance on all stakeholders.

Keywords: Transmission, Gram Negative, Multidrug-resistance, Blood Borne Infections, Wards, Lagos.

INTRODUCTION

There have been increasing incidence of Gram negative organisms over GBS in the etiology of early onset neonatal sepsis following the introduction of the intra-partum antibiotic prophylaxis against group B Streptococcus (GBS) (CDC, 1996). It has also been established that whereas Gram positive organisms were the major culprits in the etiology of neonatal sepsis in developed countries, the reverse was the case in developing world, including Nigeria where Gram negative organisms were the major isolates with *Enterobacteriaceae* predominating (Plazek & Whitelaw, 1983). A study in India showed that multi drug resistant Gram negative bacilli were major causes of early and late onset neonatal sepsis and that these organisms were well distributed in the community (Viswanathan *et al.*, 2012). A related study in Tanzania reported high neonatal morbidity and mortality resulting from culture positive blood borne infections caused by multidrug resistant Gram negative bacteria (Kayange, Kamugisha, Mwizamholya, Jeremiah, & Mshana, 2010).

Most Gram negative bacteria particularly species of the *Enterobacteriaceae* family were known to elaborate extended spectrum β-lactamases (ESBL) a

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group of enzymes that conferred resistance to third generation cephalosporins and aztreonam but not carbapenems and they are inhibited by β –lactamases inhibitors such as clavulanic acid (Olowe & Aboderin, 2010). Organisms elaborating ESBL were identified to coexist with multidrug resistance to other classes of antibiotics such as gentamicin, ampicillin (Viswanathan *et al.,* 2012) cotrimoxazole, tetracyclines and fluoroquinolones (Morosini *et al.,* 2006). This development is worrisome, considering that ampicillin – gentamicin or gentamicin-cefotaxime combinations are readily the empirical antibiotic regimen for the treatment of suspected bacterial neonatal blood borne infections and or meningitis. Antimicrobial options for the treatment of infections caused by organisms elaborating ESBL was limited to few expensive antibiotics, not readily available in developing countries and not affordable to many patients even when found. Treatment outcome was poor and characterized by increasing disease burden (Blomberg *et al.,* 2005), prolonged hospital stay, increase cost of treatment, loss of man-hours at work and high mortality (Schwaber & Carmeli, 2007) due to high rate of treatment failure.

Portal of entry of these organisms within the time thrust of this study was either vertical (mother to newborn) or horizontal (exogenously through early birth intervention procedures such as vaginal examinations, forceps delivery, intravenous line /parenteral agents administration to the newborn, cleaning and suctioning of baby etc.).

Maternal, newborn and child health (MNCH) was of immense concern in global development agenda. Poor MNCH was yet a significant feature in many low and middle income countries including Nigeria. This posed an impedance to the realization of the Sustainable Development Goal (SDGs). Addressing MNCH was contributory to poverty reduction, economic growth and productivity and more stable societies (Singh, Darroch, Ashford, & Vlassof, 2009). On the strength of the aforementioned, the thrust of this study was focused on newborn health with the objective to determine the percentage horizontal transmission of blood borne Gram negative infections to neonates within the first 7 days of postnatal life, in the obstetric wards of the Lagos University Teaching Hospital, Lagos, Nigeria. It was with a view to assessing the degree of compliance with the prevailing infection control protocols in the wards and recommending where need be, appropriate remedial measures.

The Specific Objectives Of The Study Were To Determine.

- The antibiogram of Gram negative isolates from the newborn babies who showed symptoms and signs of sepsis within the first 7 days of postnatal life.
- The percentage ESBL- production of the Gram negative blood isolates from the newborn babies with sepsis within first 7 days of postnatal life.
- The plasmid genes array (^{bla}TEM, ^{bla}SHV, ^{bla}CTX-M and ^{bla}OXA) of maternal vagina isolates and the Gram negative blood isolates from their respective newborn babies who had sepsis within the first 7 days of postnatal life.

MATERIALS AND METHODS

Study Design

This was a prospective study, carried out among pregnant women in labor and their newborn babies who developed symptoms and signs of sepsis within 7 days of delivery. The study was carried out in the obstetric wards of the Lagos University Teaching hospital (LUTH), Lagos January 2014 and May 2015, having received written approval from the LUTH Health Research Ethics committee (HREC).

High vaginal swabs were collected from the pregnant women in first stage of labor and cultured. Isolates of *E.coli* and *K. pneumoniae* were identified and documented and used as surrogates for species of *Enterobacteriaceae* colonizers. The newborn babies were monitored for symptoms and signs of sepsis for 7 days from the time of delivery. Newborn babies discharged from the hospital before the expiration of this period were monitored by phone contact with their mothers who were earlier educated on symptoms of sepsis. A newborn was asked to be brought back to hospital for review once a mother reported any symptom of sepsis. Blood culture was carried out on all newborn babies suspected of sepsis and all Gram negative isolates were documented and their antibiogram determined. The plasmid genes profile of the maternal vagina colonizing and the blood isolates of their respective newborn babies were determined using a thermocycler (A&E Laboratories UK model cyl- 005-1) with a view to comparing the 2 and finding out the similarities and differences in gene distribution. Structured questionnaires were used to collect socio-demographic data from the mothers.

Inclusion / Exclusion Criteria

Enlisted into the study were pregnant women of gestational ages ≥28 weeks who gave consent who had no fever through the period of pregnancy or had fever that responded to antimalarial treatment. Excluded from the study were pregnant women who had preterm labor below 28 weeks gestation or those ≥28 weeks gestation who had fever during the period of pregnancy that never responded to antimalarial treatment. Women who refused consent and or delivered by Caesarean section were also excluded alongside with their babies.

Study Area

This work was carried out in LUTH, a 761 bedded tertiary hospital (Wikipedia, 2018) located in the metropolitan city of Lagos, South West Nigeria.

Lagos covered a land mass of 3,577 sq.km and had a population of 9,113,605 (Wikipedia, 2018). The hospital is patronized by inhabitants of Lagos and adjoining states as well occasional referrals from bordering countries.

Study Population

Participants in this study were pregnant women of gestational ages ≥28 weeks who were in labour at the Lagos University Teaching Hospital, Lagos between January 2014 and May, 2015 and their newborn babies who had symptoms and signs of sepsis within 7 day of postnatal life.

Sample Size

Sample size was calculated based on this formula (Daniel & Cross, 2013) : $N = Z^2 pq/d^2$, where N = sample size, p = 20.8% (the local ESBL-PE prevalence (Aibinu, Odugbemi, & Mee, 2003), Z = critical value at 95% confidence level, set at 1.96 and q = 1-p, d = precision, at 5%. When calculated, N = 253, however, a sample size of 350 was used to make up for non-response cases.

Sample Collection and Transport

High Vaginal Swabs (HVS)

High vaginal swab was collected by first cleaning the vagina orifice with sterile sanitary pad. The vagina walls were then parted with sterile speculum and the posterior vagina wall swabbed with sterile swab stick. Sample was labeled and sent to the special pathogens laboratory of the hospital for immediate processing.

Blood Culture Samples

A blood culture samples were aseptically collected from newborn babies who showed symptoms and signs of sepsis within the first days of postnatal life and inoculated into BACTEC PEDS/F pediatric culture bottle, mixed, labeled and taken to the automated BACTEC incubator room for culture.

Samples Processing

All samples were processed in level 2 biosafety cabinet. Maternal HVS samples were inoculated on MacConkey agar plates and labeled. Time of commencement of incubation was indicated on the plates and thereafter they were incubated for 18 hours at 37^0C in ambient air. Based on morphological characteristics and Gram stain reactions, suspected colonies of E. coli and *K. pneumoniae* were sub-cultured on nutrient agar and re-incubated at37^OC for 18 hours. This was meant to provide discrete growths of these colonies on non- inhibitory media for Microbact identification procedures, other biochemical identification processes and antibiotic sensitivity testing.

Inoculated BACTEC PEDS/F pediatric culture bottles were incubated aerobically at 37^0c using BD BACTEC 9050 incubator (Becton Dickinson Inc. New Jersey, USA). Incubated samples were monitored daily for growth for a maximum period of 5 days. Those that flagged positive were manually sub-cultured on MacConkey, chocolate and blood agar plates at 37^OC for 18 hours. Isolates from HVS and blood culture were Gram stained and only Gram negative organisms were processed further. Identification of isolates to specie level was by use of microbact™ biochemical identification kits (Oxoid ltd, Basingstoke Hants, UK).

Identification of isolates using microbact™ biochemical identification kits

Oxidase test was done on all Gram negative isolates from maternal HVS and newborn blood isolates. The 12A and 24E microbact test strips were used to process oxidase negative and positive groups of organisms respectively. Suspension of the isolates and controls were made to match 0.5% Mcfarland standard and 4 drops of each suspension were dispensed into each test well and tilted 2 times to mix. Wells 1(Lysine), 2 (Ornithine) and 3 (H_2S) on the 12A test strip and well 24 (Arginine) on the 24E test strip were then overlaid with mineral oil to create anaerobiosis. The inoculated strip(s) were incubated for 18hours at 37^OC in ambient air.

At The End of Incubation:

In test strip 12A: 2 drops of indole reagent was added into well 8 and colour change read within 2minutes, a drop each of VPi and VPii were added to well 10 and colour change read within 15 – 30 minutes, a drop of TDA was added to well 12 and colour change read immediately.

In test strip 24E: changes in well 13 (gelatin) was read after 24hours. Hydrolysis of the gelatin was indicated by the dispersal of black particles throughout the well. The 24E test strip, was allowed to incubate for up to 48hours to allow for identification of denitrifying Gram negative (glucose non fermenters) bacteria. The color reaction in each well was read as either negative or positive with assigned numerical values using the Microbact color chart. The numerical values for each isolate were summed up into a code which was keyed into the microbact computer aided package to give the identity of the isolate.

Antibiotic susceptibility test

Antibiotic susceptibility testing was by the Kirby Bauer disk diffusion technique according to Clinical Laboratory Standard Institute (CLSI) guidelines ("Clinical Laboratory Standard Institute. Performance Standard for Antimicrobial Disc Susceptibility Test; Approved Standard- Tenth edition.," 2009). It was performed only on blood isolates from newborn babies with sepsis. The following antibiotics were used, namely Amoxicillin/

clavulanate (20+10µg) Ceftazidime (30µg), Cefotaxime (30µg), Cefepime (30µg), Cefoxitin (30µg), Levofloxacillin (5µg), Piperacillin/tazobactam (110µg), Aztreonam (30µg),Gentamicin (10µg), Meropenem (10µg), Imipenem (10µg) and Ertapenem (10µg). *Enterobacteriaceae* species or miscellaneous Gram negative bacteria with zones of inhibition to ceftazidime or cefotaxime or both less than 22mm and 27mm respectively were suspected to be ESBL- producing . Isolates so suspected were then screened by the double disks synergy technique and confirmed by the combined disks method according to CLSI criteria.

Phenotypic combined disks ESBL confirmatory test

Used as controls were E.coli ATCC 35218 (positive) and E.coli ATCC 25922 (negative) ESBL controls. Suspensions of isolate and controls were made to match 0.5% Macfaland standard and inoculated on separate Mueller hinton agar plates to make a thin lawn. Paired antibiotic disks of (i) single cefotaxime and combined cefotaxime/clavulanate disks and (ii) single ceftazidime and combined ceftazidime/clavaulanate disks were implanted on the on the separate plates, 30mm apart. They were incubated at 35ºC in ambient air for 18 hours. Following incubation, zones of inhibition around a single disk and its respective combination were measured and a difference in zone diameter of ≥5mm was confirmatory for ESBL-production.

PCR Plasmid genes amplifications of maternal vagina isolates and the blood isolates of the respective newborn babies with sepsis

PCR amplifications for ^{bla}TEM, ^{bla}SHV , ^{bla}CTX-M and ^{bla} OXA genes was performed on a thermocycler (A&E Laboratories UK model cyl- 005-1) using primer pairs (Table1).The reaction volume of 25µl was used consisting of 10X PCR buffer, 10mM $MgCl_2$, 10 mM dNTP's mixture, 5 U/µL of Taq DNA polymerase (Fermentas, USA), 10pmol of each primer set and 5ng of extracted plasmid DNA from samples, negative (*E.coli ATCC25922*) and positive (*ATCC 35218)* controls. Amplifications was done following an initial denaturation at 96^{O}C for 5minutes, 35 cycles at 96^{O}C for 60S, 60^{O}C for 60S (SHV), 58^{O}C for 60S (TEM& OXA) and 50^{O}C for 6Os (CTX-M), followed by 72^{O}C for 60S and a final period of extension at 72^{O}C for 10 minutes.

Plasmid DNA electrophoresis

Amplified gene products (10µL) along with control were separated using 1.5% agarose gel electrophoresis in TAE buffer (40mM Tris -acetate buffer 2mM EDTA [p^{H} 8.3]) performed at 70V for 1.5 hours. Gel was stained with 0.5µg/ml of ethidium bromide for 45 minutes and destained with water for 20 minutes.

Stained gel was examined under ultraviolet (UV) light transilluminator (Clinix Japan, model 1570) in a photodocumentary system. Major bands corresponding to the major band sizes were considered in the analysis. A DNA ladder digest of 1kilobase pair (Fermentas USA) was used as molecular weight marker (Figure 1)

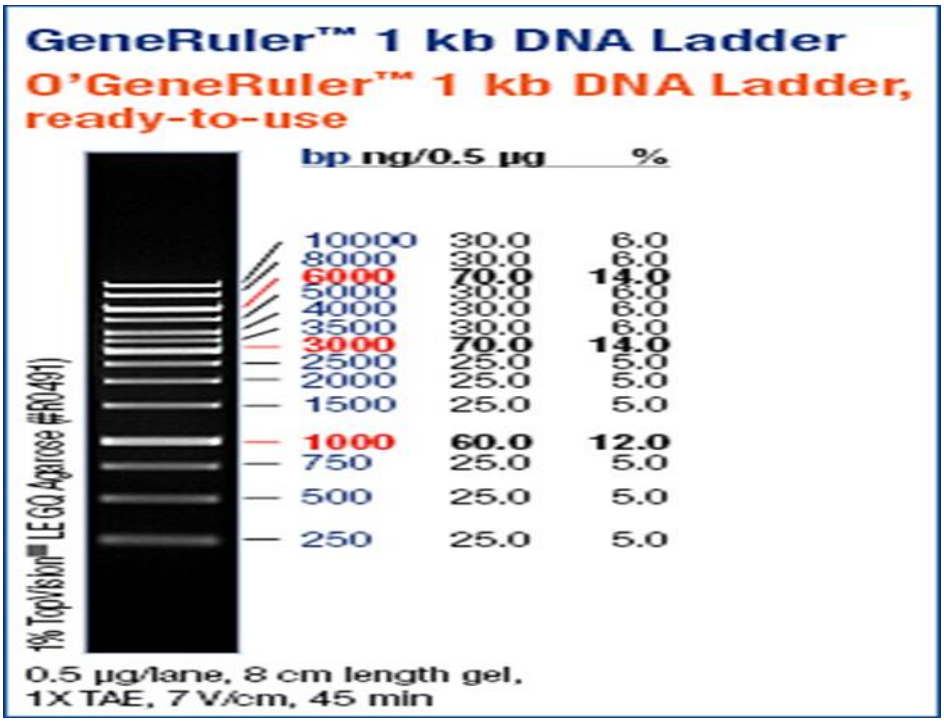

Figure 1. DNA ladder digest of 1kbp (Fermentas USA)

TABLE 1. Primers used for the study (Ogbolu *et al.*, 2013)**.**

Primers	Oligonucleotide sequence (5' to 3')		Expected size (bp)
TEM-F	ATGAGTATTCAACATTTCCG		
TEM-R	CTGACAGTTACCAATGCTTA		517
SHV-F SHV-R	GGTTATGCGTTATATTCGCC TTAGCGTTGCCAGTGCTC		393
CTX-M1	ATGTGCAGYACCAGTAARGT		585
CTX-M2 OXA OXA	TGGGTRAARTARGTSACCAGA ATATCTCTACTGTTGCATCTCC AAACCCTTCAAACCATCC		620

Determination of Relationship

A blood born infection is considered to be transmitted from mother to her newborn baby if on amplification and electrophoresis, the plasmid DNA array of the maternal vagina isolate is same with the plasmid DNA array of the blood culture isolate from her newborn baby who had sepsis. If on the contrary, the infection was considered as being horizontally transmitted to the newborn from the environment.

Data Analysis

SPSS software (version 19.0, SPSS Inc. Chicago, IL. USA) was used for data entry and analysis. Continuous variables were represented as mean ± Standard deviation (SD). Categorical variables were represented as actual numbers or percentages or bar or pie charts. Categorical data were compared using chi square and p- value < 0.05were considered significant for all tests.

RESULTS

Socio-Demographic Data

The hospital recorded a total of 831 deliveries during the period January 2014 to May 2015 that the study lasted. Four hundred and three (48.5%) of the total deliveries were vaginal (SVD) whereas 428 (51.5%) were by Caesarian sections (CS). Three hundred and fifty (42.1%) of the total number of deliveries met the inclusion criteria and were so recruited, 13 (1.6%) missed on grounds of failed monitoring, 40 (4.8%) did not meet the inclusion criteria either due to extreme prematurity, still birth or both. All deliveries by CS were excluded.

Every subject was enrolled once. The women were ages 16 – 46 years (average-29.3± 1.69 years). Most of the women 200 (57.1%), had tertiary education, 148 (42.3%) stopped at secondary education and 2 (0.6) had only primary education. They were mostly civil servants, 156 (44.6%), 70(20%) were of the business class, 113 (32.2%) were house-wives and 11(3.3%) belonged to other professions.

They were mostly civil servants, 156 (44.6%), 70(20%) were of the business class, 113 (32.2%) were house-wives and 11(3.3%) belonged to other professions.

The pregnancies were of gestational ages 28-42 weeks (average- 38.5 ±0.41weeks), 34 (9.7%) were preterm, 316 (90.3%) were term, and none was post term. There were 304 (86.9%) booked cases while 46 (13.1%) did not book for antenatal care. In the course of the index pregnancy, 40 (11.4%) of the women had previous hospital admission, 183 (52.3%) had fever, there was history of previous antibiotics use in 103 (29.4%) and vaginal discharge in 89 (25.4%) of the women.

Demographic Data Of Newborn Babies Suspected Of Sepsis.

Out of the total of 350 newborn babies delivered, 57(16.3%) who were suspected of sepsis within the first 7 days of postnatal life, based on defined criteria were listed in the study. Thirty two (56.1%) were females and 25 (43.9%) were males. Their body weights at birth were as follows 49 (86%) had normal weight (2.5-4.0kg), 1 (1.8%) was overweight (4.6kg), 7 (12.3%) had low birth weight (<2.5kg). Total time spent in labour was normal for all the deliveries (< 18hours, average 8.9 ±0.365 hrs). Placental weights were within the range of 300-1600g (Average 985±0.873g).

Percentage of Newborn Babies with Culture Positive Sepsis

A total of 57(16.3%, n=350) newborn babies were suspected of sepsis based on defined manifestations. Twenty eight (49.1%, n=57) of the babies were culture positive and 29(50.9%, n=57) were culture negative. Twelve (21.0%, n= 57) of the babies grew Gram negative bacilli (GNB) from blood while 16 (28.1%, n=57) were Gram positive cocci. Gram positive growths were not processed further. There was no record of polymicrobial sepsis.

Antibiogram of the blood isolates from the newborn babies with sepsis within the first 7 days of postnatal life.

The pattern of antibiotic susceptibility of the Gram negative blood isolates from the newborn babies with sepsis was characterized by widespread resistance as shown in Table 2. The percentage resistance to selected antibiotics among these blood isolates was as follows:

Amoxicillin clavulanate 91.7%, cefotaxime 83.4%, gentamicin 66.7%, levofloxacin 41.7%, ceftazidime 75.0%, cefepime 41.7%, meropenem 50%, ertapenem 33.3%, imipenem 0.0%, piperacillin tazobactam 0.0% and cefoxitin 0.0%.

Table2. Antibiotic susceptibility patterns of Gram negative blood isolates from newborn babies with sepsis.

Antibiotics	No (%) Sensitivity	No (%) intermediate sensitivity	No (%) resistance
Amoxicillin/clavulanate	1 (8.3)	0 (0)	11 (91.7)
Ceftazidime	2 (16.7)	1 (8.3)	9 (75.0)
Cefotaxime	1 (8.3)	1 (8.3)	10 (83.4)
Cefepime	6 (50.0)	1 (8.3)	5 (41.7)
Cefoxitin	12 (100.0)	0 (0)	0 (0)
Levofloxacin	6 (50.0)	1 (8.3)	5 (41.7)
Aztreonam	3 (25.0)	1 (8.3)	8 (66.7)
Piperacillin/Tazobactam	11 (91.7)	1 (8.3)	0 (0)
Gentamicin	4 (33.3)	0 (0)	8 (66.7)
Meropenem	6 (50.0)	0 (0)	6 (50.0)
Imipenem	12 (100.0)	0 (0)	0 (0)
Ertapenem	8 (66.7)	0 (0)	4 (33.3)

Percentage ESBL-production by blood isolates from the newborn babies with Gram negative sepsis within the first 7 days of postnatal life

Out of the total of 57 newborn babies suspected of sepsis, 12 (21.0%, n=57) were culture positive for Gram negative sepsis. Eleven of these babies (19.3%, n=57), yielded ESBL-producing isolates while only 1 baby (1.7%, n= 57) had ESBL- negative blood borne infection. Four (7.0%, n=57) of the babies grew ESBL- positive *Klebsiella pneumoniae* and 3 (5.3%, n=57) grew ESBL-producing E.coli (Table 3). Percentage ESBL production by the Gram negative isolates from the newborn babies with sepsis within the first 7 days of neonatal life was 19.3% (n=57).

Table 3 Percentage ESBL distribution among blood isolates from babies suspected of sepsis

Isolates	No (%)	ESBL Status	
		Negative	Positive
E.coli	4 (7.0)	1 (1.7)	3 (5.3%)
K. pneumoniae	4 (7.0)	-	4 (7.0)
Enterobacter agglomerans	2 (3.5)	-	2 (3.5)
Proteus staurti	1 (1.7)	-	1 (1.7)
Pseudomonas aeruginosa	1 (1.7)	-	1 (1.7)
Gram +ve growth	16(28.1)	-	-
No growth	29(50.9)	-	-
Total	57(100)	1(1.7)	11(19.3)

Determination of percentage horizontal transmission of neonatal blood borne infections

There were 12 (21%, n = 57) established culture positive Gram negative sepsis during the first 7 days of postnatal life based on defined criteria. The plasmid DNA arrays of mothers and their newborn babies were partly as shown in figures 2-5. Nine (75%, n= 12) of the blood isolates had plasmid DNA arrays that varied with the plasmid DNA arrays of the vagina isolates of their respective mothers (Table 4). In this study therefore 75% of the multidrug resistant Gram negative neonatal blood borne infections within first 7 days of postnatal life were horizontally transmitted. On the other hand, 3 (25%, n=12) of the blood isolates had plasmid DNA arrays that were same with the plasmid DNA arrays of the vagina isolates of their respective mothers (Table 4), indicating 25% vertical transmission of infections to newborns.

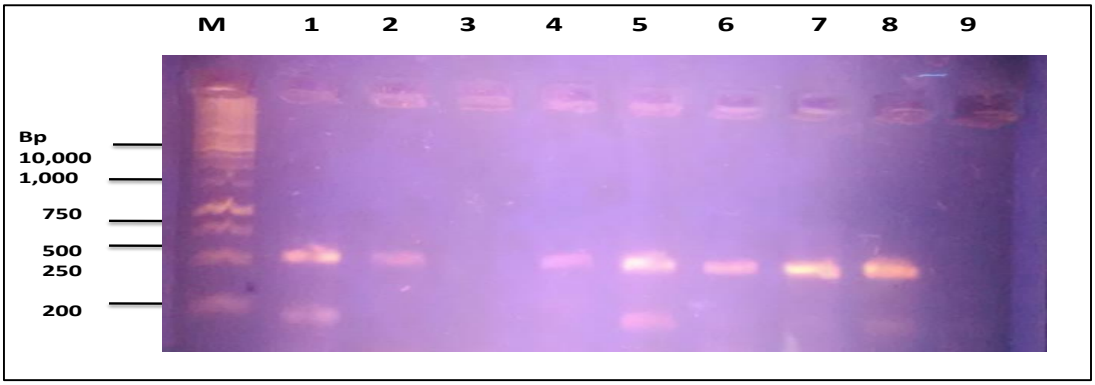

Figure 2. TEM plasmid gene electrophoresis of Paired maternal vagina isolates in pregnancy (odd) to the blood isolates of their respective newborn babies (even) lane numbers 19-24, controls and a molecular standard ladder.

Lane M shows bands for 10,000 base pair (1Kb) molecular weight standard ladder. Lane numbers 20,21,22 showed positive amplification band of 517 bp corresponding to the expected band size for TEM gene in the bacterial species tested. Lane numbers 19,23 and 24 showed no positive bands for TEM gene in the affected species tested.

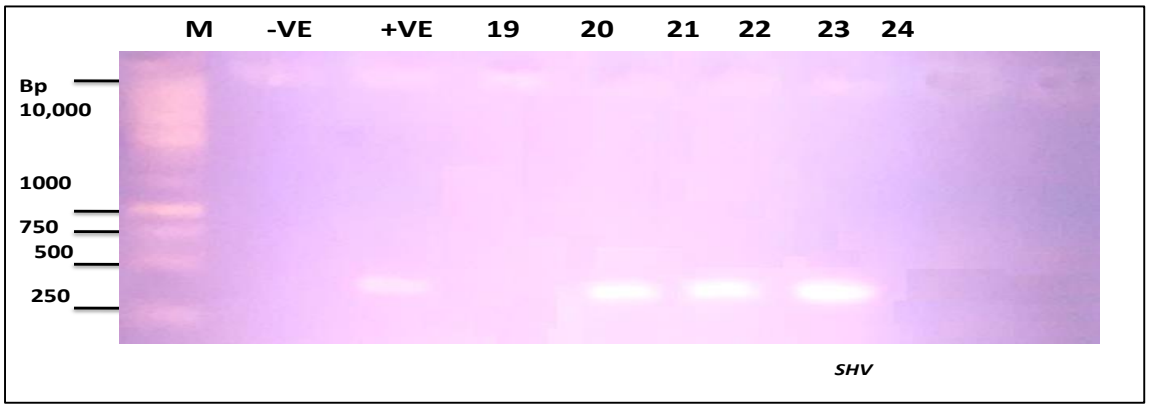

Figure 3. SHV Figure 4.4: SHV plasmid gene electrophoresis of Paired maternal vagina isolates in pregnancy (odd) to the blood isolates of their respective newborn babies (even) lane numbers 19 – 24, controls and a standard molecular ladder .

Lane M showed bands for 10,000 base pair (1Kb) molecular weight standard ladder. Lane -ve was negative control, +ve was positive control. Lanes 20, 21 and 22 showed positive amplification band of 393 bp corresponding to the expected band size for SHV gene in the *isolates* tested. Lane numbers 19, 23 and 24 showed no positive bands for SHV gene in the isolate tested.

Figure 4. CTX plasmid gene electrophoresis of Paired maternal vagina isolates in pregnancy (odd) to the blood isolates of their respective newborn babies (even) lane numbers 19 – 24, controls and a standard molecular ladder.

Lane M shows bands for 10,000 base pair (1Kb) molecular weight standard ladder. Lane -ve is negative control, +ve is positive control. Lanes 21 and 22 showed positive amplification band of 585 bp corresponding to the expected band size for CTX gene in the isolates tested. Lane numbers 19,20, 23-25 showed no positive bands for CTX gene in the isolates tested.

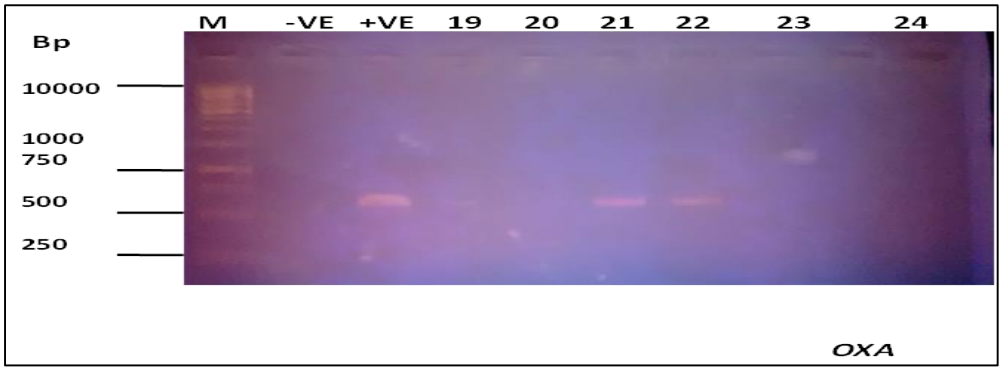

Figure 5. OXA plasmid gene electrophoresis of Paired maternal vagina isolates in pregnancy (odd) to the blood isolates of their respective newborn babies (even) lane numbers 19- 24, controls and a standard molecular ladder .

Lane M shows bands for 10,000 base pair (1Kb) molecular weight standard ladder. Lane number - VE is negative control, +VE is positive control, lane numbers 21, 22 shows positive amplification band of 620 bp corresponding to the expected band size for OXA gene in the isolates tested. Lane numbers 19, 20, 23 - 25, showed no positive bands for OXA gene in the isolates tested.

Table 4. Comparison of the plasmid DNA arrays of the maternal vagina colonizing isolates in pregnancy with the blood isolates of their respective newborn babies who had sepsis during the first 7 days of postnatal life.

Molecular lab no.	Sample no.	Isolates	Plasmid DNA profile				Remarks
			TEM	SHV	CTX-M	OXA	
1	5M	K. pneu.	+*	-	+	+	HT.
2	5B	E.agglo.	+	-	+	+	
3	347M	E. coli	-	-	+	+	HT.
4	347B	E. coli	+	-	+	+	
5	279M	K. pneu.	+*	+	-	+	HT.
6	279B	K. pneu.	+	+	+	+	
7	131M	K. pneu.	+	+	+	-	HT.
8	131B	E.agglo.	+*	+	+	-	
9	133M	E. coli	-	-	-	-	HT.
10	133B	E. coli	+	-	+	+	
11	155M	K. pneu.	+	+	+	-	VT.
12	155B	K. pneu.	+	+	+	-	
13	236M	E.coli	+	+	+	+	HT.
14	236B	E. coli	-	-	-	-	
15	333M	K.pneu.	+	+	+	+	VT.
16	333B	K. pneu.	+	+	+	+	
17	244M	E. coli	-	+	+	-	HT.
18	244B	P.staurti	+	+	-	-	
19	105M	E. coli	-	-	-	-	HT.
20	105B	P.aerugi.	+	-	-	-	
21	125M	K.pneu.	+	+	-	+	HT.
22	125B	K.pneu.	+	+	+	+	
23	274M	E. coli	-	-	-	-	VT.
24	274B	E. coli	-	-	-	-	

Key: HT = Horizontal transmission
VT = Vertical transmission

DISCUSSIONS

Early onset neonatal sepsis remained an immense concern to health providers, being a major source of morbidity and mortality to the newborn (Shaw, Shaw, & Thapaliala, 2007). In the developed countries , Gram positive organisms appeared to be the major culprits whereas in the developing countries, the Gram negative organisms predominated as etiologic agents of early onset neonatal sepsis (Plazek & Whitelaw, 1983).

In this study, of the total number of newborn babies (n = 57), suspected of sepsis, 28 (49.1%, n=57) had positive blood culture. Gram negative bacilli were

isolated from the blood of 12 (21.0%, n= 57) of these babies. It is reasoned that these Gram negative organisms might be acquired vertically from the vagina and fecal flora of the mother and also horizontally acquired from contaminants in the environment where the delivery took place (Mahmood, Karamat, & Butt, 2002). This percentage involvement of Gram negative bacilli in the etiology of early onset neonatal sepsis was less than the 68% reported in a study in India (Viswanathan *et al.*, 2012). *Klebsiella pneumoniae* (7.0%, n= 57) and *E.coli* (7.0%, n=57) were the predominant isolates in this study followed by *Enterobacter* (3.5%, n= 57). This finding was similar to the reports of (Litzow *et al.*, 2009) and (Rahman, Hameed, Roghani, & Ullah, 2002) who reported *Klebsiella pneumoniae* as the commonest isolate in their studies. *Enterobacter* has been widely reported as the second most isolated organism in early onset neonatal sepsis (Kayange *et al.*, 2010; Litzow *et al.*, 2009; Kumhar & Ramachandran, 2002; Greenberg *et al.*, 1997). This is similar to the finding in this study in which *Enterobacter* speie (3.5% n=57) is reported as the second most dominant isolate.

The antibiogram (Table 2) of the blood isolates from babies with sepsis in the early postnatal life showed widespread multidrug resistance, particularly to the third generation cephalosporins. This degree of expression of multidrug resistance suggested hospital-acquired organisms. This finding was similar to the reports of a study in India where over 80% of Gram negative isolates of early onset neonatal sepsis were multidrug resistant to ampicillin, third generation cephalosporins and gentamicin (Viswanathan *et al.*, 2012). It was a disturbing observation that antibiotics readily used in emergency empirical intervention in neonatal sepsis for example Cefotaxim, gentamicin etc were those mostly affected in this expression of multidrug resistance.

Of the 57 babies suspected of sepsis, 11(19.3%) yielded blood isolates that were ESBL-producing. *Klebsiella pneumoniae* was the most frequently isolated (7.0%) and predominantly (7.0%) ESBL-producing, followed by E.coli with same prevalence (7.0%) but (5.3%) ESBL –producing. The overall ESBL – prevalence of 19.3% (n = 57) as recorded in this study was less than the 44.7% documented in a study in India (Sharma *et al.*, 2016) which also recorded *Klebsiella pneumoniae* as the predominant ESBL-producer.

A 75% horizontal transmission of infection was recorded in this study. These were infections arising from organisms acquired from the obstetric ward environment in the course of delivery and needed resuscitative interventions bordering on body surfaces cleaning of babies, suctioning, severing of cords, intravenous drug administrations and other perinatal instrumentations. There is hence need for re-appraisal of infection control protocols in the wards.

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