Clinical Infectious Diseases

Carriage and acquisition of Extended Spectrum Beta-Lactamase producing Enterobacterales among neonates admitted to hospital in Kilifi, Kenya

--Manuscript Draft--

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Abstract:	Background Infections caused by extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) among hospitalized neonates in sub-Saharan Africa pose significant clinical challenges. However, sources of infection and risk factors for transmission are not clearly defined in this setting. Methods In a cohort study of consecutive neonatal admissions to Kilifi County Hospital (KCH) from July 2013-August 2014, we estimated ESBL-E carriage prevalence on admission using rectal swab cultures and identified risk factors using logistic regression. Using

	twice-weekly follow up swabs, we estimated the incidence and identified risk factors for ESBL-E acquisition in hospital using Poisson regression. Results The prevalence of ESBL-E carriage at admission was 10% (59/569). Caesarean section delivery, older neonatal age, and smaller household size were significant risk factors. Of the 510 infants admitted without ESBL-E carriage, 238 (55%) acquired carriage during their hospital stay. The incidence of acquisition was 21.4% (95% CI 19.0, 24.0) per day. The rate was positively associated with the number of known neonatal ESBL-E carriers and with the total number of neonates on the same ward. Conclusions Carriage of ESBL-E was common among neonates on admission and acquisition in hospital was rapid. The dissemination and selection of ESBL-E appears to be driven by hospital exposures; operative delivery and neonatal ward patient density. Further attention to infection control, patient crowding and carriage surveillance is warranted.
Response to Reviewers:	Response to reviewer 1: A very clear and well written manuscript highlighting the rapidity of acquisition of ESBL coliforms in hospitalized neonates. It will be of interest to paediatric infection specialists and neonatologists. I have a few minor technical queries / comments on the methods: - Can the authors include the year / version number of the CLSI guidelines in the text for clarity. Response: We used the CLSI guideline for the year 2014 and reference to it was overlooked during writing up the manuscript. We have updated this in the text and references. - The method for detection of ESBL in Enterobacter needs clarification since this genus is not included in the CLSI guidance and the presence of AmpC complicates things. Response: The reviewer is correct; phenotypic ESBL testing is problematic for Enterobacter species due to AmpC beta-lactamase production. We have described the limitation of our methods in terms of Enterobacter species that may give false negative results in phenotypic ESBL testing in the discussion as follows: “Our prevalence and incidence estimates may also be underestimates since stool culture may be more sensitive than rectal swab culture, a single sample is less sensitive than multiple samples for culture and some Enterobacter spp, which are known to produce AmpC beta-lactamases, may have tested falsely-negative for ESBL by the phenotypic method used.” We do have whole-genome sequencing data for 19 Enterobacter species from this study, that were ESBL positive using the CLSI phenotypic assay for E. coli and K. pneumoniae, and can report that all of them possessed bla CTX-M-15 in addition to an AmpC cephalosporinase. - Please provide a definition of "multi-drug resistant" (results line 172). Response: We defined multi-drug resistance as resistance to at least one agent in three or more antimicrobial categories as recommended by Magiorakos et al. 2012. - I am not a statistician but am aware of the problems of over-dispersion in Poisson regression modelling: was this checked for? Response: The reviewer is correct that over dispersion is sometimes an issue when using Poisson models. It arises, for example, when there are multiple events per person and heterogeneity between individuals in the rate of acquisition. Under these circumstances the variability in the number of events per person is greater than that predicted by the Poisson model (i.e., the data are over-dispersed relative to the Poisson distribution). However, our analysis is different because we modelled time to first acquisition (i.e., maximum of 1 event per person). In this situation it is impossible to identify heterogeneity and hence over-dispersion does not play a role.

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**This study is a prospective study estimating ESBL-E carriage prevalence from rectal swab with a robust systematic approach (day 0, 2, 4, 6, and twice weekly thereafter until an ESBL-E was isolated or until hospital discharge or death, whichever came first). In addition to the epidemiological and clinical data, the number of neonates in each room, bed-location and antimicrobial use of all participants was recorded daily.

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15th October, 2018.

Dear Dr. Kathryn M. Edwards,

Associate Editor,

Clinical Infectious Diseases,

198 Madison Ave

New York NY 10016.

RE: Submission of revised manuscript CID-91249.

Thank you for your email dated 16th August 2018 enclosing reviewers comments.

We have carefully reviewed the comments and revised the manuscript accordingly. Our responses are given in a point by point manner below. We have also attached a manuscript copy with tracked changes and a clean one for review.

I hope that the changes we have made will resolve your concerns. We are more than happy to make further changes that will improve the paper and facilitate successful publication in your journal.

Thank you for your time and interest in our work and we are looking forward to hearing from you in due course.

Yours sincerely,

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Carriage and acquisition of Extended Spectrum Beta-Lactamase producing *Enterobacterales* among neonates admitted to hospital in Kilifi, Kenya

Ngure Kagia[1*], Patrick Kosgei[1], Michael Ooko[1], Leonard Wafula[1], Neema Mturi[1], Kirimi Anampiu[1], Salim Mwarumba[1], Patricia Njuguna[1], Anna C. Seale[1,2,3], James A. Berkley[1, 2], Christian Bottomley[3], J. Anthony G. Scott[1, 3], Susan C. Morpeth[1, 3, 4]

Affiliations

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Keywords

Neonates, Extended Spectrum Beta-Lactamase, Carriage, Acquisition, Risk-factors

Running title

Risk factors of carriage and rapid nosocomial acquisition of Extended Spectrum Beta-Lactamase producing *Enterobacterales* amongst neonates admitted to hospital in Kilifi, Kenya

Corresponding author*

We report a prospective hospital-based longitudinal study that estimates the ESBL-E carriage prevalence among neonates on admission, the incidence of acquisition of ESBL-E carriage in hospital and the risk factors for neonatal prevalent and incident ESBL-E carriage.

ABSTRACT

Background

Infections caused by extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-E) among hospitalized neonates in sub-Saharan Africa pose significant clinical challenges. Data on prevalence and acquisition of ESBL-E carriage among hospitalized neonates in the region are few and risk factors for transmission are not clearly defined.

Methods

In a cohort study of consecutive neonatal admissions to Kilifi County Hospital (KCH) from July 2013-August 2014, we estimated ESBL-E carriage prevalence on admission using rectal swab cultures and identified risk factors using logistic regression. Using twice-weekly follow up swabs, we estimated the incidence and identified risk factors for ESBL-E acquisition in hospital using Poisson regression.

Results

The prevalence of ESBL-E carriage at admission was 10% (59/569). Caesarean section delivery, older neonatal age, and smaller household size were significant risk factors. Of the 510 infants admitted without ESBL-E carriage, 238 (55%) acquired carriage during their hospital stay. The incidence of acquisition was 21.4% (95% CI 19.0, 24.0) per day. The rate was positively associated with the number of known neonatal ESBL-E carriers and with the total number of neonates on the same ward.

Conclusions

Carriage of ESBL-E was common among neonates on admission and acquisition in hospital was rapid. The dissemination and selection of ESBL-E appears to be driven by hospital exposures; operative delivery and neonatal ward patient density. Further attention to infection control, patient crowding and carriage surveillance is warranted.

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INTRODUCTION

Infection and carriage rates of extended-spectrum beta-lactamase producing *Enterobacterales* (ESBL-E) are on the rise globally and pose a particular threat to neonates [1–3]. Outbreaks of multi-drug resistant infections due to ESBL-E in hospitals are common[4–7] and are a growing burden, especially among neonates[3].

It is known that neonatal ESBL-E carriage can be a precursor to invasive infections[7,8] but the epidemiology of transmission in sub Saharan Africa (sSA) is poorly characterized. In sSA, data on neonatal ESBL-E infection and carriage are scarce[2,3] but there is some evidence of hospital-acquired carriage in older children. In a general pediatric ward in Madagascar, prevalence of carriage of ESBL-E in stool was found to be 21% on admission and 57% on discharge, among patients discharged ≥48 hours after admission[9]. In the community, amongst children and adults in Madagascar[10], prevalence of ESBL carriage was 10%.

At Kilifi County Hospital (KCH), we have observed sporadic outbreaks of ESBL-E bacteraemia among neonatal admissions over several years. These infections often have a poor outcome (in KCH the case-fatality risk for hospital-acquired paediatric bloodstream infections is 54%[11]). We have also observed an increase in the proportion of ESBL-producing invasive *Klebsiella pneumoniae* over a decade at Kilifi County hospital[12].

Here we report a prospective hospital-based longitudinal study at KCH to estimate the ESBL-E carriage prevalence among neonates on admission, the incidence of acquisition of ESBL-E carriage in hospital and the risk factors for neonatal prevalent and incident ESBL-E carriage.

METHODS

Study design and sampling procedure

Neonatal admissions were eligible for recruitment into the study if they were admitted to the High Dependency Unit (HDU) between 1st July 2013 and 29th August 2014 or to the neonatal rooms in the general pediatric ward between 16th August 2013 and 29th August 2014. The HDU consists of an open ward with six beds for older children and two small rooms for neonatal admissions. The neonatal rooms are of equal size and have a combined bed-capacity of eight. In the general pediatric ward, there are two neonatal rooms with a combined bed-capacity of 24, including five incubators, four small beds and fifteen cots. KCH practices comprehensive obstetric care, as defined by the World Health Organization, with caesarean section services available.

Data and clinical sample collection

Epidemiological and clinical data were collected on admission and entered in real-time into an electronic medical record system. Rectal swabs were collected on admission (day 0), at days 2, 4 and 6, and twice weekly thereafter until an ESBL-E was isolated or until hospital discharge or death, whichever came first. Rectal swabs were collected using pre-moistened viscose-tipped swabs and placed in Amies transport media (Deltalabs, Barcelona, Spain). The number of neonates in each room, bed-location and antimicrobial use of all participants was recorded daily. Blood culture is performed routinely at admission on all children hospitalized at KCH[13]. Clinical samples are collected at the discretion of the attending clinician.

Laboratory procedure

Rectal swabs were inoculated onto 5% horse blood agar and MacConkey agar supplemented with 8% gentamicin. Cefotaxime (30 ug) and ceftazidime (30 ug) antibiotic discs (Oxoid, United Kingdom) were added on the 2nd and 4th streaking zones on the blood agar plate to detect bacteria

resistant to third-generation cephalosporins. Blood agar plates were incubated in a CO_2 incubator while MacConkey agar plates were incubated in an aerobic incubator for 24 hours at 35 +/- 2^{O}C. Oxidase-negative, gram-negative rods were subcultured and identified using standard techniques (API 20E; BioMérieux, France). Antimicrobial susceptibility testing was performed using the disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2014) guidelines[14]. ESBL testing was performed for isolates that were non-susceptible to third generation cephalosporins using the double disc method[14]. External quality assurance was provided for by UK National External Quality Assessment service.

Analysis

The binomial confidence interval around a prevalence estimate is widest (for a fixed sample size) when the estimate is 50%. We calculated that a sample size of 555 neonates would be required to estimate a 50% prevalence of ESBL-E carriage with a precision of +/-5%.

Carriage on admission (prevalent carriage) was defined as a positive culture on the first rectal swab, provided it was obtained within 48 hours of admission. Logistic regression was used to determine risk factors for carriage at admission. The factors considered were: infant age, sex, maternal age, infant weight at admission, current method of feeding, place and mode of delivery, prematurity (less than or equal to 37 weeks gestation, estimated by the admitting clinician), number living in the same household, type of toilet and main source of water. Multivariable logistic regression models were fitted after confounders had been identified (Figure 1). Infant age at admission was adjusted for prematurity and place/mode of delivery, and prematurity was adjusted for age on admission.

Kaplan-Meier curves were used to describe the time to acquisition of carriage in hospital among neonates who did not have ESBL-E carriage at admission. In this analysis, follow up time, which

was measured in days after admission, was censored at the earliest of: (i) time of the first ESBL-E culture positive swab (ii) time of last swab collection if the neonate died or was discharged and had remained negative throughout the course of admission. For neonates who acquired ESBL-E, the date of acquisition was assumed to be the midpoint between the date of the last negative swab and the date of the first positive swab.

We calculated the rate of ESBL-E acquisition per 100 days at risk. Poisson regression was used to identify predictors of the acquisition rate and to test for interactions. The potential predictors were both time-invariant (e.g. weight at admission, place/mode of delivery, mother's age and age at admission) and time-varying (e.g. number of known ESBL carriers on the ward). A multivariable Poisson regression model was fitted to investigate the effect of crowding on ESBL-E acquisition. The model included as covariates the ward, the number of neonates on the ward and the number of known ESBL-E carriers on the ward.

We defined multi-drug resistance as resistance to at least one agent in three or more antimicrobial categories[15]. Statistical analyses were done with STATA 12.0 (StataCorp, College Station, TX, USA).

Study clearance and ethical considerations

The KEMRI National Ethical Review Committee approved the study (SSC 2301). Informed consent was obtained from all parents/guardians before enrollment.

RESULTS

During the study period, 1,014 neonates were admitted to Kilifi County Hospital, and the parents/guardians of 597 neonates gave consent for them to participate in the study (Figure 2).

The median age of the participants was one day (IQR 0-3 days) and the median duration of hospital stay was 5 days (IQR 3-9 days).

Of 597 who gave consent to participate, five parents/guardians withdrew consent and 23 neonates had no swab collected. The prevalence of ESBL carriage at admission was 10% (59/569). From the 59 neonates with ESBL-E carriage at admission, there were 65 isolates consisting of 31 *Klebsiella pneumoniae*, 25 *Escherichia coli*, 8 *Enterobacter cloacae* and 1 *Klebsiella oxytoca*. Multiple colonization, ie. colonization with two or more ESBL-E from one participant, was found in 6/59 neonates (10%).

Among the 510 non-carriers on admission, 55% (283/510) acquired ESBL-E during their hospital stay. The incidence of ESBL-E acquisition was 21.4 (95% CI 19.0, 24.0) per 100 child days of observation, and the median time to acquisition among these patients was 3 (IQR 1-5) inpatient days (Figure 3). Nine neonates were diagnosed with ESBL-E bacteraemia during this study, all of whom had ESBL-E isolated from fecal carriage prior to or on the same day as blood was collected.

Most ESBL-E isolates were multi-drug resistant; resistance to chloramphenicol, trimethoprim-sulfamethoxazole, quinolones and gentamicin being common, none were resistant to imipenem and only 5% were resistant to amikacin (Table 1).

Risk factors for ESBL-E carriage at admission

In the univariable analysis, variables associated with prevalent ESBL-E carriage on admission were: being born at term, older infant age at admission, having fewer than eight people living in the same house, and hospital delivery, particularly by caesarean section (CS) (Table 2 and Supplementary Table S1). Babies born prematurely were more commonly admitted directly to the

neonatal ward (107/157, 68%) than babies born at full term (114/410, 28%; P-value<0.001). After adjusting for prematurity and place/mode of delivery, increasing infant age was positively associated (p<0.001) with ESBL-E carriage at admission, with odds ratios 1.72 (95% CI 0.69 – 4.27) and 3.88 (95% CI 1.47 - 10.21) among neonates aged 1-2 days and 3-28 days respectively, relative to the odds of ESBL carriage among neonates admitted on the day of birth. Being born at term was not associated with ESBL-E carriage after adjusting for the effect mediated through age on admission. We did not estimate an adjusted OR for place/mode of delivery and number of people in the same household since the associations were not confounded (Figure 1).

Risk factors associated with acquisition of ESBL-E during hospitalization

In the univariable analysis, hospital ward on admission, the number of neonates present in the hospital, the number of other neonates admitted in the same ward, and the number of known ESBL-E carriers were associated with incident acquisition of carriage (Table 3). Both current number of known neonatal ESBL-E carriers and number of other neonates in the same ward were positively associated with carriage acquisition when simultaneously included in multivariable model (Table 4), and there was no interaction between these risk factors. In both the univariable and multivariable analyses, the number of neonates in the ward exhibited a threshold effect whereby there was a plateau effect in carriage acquisition beyond 10 patients per ward. Recorded antibiotic prescription, specifically use of third generation cephalosporins during the inpatient stay was not shown to be associated with ESBL-E acquisition.

DISCUSSION

Our study reveals that among neonates admitted to a rural Kenyan hospital, 10% were already carriers of ESBL-E. Among those neonates who were not carriers at admission, 21.4% acquired ESBL-E carriage each day of admission; thus, more than half of the neonates were colonized with ESBL within the first three inpatient days.

For babies coming in to hospital, the main risk factors for existing rectal carriage with ESBL-E were delivery in hospital via caesarean section and older infant age at admission. For those admitted without carriage of ESBL-E, the principal risk factors for acquisition in hospital were the number of other neonates in the ward and the number with ESBL-E carriage.

Delivery through caesarean section has been reported to be a significant risk factor for prolonged faecal colonization with ESBL producing *K. pneumoniae*[16] and also a determinant of intestinal microflora early in life[17,18]. Mothers undergoing caesarean section are treated with antibiotics for surgical prophylaxis, sometimes extended to treatment of wound infections[19], which may select for antibiotic-resistant enteric bacteria. In Cambodia, young hospital-born infants were found to be at a greater risk of early colonization by third generation cephalosporin resistant gram-negative rods compared to infants born at home, a health centre or other locations and subsequently admitted to hospital[20]. In Madagascar, Herindrainy *et al.* reported that low birth weight, caesarean section and use of antibiotics by mothers at delivery were independently associated with neonatal acquisition of ESBL-E during the first month of life[21].

The finding that babies coming from a large family of >8 household members were less likely to carry ESBL-E at admission was surprising. We speculate that these neonates may have a more diverse gut microbiome, which could be protective against acquisition of ESBL-E carriage. Increased neonatal age at hospital admission was associated with a greater likelihood of ESBL-E carriage, as expected, since older neonates have had more time to acquire carriage.

Overall, we isolated ESBL-E from 10% of swabs within 48 hours of admission. In a cross-sectional ESBL-E carriage study done in a Tanzanian hospital, the overall neonatal prevalence of ESBL-E carriage was 25.4% [22]. Our findings suggest that some acquisition occurs before neonates come into the paediatric wards and we can speculate that this does not only come from their

mothers but also from the procedures and settings of childbirth, particularly caesarian delivery. We did not collect data on ESBL-E carriage in mothers or maternal antibiotic use.

Among neonates admitted without carriage, 55% acquired ESBL-E during hospitalization. An ESBL-E carriage study in a tertiary hospital in Rwanda among inpatients of all ages reported that 55% of participants acquired ESBL-E carriage during hospitalization [23]. A study in Madagascar reported that 48% of pediatric non-carriers at admission acquired ESBL-E during hospitalization.

Our findings from the longitudinal study suggest that the greatest risk factors for ESBL-E acquisition in hospital were having increased numbers of existing ESBL-E carriers among the neonatal patients and a greater number of neonates admitted to the ward. We assume that increased numbers of ESBL-E carriers increase the opportunity for transmission. This finding corresponds with a prospective cohort study done in the general intensive care unit of a hospital in Greece; colonization pressure contributed significantly to acquisition of carriage of carbapenemase producing *Klebsiella pneumoniae* in hospital[24]. Intuitively, hospital crowding is expected to be associated with higher rates of ESBL-E transmission and our results support this. However, our findings suggest a threshold effect where risk plateaued after admitting more than 10-14 neonates in a ward, suggesting that transmission effects associated with crowding are complex. Restricting the number of neonatal admissions to the hospital is impractical, but this does justify allocating increased space to neonatal admissions. Fixed low healthcare staff numbers relative to numbers of patients, the cultural practice of mothers caring for each other's babies on the ward, physical proximity of adjacent neonates, and shared hygiene facilities, may all contribute to acquisition of ESBL-E carriage by neonates in hospital. As well as direct transmission between babies on the ward, nosocomial carriage acquisition directly from the hospital environment is also possible. A study in Cambodia of transmission of third-generation cephalosporin-resistant *Klebsiella pneumoniae* isolates in a newly opened neonatal unit found

that most clusters were likely to have been due to patient sources while 2 of 9 clusters could have been due to either an environmental or a patient source[25].

During the study period, nine neonates were diagnosed with ESBL-E bacteraemia. Nosocomial spread of ESBL-E carriage may result in outbreaks of ESBL-E bacteraemia in the hospital; such outbreaks have occurred in KCH in recent years [12], signifying the importance of awareness of ESBL-E carriage. There is potential for surveillance to help inform hospital infection control and to assist in averting such outbreaks. At KCH screening for carriage of ESBL-E among neonates is not routinely done, hand washing facilities frequently lack water supply, and there are no fully dedicated infection control staff.

Antibiotic use has been shown to affect the composition of gut microbiota and is associated with ESBL-E carriage and acquisition[9,16,17,24,26]. Antimicrobial stewardship services are used as part of hospital infection control services to reduce ESBL carriage in well-resourced hospitals. We were unable to detect antibiotic use as a risk factor for ESBL-E acquisition in our study. We suspect that this is mainly attributed to the fact that 93% of our participants were given antibiotics during their hospital stay and we were therefore underpowered to observe any differences (Supplementary table S2).

We did not collect data from babies after they were discharged from hospital, but patients discharged with ESBL-E carriage have been shown to spread these ESBL-E within family units and close contacts[16,27]. In a prospective cohort study of infants and their families in Norway, the median carriage duration among infants discharged with carriage of ESBL-producing *Klebsiella pneumoniae* after a hospital outbreak was 12.5 months[16]. If carriage of ESBL-E persists and intra-household transmission occurs, discharged patients may act as reservoirs of ESBL-E in the community.

Being a hospital-based study, focusing on sick newborns, our estimates of ESBL-E prevalence at admission cannot be generalized to community prevalence. It is theoretically possible that low-level ESBL-E carriage was more prevalent at admission than we were able to determine; below detection rate by culture methods, but then amplified by selection pressure from the use of antibiotics in hospital until detectable. We also were only able to recruit 68% of eligible neonates limiting the generalizability of our findings (Supplementary table S1). Of note, significantly more parents/guardians of older neonates, and neonates born in hospital by caesarean section, declined to participate in the study suggesting that our estimate of prevalence of ESBL-E carriage at admission is likely to be an underestimate. Our prevalence and incidence estimates may also be underestimates since stool culture may be more sensitive than rectal swab culture, a single sample is less sensitive than multiple samples for culture and some *Enterobacter spp,* which are known to produce *AmpC* beta-lactamases, may have tested falsely-negative for ESBL by the phenotypic method used. We did not find any carbapenem resistant Enterobacterales (CRE) in this study, but it is known that such isolates are present in Kenya[28,29]. Use of central quality assured microbiology laboratories in surveillance for ESBL-E carriage could therefore be expected to have the added benefit of an early warning system for the introduction of CRE carriage.

In conclusion, our findings reveal a high incidence of ESBL-E colonization among hospitalized neonates, which is endemic in this setting. Further work to investigate the association between ESBL-E acquisition and both caesarean section delivery and crowding, perhaps including restrictions on room capacity, and more deliberate cohorting of older neonates and those born in hospital through caesarean section is needed. Given the link between ESBL-E carriage and outbreaks of potentially fatal ESBL-E infection, our data emphasize the importance of routine surveillance and hospital infection control.

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405

Table 1: Non-susceptibility profile for *Klebsiella pneumoniae, Escherichia coli* and *Enterobacter cloacae* carriage isolates

Antibiotic tested	Timing of admission	*Klebsiella pneumoniae*		*Escherichia coli*		*Enterobacter cloacae*	
	on admission	N=31		N=25		N=8	
	after admission	N=196		N=73		N=42	
		n	**%**	**n**	**%**	**n**	**%**
Chloramphenicol	on admission	11	35.5	3	12.0	7	87.5
	after admission	66	33.7	17	23.3	33	78.6
Ciprofloxacin	on admission	13	41.9	16	64.0	5	62.5
	after admission	99	50.5	64	87.7	25	59.5
Cotrimoxazole	on admission	31	100	22	88.0	7	87.5
	after admission	193	98.5	70	95.9	38	90.5
Gentamicin	on admission	30	96.8	11	44.0	7	87.5
	after admission	192	98.0	58	79.5	38	90.5
Amikacin	on admission	2	6.5	1	4.0	0	0
	after admission	19	9.7	3	4.1	2	4.8
Imipenem	on admission	0	0	0	0	0	0
	after admission	0	0	0	0	0	0

On admission: ≤48 hours after admission

After admission: >48 hours after admission

All isolates were Extended Spectrum beta-lactamase producing *Enterobacterales*

Table 2. Univariable analysis of risk factors for Extended Spectrum Beta-lactamase producing *Enterobacterales* colonization at admission

Variable	n	N	%	OR	95% CI	P-value
Prematurity						**0.017**
No	50	410	12.2	1		
Yes	9	157	5.7	0.44	0.21 - 0.91	
Weight at admission						0.083
<2.5Kgs	20	250	8.0	1		
≥2.5Kgs	39	313	12.5	1.64	0.93 - 2.89	
Sex						0.327
Males	31	333	9.3	1		
Females	28	236	11.9	1.31	0.76 - 2.25	
Age at admission						**<0.001**
0 days	8	221	3.6	1		
1-2 days	16	177	9.0	2.65	1.11 - 6.33	
3-28 days	35	171	20.5	6.85	3.09 - 15.21	
Place/mode of delivery						**0.002**
Community	6	116	5.2	1		
Hospital non-CS	32	339	9.4	1.91	0.78 - 4.69	
Hospital CS	21	105	20.0	4.58	1.77 - 11.86	
Mother's age						0.780
<18 years	6	54	11.1	1		
18-35 years	47	455	10.3	0.92	0.37 - 2.27	
>35 years	4	53	7.6	0.65	0.17 - 2.46	
Main water source						0.153
Tap in the compound	27	179	15.1	1		
Tap in community	24	245	9.8	0.61	0.34 - 1.10	
Borehole in community	6	54	11.1	0.70	0.27 - 1.81	
Natural source	1	33	3.0	0.18	0.02 - 1.34	
Water vendor	1	18	5.6	0.33	0.04 - 2.59	
Current feed						0.251
Breastfeeding	39	315	12.4	1		
No breastfeeding	17	187	9.1	0.71	0.39 - 1.29	
Type of toilet						0.169
Toilet in house	16	122	13.1	1		
Toilet shared in compound/community	37	333	11.1	0.83	0.44 - 1.55	
None	6	101	5.9	0.42	0.16 - 1.11	
Number of people living in the same house						**0.012**
1-4	29	227	12.8	1		
5-7	22	167	13.2	1.04	0.57 - 1.88	
8-40	8	162	4.9	0.35	0.16 - 0.80	

CS Caesarean section;
CI Confidence interval

at admission: swabs collected within 48 hours after admission

Missing data: Prematurity n=2, Weight at admission n=6, Place/mode of delivery n=9, Mother's age n=7, Main water source n=40, Current feed n=67, Type of toilet n=13, Number of people living in the same house n=13

CS Caesarean section
CI Confidence interval

Table 3. Univariable analysis of risk factors for acquisition of Extended Spectrum Beta-lactamase producing *Enterobacterales* (ESBL-E) colonization in hospital

Variable	Events	Person days	Rate[§]	Rate ratio	95% CI	P-value
Prematurity						0.948
No	171	802.5	21.31	1		
Yes	112	521.5	21.48	1.01	0.79 - 1.28	
Weight at admission						0.979
<2.5kgs	153	709	21.58	1		
≥2.5kgs	126	582	21.65	1.00	0.79 - 1.27	
Sex						0.155
Males	168	731	22.98	1		
Females	115	594	19.36	0.84	0.66 - 1.07	
Age at admission						0.769
0 days	127	622	20.42	1		
1-2 days	82	364	22.53	1.10	0.84 - 1.46	
3-28 days	74	339	21.83	1.07	0.80 - 1.42	
Place/mode of delivery						0.185
Community	74	354	20.90	1		
Hospital, non-CS	160	769	20.81	1.00	0.76 - 1.31	
Hospital, CS	47	166.5	28.23	1.35	0.94 - 1.95	
Mother's age						0.583
<18 years	27	146.5	18.43	1		
18-35 years	228	1060	21.51	1.17	0.78 - 1.74	
>35 years	25	102	24.51	1.33	0.77 - 2.29	
Treated with antibiotics						0.566
No	20	82.5	24.24	1		
Yes	263	1242.5	21.17	0.87	0.55 - 1.38	
Treated with ampicillin and gentamicin						0.403
No	35	143	24.48	1		
Yes	248	1182	20.98	0.86	0.60 - 1.22	
Treated with third-generation cephalosporins						0.44
No	206	937	21.99	1		
Yes	77	388	19.85	0.90	0.69 - 1.17	
Duration of antibiotic use						0.627
1-3 days	24	133.5	17.98	1		
4-7 days	135	624	21.63	1.20	0.78 - 1.86	
>7 days	104	468	22.22	1.24	0.79 - 1.93	

Variable	Events	Person days	Rate[§]	Rate ratio	95% CI	P-value
Number of neonates present in the hospital per day						**0.001**
1-19	71	465.5	15.25	1		
20-32	163	662	24.62	1.61	1.22 - 2.13	
33-45	49	197.5	24.81	1.63	1.13 - 2.34	
Number of known ESBL-E carriers per day						**<0.001**
0-4	22	260.5	8.45	1		
5-9	88	477	18.45	2.18	1.37 - 3.49	
10-14	122	407	29.98	3.55	2.25 - 5.59	
15-21	51	180.5	28.25	3.35	2.03 - 5.52	
Number of neonates on the same ward						**<0.001**
0-4	14	146	9.59	1		
5-9	49	357	13.73	1.43	0.79 - 2.59	
10-14	70	282.5	24.78	2.58	1.46 – 4.59	
15-20	81	290	27.93	2.91	1.65 – 5.14	
21-29	69	249.5	27.66	2.88	1.62 – 5.12	
Ward at admission						**0.003**
HDU	45	302	14.90	1		
General pediatric	236	1004	23.51	1.58	1.15 - 2.17	
Current feed						0.599
Breastfeeding	140	661.5	21.16	1		
No breastfeeding	111	490.5	22.63	1.07	0.83 - 1.37	
Type of toilet						0.943
Toilet in house	57	248	22.98	1		
Toilet shared with community	162	733	22.10	0.96	0.71 - 1.30	
None	59	255.5	23.09	1.00	0.70 - 1.45	
Main water source						0.327
Tap in compound	87	328.5	26.48	1		
Tap in community	124	554	22.38	0.85	0.64 - 1.11	
Borehole in community	26	141	18.44	0.70	0.45 - 1.08	
Natural source	19	84.5	22.49	0.85	0.52 - 1.39	
Water vendor	7	47	14.89	0.56	0.26 - 1.21	
Number of people living in the same house						0.227
1-4	112	440	25.45	1		
5-7	85	425.5	19.98	0.78	0.59 - 1.04	
8-40	81	371	21.83	0.86	0.64 - 1.14	

CS Caesarean section; HDU High dependency unit;
§ Rate per 100 person day

Table 4: Multivariable analysis of risk factors for acquisition of Extended Spectrum Beta-lactamase producing *Enterobacterales* (ESBL-E) colonization in hospital

	Rate ratio	95% CI	P-value
Number of known ESBL-E carriers [§]			
0-4	1		**<0.001**
5-9	1.77	1.09 - 2.88	
10-14	2.80	1.70 - 4.59	
15-21	2.57	1.48 – 4.48	
Number of neonates on the same ward [§§]			**0.025**
0-4	1		
5-9	1.35	0.74 - 2.46	
10-14	2.13	1.16 - 3.90	
15-20	1.90	1.04 - 3.48	
21-29	1.76	0.93 - 3.31	

[§] Rate ratio adjusted for number of neonates on the same ward and ward at admission.
[§§] Rate ratio adjusted for number of known ESBL-E carriers and ward at admission.

FIGURE LEGENDS

Figure 1: Causal diagram for determinants of Extended Spectrum Beta-lactamase producing *Enterobacterales* carriage at admission

Footnote:

Only variables that had plausible interactions as shown in the casual diagram were included in the multivariable model

Figure 2: Flow of subjects recruited in the study

Footnote: Figure 2.

Among the n=177 not eligible neonatal admissions: 57 were admitted to the general pediatric ward before the study rolled out in that ward, 42 died before consenting, 30 not consented because there was no competent adult available to provide consent on behalf of the child within the first 48 hours of admission, 21 were discharged on admission, 5 were cases of readmission and their ESBL-E carriage status was known, 5 were not followed up after admission, 4 absconded and could not be traced after admission, 4 were considered too tiny for a sample to be collected, 3 were admitted for elective surgery and consenting was not done, 2 had congenital abnormalities and were not approached to participate, 2 could not be enrolled because the parent was a minor, 1 was not consented because the mother could not be approached for consenting, and 1 was enrolled after recruitment had stopped. +ve; positive and –ve; negative.

Figure 3: Kaplan-Meier estimate of Extended Spectrum Beta-lactamase producing *Enterobacterales* (ESBL-E) carriage acquisition as a function of days after admission among 510 neonates who were ESBL-E carriage negative at admission

Figure 1 (.tif and .eps files only)

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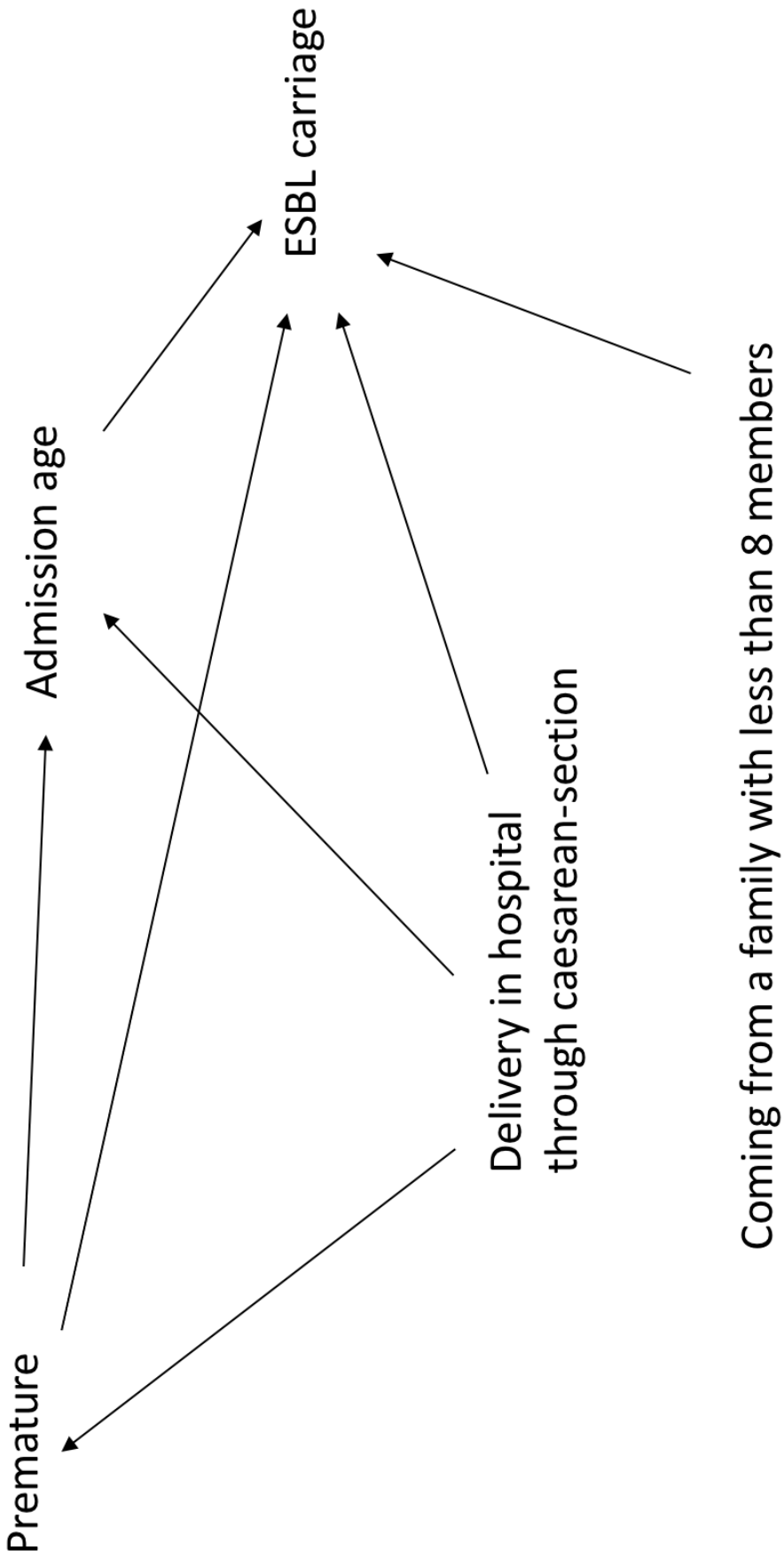

Figure 2 (.tif and .eps files only)

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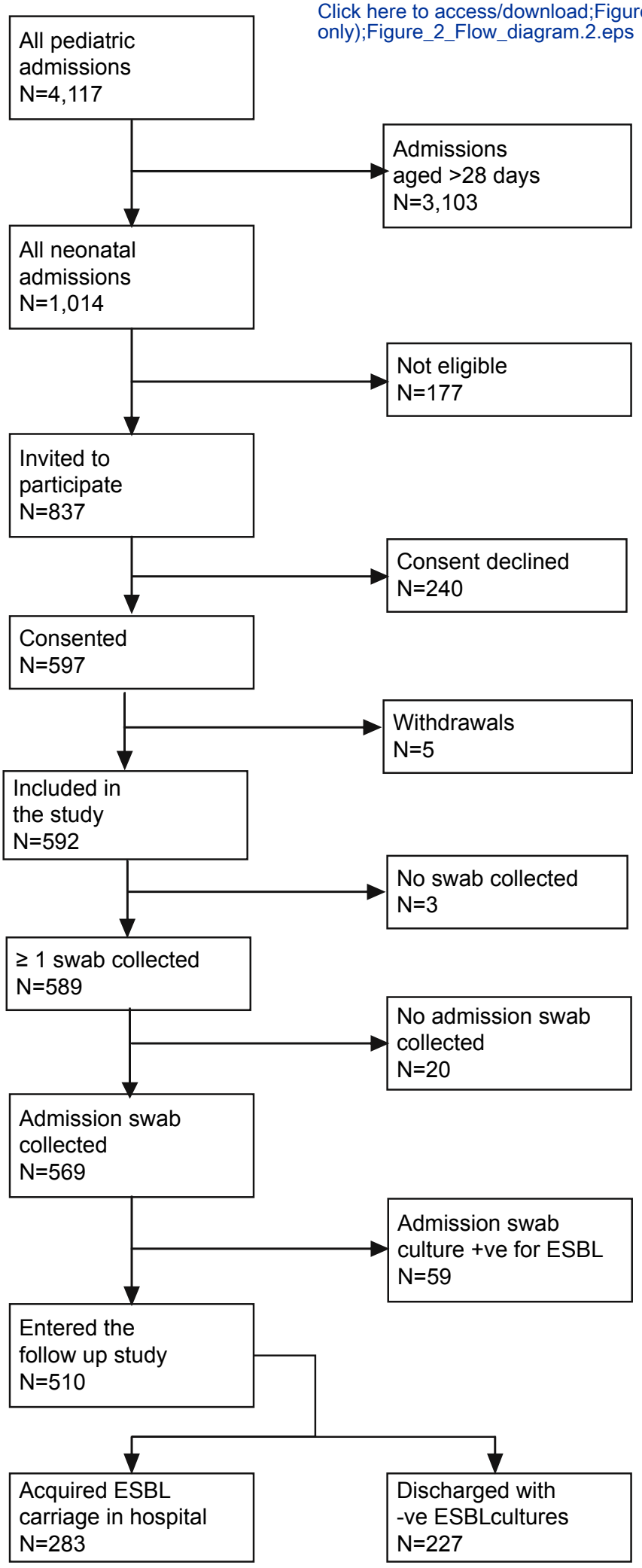

Figure 3 (.tif and .eps files only)

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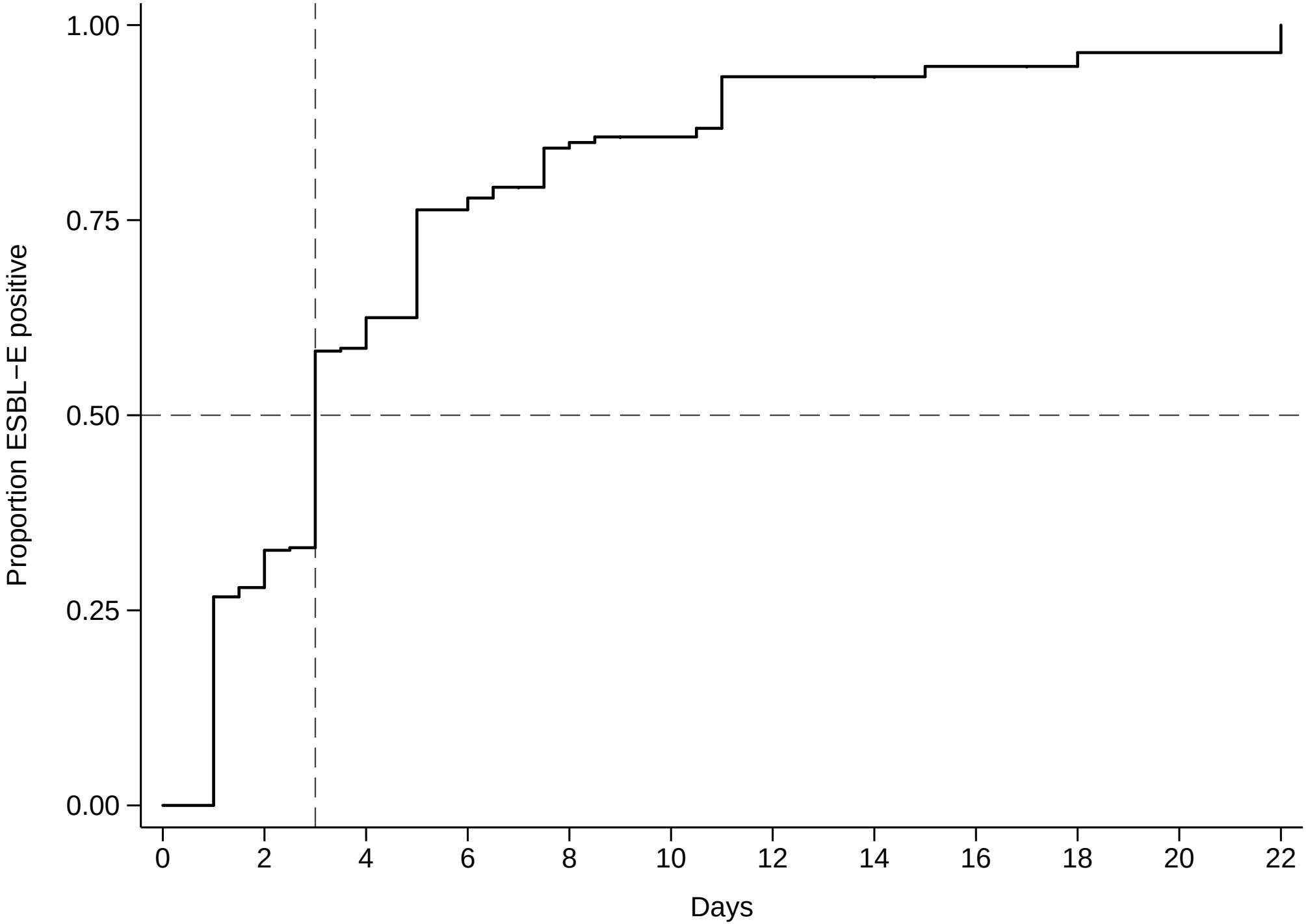

Supplementary Material

Carriage and acquisition of Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae* among neonates admitted to hospital in Kilifi, Kenya

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Table S1. Characteristics of study neonates and those who were eligible but not included, either because they were not swabbed, or because they withdrew prior to the start of the study, or because their parents did not provide consent.

	Included in study (N=569)		Excluded from study (N=268)		
Characteristic	**n**	**%**	**n**	**%**	**P - value**
Sex					0.695
Male	333	58.5	153	57.1	
Females	236	41.5	115	42.9	
Age at admission					0.454
0 days	221	38.8	93	34.7	
1-2 days	177	31.1	93	34.7	
3-28days	171	30.1	82	30.6	
Weight at admission					**0.026**
<2.5kgs	250	44.4	96	36.2	
≥2.5kgs	313	55.6	169	63.8	
Prematurity					**0.004**
Yes	157	27.7	49	18.5	
No	410	72.3	216	81.5	
Place/mode of delivery					**0.005**
Community	116	20.7	30	11.4	
Hospital, non-CS	339	60.5	175	66.5	
Hospital, CS	105	18.8	58	22.1	
Mother's age					0.098
<18 years	54	9.6	14	5.2	
18-35 years	455	81.0	228	85.4	
>35 years	53	9.4	25	9.4	

CS Caesarean section

Missing data:
Included in the study; Weight n=6, Prematurity n=2, Place/mode delivery n=9, Mother's age n=7 and
Excluded from the study; Weight n=3, Prematurity n=3, Place/mode delivery n=5, Mother's age n=1

Table S2 Summaries on Antibiotics given for the 510 neonates recruited into the study

	n	%
Antibiotics given	475	93.1
Given ampicillin and gentamicin	455	89.2
Treated with a third generation cephalosporin	116	22.8

Carriage and acquisition of Extended Spectrum Beta-Lactamase producing *Enterobacterales* among neonates admitted to hospital in Kilifi, Kenya

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Neonates, Extended Spectrum Beta-Lactamase, Carriage, Acquisition, Risk-factors

Running title

Risk factors of carriage and rapid nosocomial acquisition of Extended Spectrum Beta-Lactamase producing *Enterobacterales* amongst neonates admitted to hospital in Kilifi, Kenya

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We report a prospective hospital-based longitudinal study that estimates the ESBL-E carriage prevalence among neonates on admission, the incidence of acquisition of ESBL-E carriage in hospital and the risk factors for neonatal prevalent and incident ESBL-E carriage.

ABSTRACT

Background

Infections caused by extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-E) among hospitalized neonates in sub-Saharan Africa pose significant clinical challenges. Data on prevalence and acquisition of ESBL-E carriage among hospitalized neonates in the region are few and ~~However, sources of infection and~~ risk factors for transmission are not clearly defined ~~in this setting~~.

Methods

In a cohort study of consecutive neonatal admissions to Kilifi County Hospital (KCH) from July 2013-August 2014, we estimated ESBL-E carriage prevalence on admission using rectal swab cultures and identified risk factors using logistic regression. Using twice-weekly follow up swabs, we estimated the incidence and identified risk factors for ESBL-E acquisition in hospital using Poisson regression.

Results

The prevalence of ESBL-E carriage at admission was 10% (59/569). Caesarean section delivery, older neonatal age, and smaller household size were significant risk factors. Of the 510 infants admitted without ESBL-E carriage, 238 (55%) acquired carriage during their hospital stay. The incidence of acquisition was 21.4% (95% CI 19.0, 24.0) per day. The rate was positively associated with the number of known neonatal ESBL-E carriers and with the total number of neonates on the same ward.

Conclusions

Carriage of ESBL-E was common among neonates on admission and acquisition in hospital was rapid. The dissemination and selection of ESBL-E appears to be driven by hospital exposures; operative delivery and neonatal ward patient density. Further attention to infection control, patient crowding and carriage surveillance is warranted.

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INTRODUCTION

Infection and carriage rates of extended-spectrum beta-lactamase producing *Enterobacterales* (ESBL-E) are on the rise globally and pose a particular threat to neonates [1–3]. Outbreaks of multi-drug resistant infections due to ESBL-E in hospitals are common[4–7] and are a growing burden, especially among neonates[3].

It is known that neonatal ESBL-E carriage can be a precursor to invasive infections[7,8] but the epidemiology of transmission in sub Saharan Africa (sSA) is poorly characterized. In sSA, data on neonatal ESBL-E infection and carriage are scarce[2,3] but there is some evidence of hospital-acquired carriage in older children. In a general pediatric ward in Madagascar, prevalence of carriage of ESBL-E in stool was found to be 21% on admission and 57% on discharge, among patients discharged ≥48 hours after admission[9]. In the community, amongst children and adults in Madagascar[10], prevalence of ESBL carriage was 10%.

At Kilifi County Hospital (KCH), we have observed sporadic outbreaks of ESBL-E bacteraemia among neonatal admissions over several years. These infections often have a poor outcome (in KCH the case-fatality risk for hospital-acquired paediatric bloodstream infections is 54%[11]). We have also observed an increase in the proportion of ESBL-producing invasive *Klebsiella pneumoniae* over a decade at Kilifi County hospital[12].

Here we report a prospective hospital-based longitudinal study at KCH to estimate the ESBL-E carriage prevalence among neonates on admission, the incidence of acquisition of ESBL-E carriage in hospital and the risk factors for neonatal prevalent and incident ESBL-E carriage.

METHODS

Study design and sampling procedure

Neonatal admissions were eligible for recruitment into the study if they were admitted to the High Dependency Unit (HDU) between 1st July 2013 and 29th August 2014 or to the neonatal rooms in the general pediatric ward between 16th August 2013 and 29th August 2014. The HDU consists of an open ward with six beds for older children and two small rooms for neonatal admissions. The neonatal rooms are of equal size and have a combined bed-capacity of eight~~, although doubling up of neonates in beds or cots is frequently necessary at KCH.~~ . In the general pediatric ward, there are two neonatal rooms with a combined bed-capacity of 24, including five incubators, four small beds and fifteen cots. KCH practices comprehensive obstetric care, as defined by the World Health Organization, with caesarean section services available.

Data and clinical sample collection

Epidemiological and clinical data were collected on admission and entered in real-time into an electronic medical record system. Rectal swabs were collected on admission (day 0), at days 2, 4 and 6, and twice weekly thereafter until an ESBL-E was isolated or until hospital discharge or death, whichever came first. Rectal swabs were collected using pre-moistened viscose-tipped swabs and placed in Amies transport media (Deltalabs, Barcelona, Spain). The number of neonates in each room, bed-location and antimicrobial use of all participants was recorded daily. Blood culture is performed routinely at admission on all children hospitalized at KCH[13]. Clinical samples are collected at the discretion of the attending clinician.

Laboratory procedure

Rectal swabs were inoculated onto 5% horse blood agar and MacConkey agar supplemented with 8% gentamicin. Cefotaxime (30 ug) and ceftazidime (30 ug) antibiotic discs (Oxoid, United

Kingdom) were added on the 2nd and 4th streaking zones on the blood agar plate to detect bacteria resistant to third-generation cephalosporins. Blood agar plates were incubated in a CO_2 incubator while MacConkey agar plates were incubated in an aerobic incubator for 24 hours at 35 +/- 2^{O}C. Oxidase-negative, gram-negative rods were subcultured and identified using standard techniques (API 20E; BioMérieux, France). Antimicrobial susceptibility testing was performed using the disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2014) guidelines[14]. ESBL testing was performed for isolates that were non-susceptible to third generation cephalosporins using the double disc method[14]. External quality assurance was provided for by UK National External Quality Assessment service.

Analysis

The binomial confidence interval around a prevalence estimate is widest (for a fixed sample size) when the estimate is 50%. We calculated that a sample size of 555 neonates would be required to estimate a 50% prevalence of ESBL-E carriage with a precision of +/-5%.

Carriage on admission (prevalent carriage) was defined as a positive culture on the first rectal swab, provided it was obtained within 48 hours of admission. Logistic regression was used to determine risk factors for carriage at admission. The factors considered were: infant age, sex, maternal age, infant weight at admission, current method of feeding, place and mode of delivery, prematurity (less than or equal to 37 weeks gestation, estimated by the admitting clinician), number living in the same household, type of toilet and main source of water. Multivariable logistic regression models were fitted after confounders had been identified (Figure 1). Infant age at admission was adjusted for prematurity and place/mode of delivery, and prematurity was adjusted for age on admission.

Kaplan-Meier curves were used to describe the time to acquisition of carriage in hospital among neonates who did not have ESBL-E carriage at admission. In this analysis, follow up time, which was measured in days after admission, was censored at the earliest of: (i) time of the first ESBL-E culture positive swab (ii) time of last swab collection if the neonate died or was discharged and had remained negative throughout the course of admission. For neonates who acquired ESBL-E, the date of acquisition was assumed to be the midpoint between the date of the last negative swab and the date of the first positive swab.

We calculated the rate of ESBL-E acquisition per 100 days at risk. Poisson regression was used to identify predictors of the acquisition rate and to test for interactions. The potential predictors were both time-invariant (e.g. weight at admission, place/mode of delivery, mother's age and age at admission) and time-varying (e.g. number of known ESBL carriers on the ward). A multivariable Poisson regression model was fitted to investigate the effect of crowding on ESBL-E acquisition. The model included as covariates the ward, the number of neonates on the ward and the number of known ESBL-E carriers on the ward.

We defined multi-drug resistance as resistance to at least one agent in three or more antimicrobial categories[15]. Statistical analyses were done with STATA 12.0 (StataCorp, College Station, TX, USA).

Study clearance and ethical considerations

The KEMRI National Ethical Review Committee approved the study (SSC 2301). Informed consent was obtained from all parents/guardians before enrollment.

RESULTS

During the study period, 1,014 neonates were admitted to Kilifi County Hospital, and the parents/guardians of 597 neonates gave consent for them to participate in the study (Figure 2). The median age of the participants was one day (IQR 0-3 days) and the median duration of hospital stay was 5 days (IQR 3-9 days).

Of 597 who gave consent to participate, five parents/guardians withdrew consent and 23 neonates had no swab collected. The prevalence of ESBL carriage at admission was 10% (59/569). From the 59 neonates with ESBL-E carriage at admission, there were 65 isolates consisting of 31 *Klebsiella pneumoniae*, 25 *Escherichia coli*, 8 *Enterobacter cloacae* and 1 *Klebsiella oxytoca*. Multiple colonization, ie. colonization with two or more ESBL-E from one participant, was found in 6/59 neonates (10%).

Among the 510 non-carriers on admission, 55% (283/510) acquired ESBL-E during their hospital stay. The incidence of ESBL-E acquisition was 21.4 (95% CI 19.0, 24.0) per 100 child days of observation, and the median time to acquisition among these patients was 3 (IQR 1-5) inpatient days (Figure 3). Nine neonates were diagnosed with ESBL-E bacteraemia during this study, all of whom had ESBL-E isolated from fecal carriage prior to or on the same day as blood was collected.

Most ESBL-E isolates ~~in the study~~ were multi-drug resistant; resistance to chloramphenicol, trimethoprim-sulfamethoxazole, quinolones and gentamicin being common, none were resistant to imipenem and only 5% were resistant to amikacin (Table 1).

Risk factors for ESBL-E carriage at admission

In the univariable analysis, variables associated with prevalent ESBL-E carriage on admission were: being born at term ~~(rather than premature)~~, older infant age at admission, having fewer than eight people living in the same house, and hospital delivery, particularly by caesarean section (CS) (Table 2 and Supplementary Table S1). Babies born prematurely were more commonly admitted directly to the neonatal ward (107/157, 68%) than babies born at full term (114/410, 28%; P-value<0.001). After adjusting for prematurity and place/mode of delivery, increasing infant age was positively associated (p<0.001) with ESBL-E carriage at admission, with odds ratios 1.72 (95% CI 0.69 – 4.27) and 3.88 (95% CI 1.47 - 10.21) among neonates aged 1-2 days and 3-28 days respectively, relative to the odds of ESBL carriage among neonates admitted on the day of birth. Being born at term was not associated with ESBL-E carriage after adjusting for the effect mediated through age on admission. We did not estimate an adjusted OR for place/mode of delivery and number of people in the same household since the associations were not confounded (Figure 1).

Risk factors associated with acquisition of ESBL-E during hospitalization

In the univariable analysis, hospital ward on admission, the number of neonates present in the hospital, the number of other neonates admitted in the same ward, and the number of known ESBL-E carriers were associated with incident acquisition of carriage (Table 3~~). and Supplementary table S2).~~ Both current number of known neonatal ESBL-E carriers and number of other neonates in the same ward were positively associated with carriage acquisition when simultaneously included in multivariable model (Table 4), and there was no interaction between these risk factors. In both the univariable and multivariable analyses, the number of neonates in the ward exhibited a threshold effect whereby there was a plateau effect in carriage acquisition beyond 10 patients per ward. Recorded antibiotic prescription, specifically use of third generation cephalosporins during the inpatient stay was not shown to be associated with ESBL-E acquisition.

DISCUSSION

Our study reveals that among neonates admitted to a rural Kenyan hospital, 10% were already carriers of ESBL-E. Among those neonates who were not carriers at admission, 21.4% acquired ESBL-E carriage each day of admission; thus, more than half of the neonates were colonized with ESBL within the first three inpatient days.

For babies coming in to hospital, the main risk factors for existing rectal carriage with ESBL-E were delivery in hospital via caesarean section and older infant age at admission. For those admitted without carriage of ESBL-E, the principal risk factors for acquisition in hospital were the number of other neonates in the ward and the number with ESBL-E carriage.

Delivery through caesarean section has been reported to be a significant risk factor for prolonged faecal colonization with ESBL producing *K. pneumoniae*[16] and also a determinant of intestinal microflora early in life[17,18]. Mothers undergoing caesarean section are treated with antibiotics for surgical prophylaxis, sometimes extended to treatment of wound infections[19], which may select for antibiotic-resistant enteric bacteria. In Cambodia, young hospital-born infants were found to be at a greater risk of early colonization by third generation cephalosporin~~s~~ resistant gram-negative rods compared to infants born at home, a health centre or other locations and subsequently admitted to hospital[20]. In Madagascar, Herindrainy *et al.* reported that low birth weight, caesarean section and use of antibiotics by mothers at delivery were independently associated with neonatal acquisition of ESBL-E during the first month of life[21]. ~~Hospital born infants through caesarean section delivery have been found to be at a higher risk of ESBL-E carriage in Lebanon[22].~~

The finding that babies coming from a large family of >8 household members were less likely to carry ESBL-E at admission was ~~initially~~ surprising. We speculate that these neonates may have a more diverse gut microbiome, which could be protective against acquisition of ESBL-E carriage.

Increased neonatal age at hospital admission was associated with a greater likelihood of ESBL-E carriage, as expected, since older neonates have had more time to acquire carriage.

Overall, we isolated ESBL-E from 10% of swabs within 48 hours of admission. In a cross-sectional ESBL-E carriage study done in a Tanzanian hospital, the overall neonatal prevalence of ESBL-E carriage was 25.4% [22]. Our findings suggest that some acquisition occurs before neonates come into the paediatric wards and we can speculate that this does not only come from their mothers but also from the procedures and settings of childbirth, particularly caesarian delivery. We did not collect data on ESBL-E carriage in mothers or maternal antibiotic use.

Among neonates admitted without carriage, 55% acquired ESBL-E during hospitalization. An ESBL-E carriage study in a tertiary hospital in Rwanda among inpatients of all ages reported that 55% of participants acquired ESBL-E carriage during hospitalization [23]. A study in Madagascar reported that 48% of pediatric non-carriers at admission acquired ESBL-E during hospitalization. ~~[9]; while in a re-nutrition center in Niger, 94% of recovering malnourished children acquired ESBL-E during treatment with ceftriaxone, although only a few participants were sampled at discharge [25].~~

Our findings from the longitudinal study suggest that the greatest risk factors for ESBL-E acquisition in hospital were having increased numbers of existing ESBL-E carriers among the neonatal patients and a greater number of neonates admitted to the ward. We assume that increased numbers of ESBL-E carriers increase the opportunity for transmission. This finding corresponds with a prospective cohort study done in the general intensive care unit of a hospital in Greece; colonization pressure contributed significantly to acquisition of carriage of carbapenemase producing *Klebsiella pneumoniae* in hospital[24]. Intuitively, hospital crowding is expected to be associated with higher rates of ESBL-E transmission and our results support this.

However, our findings suggest a threshold effect where risk plateaued after admitting more than 10-14 neonates in a ward, suggesting that transmission effects associated with crowding are complex. Restricting the number of neonatal admissions to the hospital is impractical, but this does justify allocating increased space to neonatal admissions. Fixed low healthcare staff numbers relative to numbers of patients, the cultural practice of mothers caring for each other's babies on the ward, physical proximity of adjacent neonates, and shared hygiene facilities, may all contribute to acquisition of ESBL-E carriage by neonates in hospital. As well as direct transmission between babies on the ward, nosocomial carriage acquisition directly from the hospital environment is also possible. A study in Cambodia of transmission of third-generation cephalosporin-resistant *Klebsiella pneumoniae* isolates in a newly opened neonatal unit found that most clusters were likely to have been due to patient sources while 2 of 9 clusters could have been due to either an environmental or a patient source[25].

During the study period, nine neonates were diagnosed with ESBL-E bacteraemia. Nosocomial spread of ESBL-E carriage may result in outbreaks of ESBL-E bacteraemia in the hospital; such outbreaks have occurred in KCH in recent years [12], signifying the importance of awareness of ESBL-E carriage. There is potential for surveillance to help inform hospital infection control and to assist in averting such outbreaks. At KCH screening for carriage of ESBL-E among neonates is not routinely done, hand washing facilities frequently lack water supply, and there are no fully dedicated infection control staff. ~~. In this rural setting, most mothers use reusable cloth diapers and use a shared ablution block in the hospital where they wash soiled nappies for reuse.~~

Antibiotic use has been shown to affect the composition of gut microbiota and is associated with ESBL-E carriage and acquisition[9,16,17,24,26]. Antimicrobial stewardship services are used as part of hospital infection control services to reduce ESBL carriage in well-resourced hospitals. We were unable to detect antibiotic use as a risk factor for ESBL-E acquisition in our study. We

suspect that this is mainly attributed to the fact that 93% of our participants were given antibiotics during their hospital stay and we were therefore underpowered to observe any differences (Supplementary table S2).

We did not collect data from babies after they were discharged from hospital, but patients discharged with ESBL-E carriage have been shown to spread these ESBL-E within family units and close contacts[16,27]. In a prospective cohort study of infants and their families in Norway, the median carriage duration among infants discharged with carriage of ESBL-producing *Klebsiella pneumoniae* after a hospital outbreak was 12.5 months[16]. ~~Another study reported the median time to ESBL-E clearance post discharge to be 6.6 months[30] from carriage data of readmitted adult and paediatric patients.~~ If carriage of ESBL-E persists and intra-household transmission occurs, discharged patients may act as reservoirs of ESBL-E in the community.

Being a hospital-based study, focusing on sick newborns, our estimates of ESBL-E prevalence at admission cannot be generalized to community prevalence. It is theoretically possible that low-level ESBL-E carriage was more prevalent at admission than we were able to determine; below detection rate by culture methods, but then amplified by selection pressure from the use of antibiotics in hospital until detectable. We also were only able to recruit 68% of eligible neonates limiting the generalizability of our findings (Supplementary table S1~~3~~). Of note, significantly more parents/guardians of older neonates, and neonates born in hospital by caesarean section, declined to participate in the study suggesting that our estimate of prevalence of ESBL-E carriage at admission is likely to be an underestimate. Our prevalence and incidence estimates may also be underestimates since stool culture may be more sensitive than rectal swab culture, a single sample is less sensitive than multiple samples for culture~~s~~ and some *Enterobacter spp,* which are known to produce *AmpC* beta-lactamases, may have tested falsely-negative for ESBL by the phenotypic method used. We did not find any carbapenem resistant Enterobacterales (CRE) in

this study, but it is known that such isolates are present in Kenya[28,29]~~, and globally are on the rise~~. Use of central quality assured microbiology laboratories in surveillance for ESBL-E carriage could therefore be expected to have the added benefit of an early warning system for the introduction of CRE carriage.

In conclusion, our findings reveal a high incidence of ESBL-E colonization among hospitalized neonates, which is endemic in this setting. Further work to investigate the association between ESBL-E acquisition and both caesarean section delivery and crowding, perhaps including restrictions on room capacity, and more deliberate cohorting of older neonates and those born in hospital through caesarean section is needed. Given the link between ESBL-E carriage and outbreaks of potentially fatal ESBL-E infection, our data emphasize the importance of routine surveillance and hospital infection control.

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