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Body Surfaces Colonization of Newborn Babies with ESBL-producing *Enterobacteriaceae* on Delivery at Lagos University Teaching Hospital, Lagos, Nigeria

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Abstract: Considering that body surface colonization of the new born by multi-drug resistant bacterial strains could predispose to early onset neonatal sepsis (EONS) this study was embarked upon at the Lagos University Teaching Hospital (LUTH), Lagos to investigate the percentage body surfaces colonization of the newborn and the vagina of their respective mothers by extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-PE). The study also evaluated the percentage of babies so colonized, who manifested with signs and symptoms of sepsis in the first 7 days of postnatal life. The antibiotic susceptibility, resistance patterns and risk factors for ESBL- colonization of the newborn in pregnancy were also determined. This investigation was conducted amongst 350 newborn babies delivered in the labour wards of LUTH, Lagos and their mothers, between January 2014 and May, 2015. Structured questionnaires were used to collect mothers' socio-demographic data. The body surfaces swabs from the newborns and maternal high vagina swabs were cultured and isolates were tested both for antibiotic susceptibility and ESBL production. The babies whose body surfaces were colonized by *Enterobacteriaceae* were monitored for the first 7 days of postnatal life for signs and symptoms of EONS. Multivariate logistic regression model was constructed to determine the risk factors for newborn ESBL-PE colonization. A total of 243 (69.4%) babies were colonized by *Enterobacteriaceae*, 187 (53.4%) were non-ESBL-PE, 56 (16%) of the Isolates were conformed ESBL-PE. Out of the 56 confirmed cases ESBL-PE, 11 (19.6%) on postnatal monitoring, manifested with signs and symptoms of EONS. Maternal vagina ESBL-PE colonization in pregnancy was an independent risk factor for neonatal colonization (p-<0.05). ESBL-production was associated with cross resistance to other antibiotic classes. Antenatal screening of pregnant women for vaginal colonization with ESBL-PE is recommended. Further research to determine if a correlation exists between vaginal ESBL-PE in pregnancy and EONS should be encouraged.
Keywords: Extended spectrum beta-lactamase producing enterobacteriaceae, esbl-pe, esbl, enterobacteriaceae, Escherichia coli, multi-drug resistance, newborn, neonatal sepsis, esbl colonization, bacterial colonization.

INTRODUCTION

Multi-drug resistance to existing antibiotics is a big threat and menace to healthcare system and this is even worsened by lack of development of novel antimicrobials. There has been rising frequency of infections caused by extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-PE) [1-3].

Though much of these ESBL-PE are acquired nosocomially, there is an increasing emergence of community-associated acquisition [4-6]. There has been documented evidence reporting occurrence of transmission of ESBL-PE within families and households [7, 8]. Before 1990, most of the ESBL-PE isolated from clinical samples were of SHV and TEM type, but since 2000, CTX-M has become the commonest genetic determinant of resistance [9-12.

Several studies have demonstrated a number of risk factors associated with ESBL-PE colonization/infection in the neonates which include low birth weight, prolonged hospital stay, previous use of oxyimino-antibiotics and caesarean-section [13-16]. Although different risk factors have been implicated for new-born bacterial colonization and early-onset neonatal sepsis, most of the bacteria causing sepsis in

this group of people are colonizers of the maternal birth canal [17, 18].

The mode of transmission and impact of colonization of ESBL-PE in the neonates are public health concern. Neonatal colonization with ESBL-PE may be a mere transient phenomenon, in that case, it poses no problem, in the other hand, it may result in neonatal sepsis. It may also lead to ESBL-PE outbreak in the neonatal unit. ESBL-PE have been identified in many resource-poor countries as the commonest cause of neonatal sepsis [19]. In many countries with very high rate of under-5 year mortality, bacterial infections are leading cause of neonatal deaths [20]. The rise in the frequency of multidrug-resistant bacterial organisms such as ESBL-PE in neonatal wards limits treatment options with attendant treatment delay and treatment failure which ultimately culminates in high morbidity and mortality rates [21].

In Nigeria, the situation regarding colonization pattern, risk factors and antibiotic resistance in most neonatal wards are largely unknown. We therefore embarked on this study to determine the rate of neonatal body surfaces colonization with ESBL-PE soon after delivery in the labour rooms of Lagos University Teaching hospital, Lagos, Nigeria using *Escherichia coli* and *Klebsiella pneumoniae* as surrogates. We also profiled the resistance pattern of these *Enterobacteriaceae* and studied the risk factors associated with the colonization of these neonates.

MATERIALS AND METHODS

Study design

The design was a prospective cohort study involving pregnant women in labour and their newborns at the Lagos University Teaching Hospital, Lagos, Nigeria, between January 2014 and May 2015. High vaginal swabs (HVS) were collected from pregnant women in labour. Body surfaces swabs (axilla, groin, anal skin and auricular skin) of their newborns were respectively collected immediately after delivery.

The samples were cultured and isolates of *Escherichia coli* and *Klebsiella pneumoniae* were identified and tested for antibiotic susceptibility. Isolates with zones of inhibition below Clinical and Laboratory Standards Institute's (CLSI) established break-points of any or a combination of Ceftazidime and Cefotaxime were screened and confirmed for ESBL-production. . All newborn babies detected to be colonized by *Enterobacteriaceae*, whether ESBL-elaborating or not were monitored for signs and symptoms of sepsis within the first 7 days of post- natal life. Where a baby was discharged from the hospital before the expiration of this time target, mother was contacted on phone at intervals to ask about the presence of symptoms of sepsis and if need be, asked to bring her baby back to the hospital for check- up. Information on socio-demographic data and potential risk factors were obtained using a structured questionnaire.

Inclusion/exclusion criteria

Pregnant women in labour from gestational age of ≥28 weeks who gave consent and their live babies that were vaginally delivered in LUTH, Lagos were enrolled into the study. Excluded from the study were parturient women who refused consent, women with gestational ages below 28 weeks, intra-partum bleeding per vagina, newborns delivered via caesarean section and newborns cleaned before body surfaces swabs samples were collected.

Ethical consideration

The study was reviewed and approved by the Health Research Ethics Committee of the Lagos University Teaching Hospital, Lagos in a letter dated 19th August, 2013. Written informed consent was obtained from all mothers before they were enrolled into the study.

Study area

This study was conducted in Lagos University Teaching Hospital (LUTH), located in the metropolitan city of Lagos, Nigeria. LUTH, currently a 761 bedded hospital [22], services the inhabitants of Lagos and adjoining states as well as referrals from bordering West African countries of Benin Republic and Togo. Lagos state covers an area of 3,577sq.km. It is situated in the South Western part of the country. The Nigerian National Population Commission census of 2006 posted Lagos state population at 9,113,605. [23].

Study population

The study enrolled all consenting pregnant women in labour planned for vaginal delivery and their expected newborns at the Lagos University Teaching Hospital, Lagos.

Sample size

Sample size was calculated based on this Formula [24]: $N = Z^2 pq/d^2$, where N = sample size, p = 20.8% (the local ESBL-PE prevalence [25]), 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 increase the validity of the study and make up for non-response cases.

Sample collection and transport

Vaginal swab specimen

High vaginal swabs were aseptically collected from the consenting pregnant women in labour by first wiping away excess secretions or discharge from the vaginal orifice using sanitary pads. The vaginal walls were then parted using sterile disposable plastic vaginal speculum. Sterile swab sticks were then inserted into the vagina and used to swab round the posterior vaginal walls, just around the pouch of Douglas to collect high

vaginal sample. These samples were transported within 30 minutes from time of collection, to the special pathogens laboratory of the hospital for immediate processing.

Body surfaces swab specimens

The body surfaces (auricular skin, groin, axilla and a peri-anal skin) swabs of all newborns of consenting mothers were aseptically swabbed in that order using sterile swab sticks. These were done immediately after delivery, before the babies were cleaned. Samples collected were transported to the special pathogens laboratory of the hospital within 30 minutes of collection for immediate processing. Maternal HVS and newborn body surfaces samples were inoculated on separate MacConkey agar plates, incubated for 18 hours at 37°C in ambient air. Colonies suspected to be those of *E. coli* and *K. pneumoniae*, based on gram staining reactions and morphological appearance were re-inoculated on nutrient agar plates and re-incubated at 37°C for 18 hours. This was done to ensure the yield of pure discrete colonies in non-inhibitory medium for Microbact identification procedure and antibiotics susceptibility testing.

Identification of isolates using microbact biochemical identification kits (Oxoid Ltd, Basingstoke Hants, UK).

The identification of *E.coli* and *K. pneumoniae* was carried using Microbact as previously described [26]. The Microbact Gram-negative system is a standard micro-substrate system that mimics the conventional biochemical substrates used for the identification of *Enterobacteriaceae* and other Gram-negative bacilli. Organisms' identification is based on pH changes and substrate utilization to produce color panels which are interpreted into codes and read using the computer-aided identification package (a microbact accessory) to identify the isolate to specie level.

Antibiotic susceptibility test

Kirby-Bauer disk diffusion antibiotic susceptibility testing was carried out according to Clinical Laboratory Standards Institute (CLSI) guidelines on Mueller Hinton agar [27]. All antibiotic disks were first subjected to quality control susceptibility testing using *E.coli* ATCC 25922. Suspension of controls and isolates were made in sterile normal saline and to match 0.5% McFarlan's Standard turbidity. Using sterile swab sticks, different Mueller Hinton agar plates were inoculated to make thin homogenous lawn. The selected antibiotic disks were then implanted on the lawns and incubated at 35°C for 18 hours in ambient air. With a ruler, and based on stipulated break-points, zones of inhibition around each antibiotic disc were measured and interpreted as sensitive, intermediate sensitive or resistant. *Enterobacteriaceae* species with zones of inhibition around ceftazidime or cefotaxime or both less than 22mm in diameter were suspected to be ESBL-PE. Such isolates were then screened by the "double-disks synergy" technique. Those that passed as ESBL-positive by screening test were confirmed to be ESBL-PE by the combined disks method according to CLSI criteria [27].

Phenotypic ESBL –screening using double disks synergy technique

Isolates suspected to be ESBL-PE based on reduced zones of inhibition to ceftazidime and cefotaxime, following susceptibility testing were screened using the Double disks synergy technique based on CLSI criteria. Suspension of suspected ESBL-PE isolates were adjusted to the turbidity of 0.5% Mcfarland standard and inoculated on Mueller Hinton agar to form a thin homogenous lawn. Three (3) antibiotic disks were implanted on the lawn with Amoxicillin clavulanate disk in the centre and ceftazidime and cefotaxime disks placed on either sides of it at distances of 20mm centre to centre. The plates were incubated for 24 hours 35°C in ambient air. Enhancement of zone of inhibition (clavulanate effect) around ceftazidime or cefotaxime or both, on the side adjacent the amoxicillin/clavulanate was indicative of positive screening test for ESBL-production.

Phenotypic ESBL-confirmation by combined disks methods

All isolates that passed as positive in the ESBL screening test were confirmed as such with the combined disks ESBL confirmatory test[27]. E. coli ATCC 35218 (positive) and E. coli ATCC 25922 (negative) ESBL controls were used to quality control the tests. Suspension of isolates was adjusted to match the turbidity of 0.5% Mcfarland standard and this was used to inoculate the Mueller Hinton agar plate to form a thin lawn. The paired antibiotic disks were implanted on the lawn, side by side, 30 mm apart and incubated for 18 hours in ambient air at 35°C. The diameter of the zones of inhibition was measured at the end of incubation. ESBL-production was confirmed when the difference in diameter of zone of inhibition between the single disk and its combined pair was ≥ 5mm (Fig below).

Fig-1: Fig.1: ESBL phenotypic combined disks confirmatory test. Key:

1. Single and combined disks of cefotaxime and cefotaxime/clavulanate (upper)
2. Single and combined disks of ceftazidime and ceftaxidime/clavulanate (lower pair) both both showing difference in zones of inhibition of ≥ 5mm between single and paired disks.

Single and combined disks of cefotaxime and cefotaxime/clavulanate (upper). Single and combined disks of ceftazidime and ceftaxidime/clavulanate (lower pair) both both showing difference in zones of inhibition of ≥ 5mm between single and paired disks.

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- values < 0.05 were considered significant for all tests. Multivariate logistic regression analysis was used to determine the individual contributions of clinical factors to prediction of risk of ESBL-colonization of a pregnant woman and her newborn baby. Variables with p -value <0.2 on univariate analysis were entered into a forward stepwise multivariate logistic regression model. The association between independent determinants and ESBL-colonization were estimated using Odds ratios and p ≤ 0.05.

RESULTS

Socio-demographic data

Total delivery during the period was 831 comprising of 403 (48.5%) vaginal deliveries (SVD) and 428 (51.5%) Caesarian sections (CS). Three hundred and fifty vaginal deliveries (42.1%) that met the inclusion criteria for the study were recruited, 13 (1.6%) vaginal deliveries were missed and 40 (4.8%) did not meet the inclusion criteria either due to extreme prematurity, still birth or both. All the Caesarian sections were excluded from the study because they did not meet the inclusion criteria. No subject was enrolled more than once (i.e. one episode per subject).The women were ages, 16 – 46 years (average-29.3± 1.69 years) with 128 (36.6%) of them within the age group of (31-35) years. Most of the women 200 (57.1%), had tertiary education, 148 (42.3%) had secondary education and 2 (0.6) had 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 (Table 1).

The pregnancies were of gestational ages 28-42 weeks (average- 38.5 ±0.410weeks), 34 (9.7%) were preterm (< 37 weeks), 316 (90.3%) were term (37-42 weeks) and none was post term (>42 weeks). Booked cases were 304 (86.9%) while 46 (13.1%) did not book for antenatal care. No case of multiple pregnancies during the period of this study was enrolled as they were delivered by caesarian section and so did not meet the inclusion criteria.

There was positive history of previous hospital admission in 40 (11.4%) subjects, fever in 183 (52.3%), previous antibiotics use in 103 (29.4%) and vaginal discharge in 89 (25.4%) of cases (Table 1). Two mothers (0.6%) were grand multiparous (had obstetric history of 5 live births and 2 still births).

There were 350 babies who were enrolled in the study (1 baby per pregnancy), 170 (48.6%) were males and 180 (51.4%) were females. Body weights at birth were as follows 308 (88%) had normal weight (2.5-4.0kg), 6 (1.7%) were overweight (>4.0kg), 18 (5.1%) had low birth weight (<2.5kg), 16 (4.6%) had very low birth weight (<1.5kg) and 2 (0.6%) had extremely low birth weight (<1.0kg). Average weight at birth was 2.63 ± 0.532kg. Total time spent in labour was normal for all the deliveries (< 18hours, average 8.2 ±0.544 hours). Placental weights were within the range of 300-1600g (average 615±0.932g) with peak concentration of 158 (45.1%) within placental weight grouping of (700-900g).

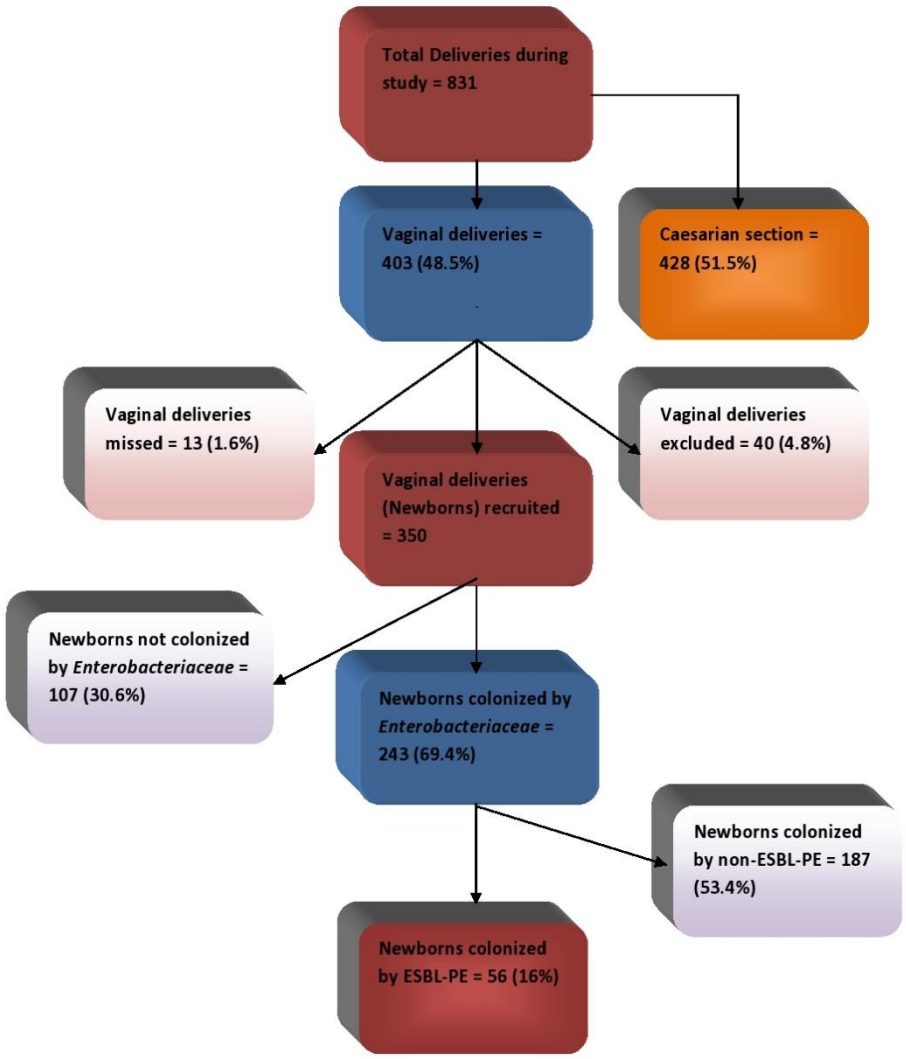

Fig-2: Flow-chart of the study participants and ESBL-PE screening

Table-1: Maternal Socio-demographic data

Age distribution(yrs)	16-20	21-25	26-30	31-35	36-40	41-45	46-50
	2(0.6%)	37(10.6%)	123(35.2%)	128(36.6%)	57(16.3%)	0(0%)	3(0.9%)
Educational sta.	Tertiary = 200(57.1)		Secondary=148(42.3)		Primary = 2(0.6)		
Occupational distribution							
	Cs 156(44.6)	Bz 70(20)	Hw113(32.3)		Oth.11(3.3)		
Previous Hospital Admission	Yes = 40 (11.4)		No = 310 (88.6)				
Antibiotics use in pregnancy		Yes = 103(29.4)			No = 247(70.6)		
Fever in pregnancy		Yes = 183 (52.3)			No = 167 (47.7)		
Vaginal discharge in pregnancy	Yes = 89 (25.4)		No = 261 (74.6)				
Antenatal care		Booked = 304(86.9)			Unbooked = 46 (13.1)		
Grand multiparity		Yes = 2 (0.6)	No =348 (99.4)				

Key: Cs = civil service, Bz = Business, Hw = House wife, Sta. = status, Disch. = discharge .Oth. = Others. Prev. Hosp. Adm = previous hospital admission, Dist. = distribution.

Percentage ESBL Colonization of the Newborn Babies

A total of 243 (69.4%) newborns were colonized by *Enterobacteriaceae*, 187 (53.4%) of the isolates were ESBL- negative and 56 (16%), were ESBL- positive out of 63 (18%) that passed screening test as positive for ESBL-production (Fig. 2). The phenotypic pattern of resistance to third generation

cephalosporins among the newborn body surface isolates was suggestive of the following distribution of ESBL-genotypes: CTX-M (43%), TEM or SHV (32%), co-existence of CTX-M with TEM or SHV or both (25%)(Fig. 3). *Klebsiella pneumoniae* was observed to be more disposed to ESBL-production than *E.coli* (X^2 = *358.653, p=0.01).*

One (0.5%) out of the 187 (53.4%) babies who were colonized by ESBL-negative *Enterobacteriaceae*, showed signs and symptoms of sepsis within first 7 days of post-natal life whereas11 (20%) of the 56 (16%) who were colonized by ESBL- positive *Enterobacteriaceae* demonstrated signs and symptoms of sepsis.

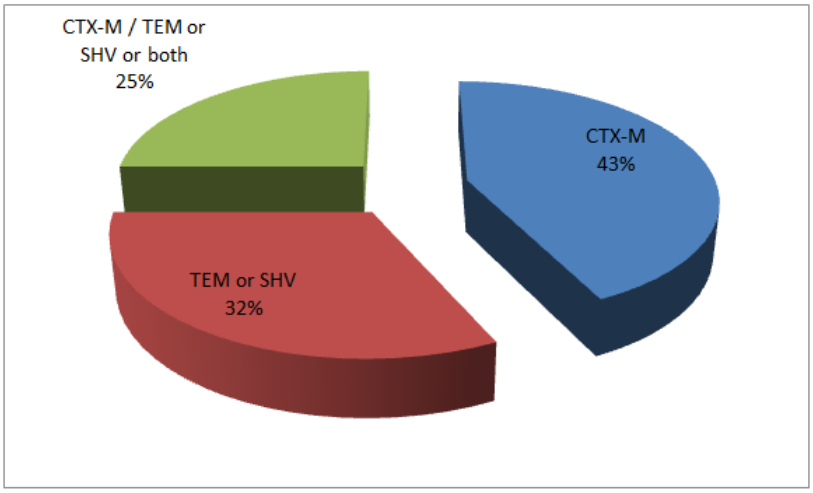

Fig-3: **Percentage Distribution of ESBL-genotypes amongst the newborn isolates of *Enterobacteriaceae* by phenotypic demonstration**

Risk factors for ESBL-PE colonization of newborn babies

A number of factors were evaluated for risk factors of body surfaces ESBL-PE colonization of newborn babies of pregnant women with vaginal ESBL-PE colonization using univariate logistic regression analysis. They included maternal vagina ESBL-PE colonization in pregnancy (OR= 000, p= 000), grand multiparity *(OR=7.46, p= 0.355*), gestational age at delivery (*OR=1.00, p= 0.586*), duration of labour (OR= 2.10, p= 0.140), weight of baby at delivery(*OR=1.37, p= 0.682*) placental weight (*OR= 2.95. p= 0.772*) and sex of baby *(OR=1.30, p= 1.60)* (Table 3) All potential risk factors (p≤ 0.2) were then entered into a forward step-wise multivariate logistic regression analysis and only maternal vagina ESBL-PE colonization in pregnancy was statistically significant as an independent risk for body surfaces ESBL-PE colonization of newborn babies *(OR = 000, p= 000)* (Table 2)

Table-2: Results of univariate / multivariate regression analysis of risk factors for body surfaces ESBL-PE colonization of newborn babies at birth

Factors	Univariate analysis		Multivariate analysis	
	OR	P-value	OR	P-value
Matern. vagina ESBL-PE colonization	0.00	0.00	0.00	0.00
Duration of labour	2.10	0.140	1.79	0.58
Birth weight	1.37	0.682		
Placental weight	2.95	0.772		
Gestational age	1.0	0.586		
Grand multiparity	7.46	0.455		
Sex of baby	1.60	1.30		

Antibiotic susceptibility pattern of *Enterobacteriaceae* isolated from the body surfaces of newborn babies

The antibiotic susceptibility pattern of *Enterobactericeae* isolated from the body surfaces of newborn babies showed that imipenem recorded the highest susceptibility rate of 99.2% followed by piperacillin/tazobactam (94.7%) and meropenem (93.4%). The least susceptibility rate of 53.1% was recorded by Amoxicillin clavulanate. Among the cephalosporins, the pattern was as follows: cefepime (88.1%), cefoxitin (87.6%), ceftazidime (78.2%) and cefotaxime (77%) (Table 4). High level of co-existence was also observed between ESBL-elaboration and resistance to other classes of antibiotics among the colonizing newborn body surfaces isolates. This was as

follows: aztreonam (71.7%), gentamicin (58.7%), levofloxacillin (47.8%) and amoxicillin/clavulanate (39.1%)(Figure 3).

Table-3: Results of univariate analysis of risk factors for body surfaces ESBL-PE colonization of newborn babies at birth

Factors	ESBL-Neg. n (%)	ESBL-Pos. n (%)	Odds ratio	P-value
Gestational age				
<37weeks	15 (8.0)	7 (12.5)	1.00	0.586
37-42 weeks	172 (92.0)	49 (87.5)		
Sex of baby				
Female	92 (49.2)	34 (60,7)	1.60	1.30
Male	95 (50.8)	22 (39.3)		
Birth weight (Average weight)				
< 2.63kg	17 (9.1)	49(87.5)	1.37	0.682
≥ 2.63 kg	170 (90.9)	7 (12.5)		
Placental weight (Average weight)				
< 615g	20 (10.7)	48 (85.7)	2.95	0.772
≥ 615 kg	167 (89.3)	8(14.3)		
Duration of labour (Hrs)				
Normal (< 18 hrs)	184(98.4)	56 (100)	2.10	0.140
Prolong (≥ 18 hrs)	3(1.6)	0 (0)		
Grand multiparity				
No	185 (98.9)	55 (98.2)	7.46	0.455
Yes	2 (1.1)	1 (1.1)		
Maternal vaginal ESBL-colonization				
No	187 (100)	0 (0)	000	000
Yes	0 (0)	56 (100)		

Table-4: Antibiotic susceptibility pattern of *Enterobacteriaceae* isolated from the body surfaces of newborn babies of the pregnant women

Antibiotics	Sensitivity n(%)	Intermediate sensitivity n(%)	Resistance n(%)	Total n(%)
Amox/clav (20 + 10µg)	129 (53.1)	27 (11.1)	87 (35.8)	243(100)
Ceftazidime (30µg)	190 (78.2)	9 (3.7)	44 (18.1)	243(100)
Cefotaxime (30µg)	187 (77.0)	7 (2.9)	49 (20.1)	243(100)
Cefepime (30µg)	214 (88.1)	9 (3.7)	20 (8.2)	243(100)
Cefoxitin (30µg)	213 (87.6)	6 (2.5)	24 (9.9)	243(100)
Levofloxaxin (5µg)	188 (77.4)	11 (4.5)	44 (18.1)	243(100)
Aztreonan (30µg)	183 (75.3)	5 (2.1)	55 (22.6)	243(100)
Piperac /Tazo (110µg)	230 (94.7)	3 (1.2)	10 (4.1)	243(100)
Gentamicin (10µg)	190 (78.2)	12 (4.9)	41 (16.9)	243(100)
Meropenem (10µg)	227 (93.4)	5 (2.1)	11 (4.5)	243(100)
Imipenem (10µg)	241 (99.2)	0 (0)	2 (0.8)	243(100)
Ertapenem (10µg)	213 (87.6)	5(2.1)	25 (10.3)	243(100)

Key: Piperac/Tazo = piperacillin tazobactam; Amox/clav = Amoxicillin clavaulanate

DISCUSSION

We aimed to gain insight into the occurrence rate of ESBL-PE colonization of the body surfaces of the newborns, risk factors fueling this colonization and overall antibiotic resistance pattern of the bacteria organisms. We used *E.coli* and *K. pneumoniae* as group representatives of ESBL-PE. Although, the study focused on the newborn ESBL-PE body surface colonization, the pre-delivery vaginal ESBL-PE colonization of the corresponding mother was also determined to enable us to find out if pre-delivery vagina colonization of the mother was a risk factor for

body surfaces colonization of her newborn. The investigation (via antibiogram and plasmid DNA profiling using PCR) showed the newborns ESBL-PE to be exactly the same as those of their mothers. A number of studies have established the spread of ESBL-PE from parents/family members to children [28, 4, 5]. In our study, the percentage body surfaces colonization with ESBL-PE in the newborn babies was 16% (56/350), same as the level of colonization with their maternal vagina in pregnancy. This depicts the maternal vagina as a common source of organisms colonizing the newborn babies in the immediate pos-delivery period. This pattern of results in the babies was expected since the timing and sites of body surfaces sample collection on delivery, did not allow any reasonable exposure of the babies to exogenous contamination. This level of ESBL-PE colonization in the newborn babies generates a lot of concern because infections arising from such organisms could pose treatment challenges since cefotaxime with or without gentamicin was readily the regimen of choice for the management of newborn Gram negative sepsis and or meningitis. This prevalence level is similar to two other studies elsewhere – 16% in Poland [29] and 15% in Morocco[30], higher than levels of 4.9% in Halle/Saale Germany[31], 5.7% in Berlin, Germany [28], 11% in an NICU in Israel [32], and lower than others [33, 34]. A most recent similar study conducted in Sri Lanka revealed a neonatal ESBL-PE colonization rate of 1.2% [12], which was much lower than the finding in our study. Ordinarily, one would have expected the ESBL-PE colonization rate in our study to be much lower than it is, considering that we sampled our respondents the same day of delivery, whereas most other similar studies sampled their subjects a couple of days from date of delivery. Perhaps, what might explain the moderately high colonization rate in our study could be differences in sampling sites. Whereas most other similar studies that had lower colonization collected only peri-anal samples, our samples were body surfaces from auricular skin, groin, axilla and peri-anal skin.

Risk factors for hospital acquired colonization or infections include ICU admission, renal failure, burns, total parenteral nutrition, use of invasive devices (urethral catheter, endotracheal tubes, central venous line) for prolonged period, third generation cephalosporin use [35, 36], frequent contact with the hands of health-care providers [37]. In the community, the following are risk factors for the acquisition of ESBL-PE colonization or infections: recurrent urinary tract infection, previous antibiotic use, diabetes mellitus, prior instrumentation to urinary tract, female sex, age, cultural background and diet in areas of high ESBL endemicity [38, 39]. However, our study did not recruit pre-morbid pregnant women and most of them were below the age of 65. Giuffre *et al.* identified low birthweight as the only risk factor for colonization in an Italian neonatal intensive care unit, NICU [13]. In another NICU in Ecuador, Nordberg *et al*. found that length of stay longer than 20 days and enteral feeding with a combination of breastfeeding and formula feeding were associated with ESBL-PE colonization [34]. A more recent study in Madagascar, employed Cox proportional hazards model to identify factors associated with first acquisition of ESBL-PE in life [16]. The study showed that low birth weight, cesarean-section and maternal use of antibiotics at delivery were independent risk factors for ESBL-PE acquisition. Though the newborn respondents in this study did not have some of the exposure risks as obtained in the above cited studies, but for the ones tested, maternal vaginal ESBL-PE colonization in pregnancy was the only independent risk factor for newborn body surfaces ESBL-PE colonization. A couple of previous studies had identified ESBL-PE colonized mother as independent risk factor for colonization of neonates with ESBL-PE [28,4, 5, 40, 41].

ESBL-producing organisms have also been identified with multidrug resistance to other classes of antibiotics such as aminoglycosides, ampicillins [42], cotrimoxazole, tetracycline and fluoroquinolones [43]. Treatment of infections arising from ESBL-PE organisms pose serious therapeutic challenges [44] as it is limited to small number of expensive drugs, not readily available in low income countries and even when present, may not be affordable to majority of patients. The outcome therefore include increasing disease burden [45], prolonged hospital stay, increase cost of treatment, loss of man-hours at work and high mortality [46] due to high rate of treatment failure.

In this study, most of the newborn body surfaces' isolates were susceptible to Imipenem 99.2% (241/243) but exhibited least susceptibility to Amoxicillin/clavulanate 53.1% (129/243). This finding is similar to the results of a related study [47] in Iraq which reported 100% susceptibility of isolates of *E. coli* to imipenem and 100% resistance to amoxicillin/clavulanate. The isolates were 93.4% and 87.6% susceptible to meropenem and ertapenem respectively, signifying gradual emergence of carbapenem resistance into the community, as was reported by Nodmann and Carrer [48] amongst others.

Significant cross-resistance of the isolated ESBL-PE to some non- cephalosporins antibiotics was observed. They included aztreonam 71.1%, gentamicin 58.7%, levofloxacillin 47.8%, and amoxicillin/clavulanate 39.1%. This finding is similar to the reports of other studies [49-51]. This development further compounded the treatment challenges associated with infections caused by ESBL-producing organisms as only few often expensive antibiotics were left to manage such cases.

Our study also revealed that 11 (20%) of the 56 (16%) newborn babies colonized by ESBL-PE manifested with signs and symptoms suggestive of

sepsis within the first 7 days of post-natal life. This finding generates anxiety as to whether there is a correlation between maternal vagina ESBL-PE colonization in pregnancy and early onset neonatal sepsis.

This study has some limitations. First, convenient sampling method was employed in the sampling of the participants; hence there could have been the possibility of selection bias. Secondly, in the study, we did not screen all ESBL-producing *enterobacteriaceae*, rather, we used *E.coli* and *K. pneumoniae* as surrogate, as such, and we might have missed some other ESPL-PE other than *E.coli* and *K.pneumoniae* from the samples. This might have resulted in under-estimated ESBL-PE prevalent rate.

In conclusion, our study revealed moderately high rate of newborn ESBL-PE colonization with maternal-neonatal transmission being a significant a significant risk factor. The ESBL-PE isolated in this study showed high level of cross resistance to other antibiotics. We consider these findings as being important since these newborns could possibly come down with ESBL-PE infections with attendant therapeutic problems. We therefore recommend that the screening of pregnant women in the labour ward prior to delivery and their newborns immediately after delivery should be made a routine practice. Strict hospital infection control and home hygiene measures should be practiced and the use of antibiotics should be more discriminatory to curtail the spread of resistant strains. We also recommend that further research be undertaken to determine whether or not a correlation exists between maternal vagina ESBL-PE colonization in pregnancy and early onset neonatal sepsis.

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