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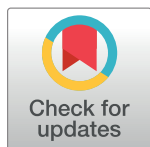


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RESEARCH ARTICLE

Acquisition of extended spectrum beta-lactamase-producing enterobacteriaceae in neonates: A community based cohort in Madagascar

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Data Availability Statement: The data underlying this study are legally restricted by both French and Malagasy regulations. The procedures carried out with the French data privacy authority (CNIL, Commission nationale de l'informatique et des libertés) do not provide for public availability of the database. Requests for data may be sent to the following points of contact: Dr. Ravelomanantena Hantamalala Vololoniaina (Ethics Committee of the Malagasy Public Health Ministry) at [hanta](mailto:hanta@hanta.mg).

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Abstract

In low and middle income countries (LMICs), where the burden of neonatal sepsis is the highest, the spread of extended spectrum beta-lactamase-producing enterobacteriaceae (ESBL-PE) in the community, potentially contributing to the neonatal mortality, is a public health concern. Data regarding the acquisition of ESBL-PE during the neonatal period are scarce. The routes of transmission are not well defined and particularly the possible key role played by pregnant women. This study aimed to understand the neonatal acquisition of ESBL-PE in the community in Madagascar. The study was conducted in urban and semi-rural areas. Newborns were included at birth and followed-up during their first month of life. Maternal stool samples at delivery and six stool samples in each infant were collected to screen for ESBL-PE. A Cox proportional hazards model was performed to identify factors associated with the first ESBL-PE acquisition. The incidence rate of ESBL-PE acquisition was 10.4 cases/1000 newborn-days [95% CI: 8.0–13.4 cases per 1000 newborn-days]. Of the 83 ESBL-PE isolates identified, *Escherichia coli* was the most frequent species ($n = 28$, 34.1%), followed by *Klebsiella pneumoniae* ($n = 20$, 24.4%). Cox multivariate analysis showed that independent risk factors for ESBL-PE acquisition were low birth weight (adjusted Hazard-ratio (aHR) = 2.7, 95% CI [1.2; 5.9]), cesarean-section, (aHR = 3.4, 95% CI [1.7; 7.1]) and maternal use of antibiotics at delivery (aHR = 2.2, 95% CI [1.1; 4.5]). Our results confirm that mothers play a significant role in the neonatal acquisition of ESBL-PE. In

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LMICs, public health interventions during pregnancy should be reinforced to avoid unnecessary caesarean section, unnecessary antibiotic use at delivery and low birth weight newborns.

Introduction

Since 2000, the number of neonatal deaths has decreased from 3.9 million to 2.7 million [1]. Nevertheless, this decline has been slower than the post-neonatal under-five mortality [2] and significant improvements in neonatal health still have to be achieved. Bacterial infections are a leading cause of neonatal deaths in countries with very high under five mortality rate [3,4]. In 2014, it has been estimated that 6.9 million cases of neonatal possible severe bacterial infection (pSBI) occurred in low or middle-income countries (LMICs) [5].

In LMICs, neonatal infections are mainly caused by Enterobacteriaceae, more specifically *Escherichia coli* and *Klebsiella pneumoniae* [6,7]. One important driver of unfavorable outcome in infections caused by these bacteria is multidrug resistance which challenges appropriate therapy [8]. Of particular concern, the extended-spectrum beta-lactamase-producing enterobacteriaceae (ESBL-PE), are the most frequently isolated multidrug resistant bacteria [6,9–11]. First reported in hospital settings, ESBL-PE emerged in the community since the early 2000s [12]. ESBL-PE are resistant to beta-lactams and cephalosporins [13]. Carbapenems remain the last resort to treat pediatric ESBL-PE infections which is onerous and often unavailable in LMICs [14–16].

Enterobacteriaceae are known to colonize the digestive tract [17]. After birth, the intestinal tract of newborns is colonized by the maternal and environment flora [18] which represents the first step for potential neonatal infections [19]. However, data regarding the acquisition of ESBL-PE during the neonatal period are scarce [20]. Bacteria that first colonize the neonatal gut are from different sources (maternal, health-care facilities, community) and the routes of transmission are not well defined [21]. The maternal carriage of ESBL-PE is likely to play a significant role in the colonization and/or infection of newborns in the first week of life [22].

In Madagascar, a recent study among pregnant women at delivery in the community showed a significant prevalence (18.5%) of ESBL-PE carriage, and one strain of *Klebsiella pneumoniae* isolated was a New Delhi metallo-beta-lactamase-1 producer, indicating that pregnant women may represent a substantial source for transmission of multiresistant *Enterobacteriaceae* to neonates [23].

The aim of this study was to understand the neonatal acquisition of ESBL-PE colonization in the community in Madagascar.

Methods

The study was reviewed and approved by the Ethics Committee of the Malagasy Public Health Ministry (no. 113-MSANP/CE 03 November 2014) before the study began and was authorized by the Institut Pasteur in Paris. Written informed consents were obtained from all participants.

Study design

This study was nested within the BIRDY program (“Bacterial Infections and antibiotic Resistant Diseases among Young children in low income countries”, <http://www.birdyprogram.org>). The study design of the BIRDY program has already detailed elsewhere [24]. Briefly, the

present study took place between October 1st, 2015 and September 30th, 2016 in two distinct areas in Madagascar: an urban area, Antananarivo, and a semi-rural area, Moramanga (110 km east from Antananarivo) (Fig 1). All pregnant women of the study areas were identified and were enrolled in the study during their third trimester of pregnancy. An active monitoring of the pregnant women was conducted to ensure the enrollment of neonates at birth.

The mother's sociodemographic, medical and obstetric characteristics and delivery information were also collected as well as the newborn's anthropometric measurements and APGAR score.



Fig 1. Study areas.

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Newborns were included at birth and systematic home visits were planned twice in the first week of life; the first visit occurring within three days after delivery and then weekly in the first month (day 0, day 3, day 7, day 14, day 21 and day 28). At each visit, stools sample or endorectal swabs were collected. We recorded using standardized questionnaires orally administered to the mothers factors potentially associated with the risk of ESBL-PE acquisition, including habitat type, number of rooms, number of household members, parity, diet and hygiene habits, contact with animals, animal ownership, animal husbandry, hospitalization and consumption of antibiotics in the previous days, latrines [12,25–28].

Maternal fresh stools were collected at delivery or shortly after delivery in case of home delivery to detect ESBL-PE. Six stools samples or endorectal swabs from the newborns were obtained during the first month of life starting with the meconium when available, then 2 others during the first week and weekly for the 3 remaining weeks of the first month of life. In case of hospitalization of the newborn, a sample was taken at the entrance and every 7 days during hospitalization. Another sample was taken before discharge from the hospital if hospitalization was less than 7 days.

Microbiological analysis

All samples were analyzed in the laboratory of Experimental Bacteriology unit at the Institut Pasteur in Madagascar. If stools were collected, 1g of sample was suspended in 9ml of physiologic water and dilution in 1/10 was prepared. If endorectal swabs were collected, the swab was suspended in 1ml of physiologic water then vortexed.

Samples were cultivated on CHROMagar ESBL (CHROMagar, Paris, France). The presence of ESBLs in isolates was confirmed by the double-disk synergy test (DDST) [29]. DDST was performed by placing the disk of cefotaxime (30 µg), ceftazidime (30 µg), and combination of amoxicillin/clavulanic acid (20 µg/10 µg) on a lawn culture of bacteria on Muller-Hinton agar plate, with a 20 mm distance between each disk from center to center. Then, plates were incubated at 37°C for 18–24 hrs. Enhancement of the inhibition zone between the disks containing clavulanic acid and cefotaxime or ceftazidime indicated the presence of ESBL production. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as internal quality control strains. Each colony morphotype was identified by mass spectrometry MALDI-TOF, Bruker. Antimicrobial susceptibility testing was performed on each isolate. Amoxicillin, ticarcillin, ticarcillin-clavulanic acid, cefalotin, amoxicillin-clavulanic acid, aztreonam, cefotaxim, ceftazidime, cefepime, imipenem, ertapenem, cefoxitin, cefuroxim, gentamicin, nalidixic acid, ciprofloxacin were tested. The phenotype interpretation was done according to the Antibigram Committee of the French Society of Microbiology (CA-SFM) guidelines 2015[29].

Statistical analysis

Stata version no.14 (Stata Corp., College Station, TX) was used for all statistical analyses. Differences in proportions and means were compared using the chi2 and the student test, respectively. A *P*-value of < 0.05 was considered statistically significant.

We made the assumption that at birth the newborn gut was not colonized by ESBL-PE [18,30]. Also, newborns that had their first sample after the 2 first weeks of life were not included in the survival analysis [31]. We defined low birth weight (LBW) as a birth weight under 2500 g.

Our primary outcome was defined as the first acquisition of ESBL-PE occurring during the first month of life of the newborn; follow-up was thus censored after the first month, or at the date of death, date of withdrawal, or date of last visit for the newborns lost to follow-up, whichever occurred first. We calculated incidence rates of the first acquisition of ESBL-PE per 1000

newborn-days. The ESBL-PE acquisition curves were obtained by the Kaplan–Meier method and compared using the log-rank statistic. To identify factors associated with the first ESBL-PE acquisition, we used a Cox proportional hazard model. We considered variables potentially associated with ESBL-PE acquisition, such as maternal ESBL-PE colonization, antibiotic consumption, and hygiene.

For all factors included in the Cox model, the proportional hazard assumption was validated using a test on Schoenfeld residuals. All factors associated with the outcome with a *P*-value <0.20 in univariate analysis were entered in the multivariate model. A backward selection procedure was applied to identify factors independently and significantly associated with the outcome. We also tested an interaction between maternal ESBL-PE colonization and cesarean delivery; and antibiotic per-partum, respectively. A *P*-value <0.05 was considered significant.

Results

Characteristics of the study population

Of the 351 pregnant women included in the study, 340 gave birth to 343 live newborns who were included in the study (Fig 2). At the end of the study, the mean number of visits was 4.96 (standard-deviation (SD) = 0.04) and 270 newborns had a complete follow-up.

Among the 1755 samples collected in neonates, 678 (39%) were stool samples and 1077 (61%) were endorectal swabs. And among the 275 maternal samples, 177 (64%) were stool samples and 98 (36%) were endorectal swabs.

Table 1 presents the general characteristics of the mothers and newborns. On average, mothers were 27.5 years of age (range 26.8–28.2), 29.1% were primigravidae. More than one-half (54.7%) attended to partial secondary school and more than two third (67.4%) were unemployed. More than 90% used outside toilets and more than one quarter (25.6%) did not have access to electricity. A total of 153 (44.6%) pregnant women gave birth at home. Of the 343 newborns included in the study, 48.1% were male. Their mean birth weight was 3067.6 g (SD = 23.8, range: [1610–4200]); and 7.3% of them had a low birth weight. Among the 308 newborns followed-up, eleven (3.6%) were hospitalized and 5 (1.6%) took antibiotics during their first month of life. Of the 275 mothers with collected stool samples, 54 (19.6%) were colonized with ESBL-PE. The majority were *Escherichia coli* (n = 28, 49.1%) and *Klebsiella pneumoniae* (n = 6, 10.5%) S1 Table.

ESBL-PE acquisition in newborns

A total of 83 ESBL-PE isolates were identified during the follow-up. *Escherichia coli* was the most frequent species (n = 28, 34.1%), followed by *Klebsiella pneumoniae* (n = 20, 24.4%). Eleven strains remained unidentified. When we considered the first acquisition only (n = 55), *Escherichia coli* and *Klebsiella pneumoniae* remained the most prevalent pathogens (n = 17, 30.9% and n = 14, 25.5%, respectively) (see S2 and S3 Tables). Overall, thirty five newborns were excluded from the survival analysis (18 with sample provided after 15th day of life, 17 withdrawals (Fig 2). There was no statistical difference between the characteristics of these newborns and those included in the survival analysis.

The overall incidence of ESBL-PE first acquisition was 10.4 per 1000 newborn-days [95% confident interval (CI) = 8.0; 13.4]. The incidences were 13.7 [8.8; 21.3] and 9.2 [6.7; 12.7] in the urban and semi-rural areas, respectively. We did not find any difference in the incidence of acquisition between the urban and semi-rural areas (log rank test, *p* = 0.22). ESBL-PE acquisition curves according to study areas, newborn's weight, mode of delivery, maternal ESBL-PE colonization status and antibiotics use during delivery are shown in Fig 3A, Fig 3B, Fig 3C, Fig

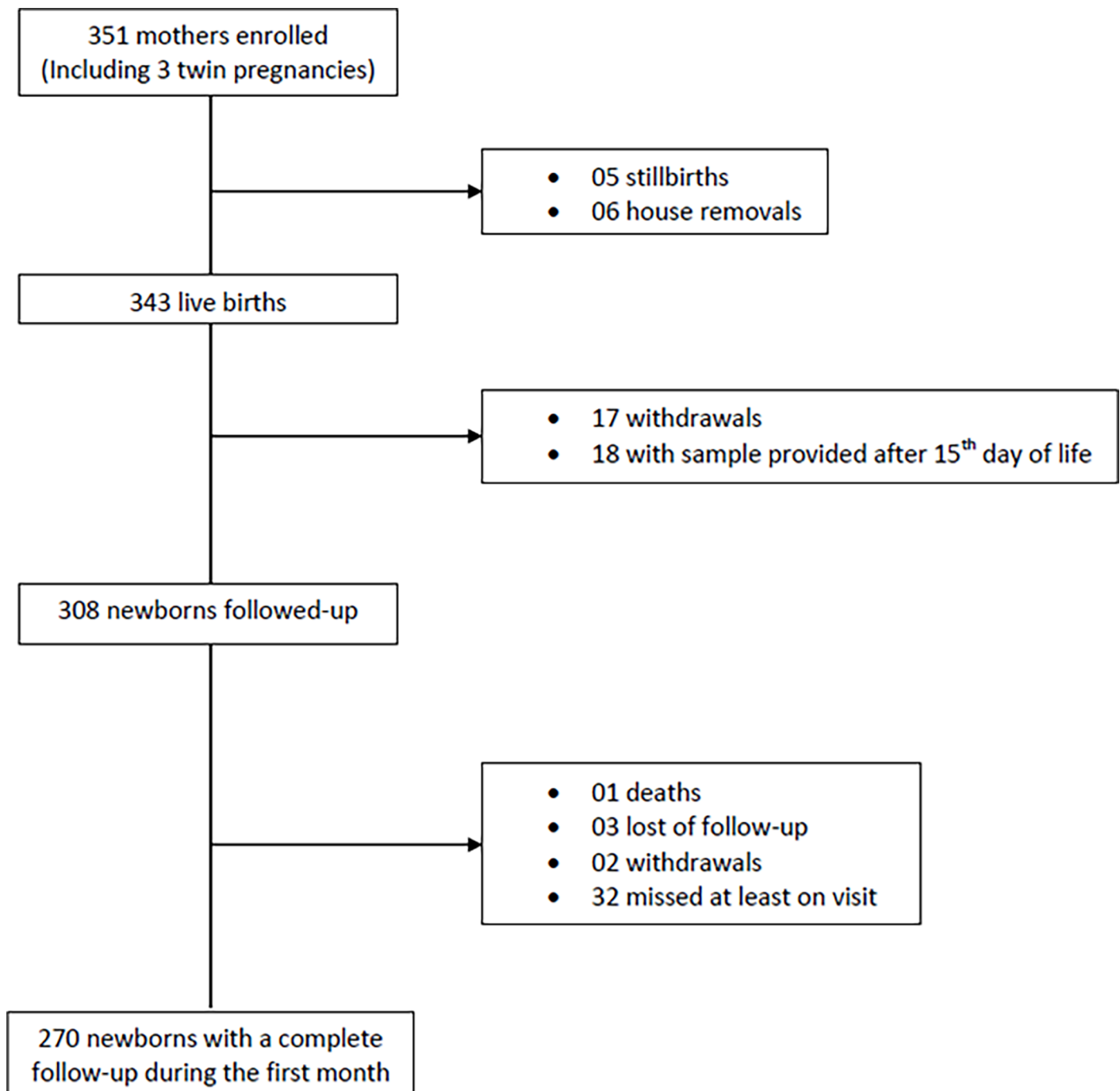


Fig 2. Flow-chart of the study participants.

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3D and Fig 3E, respectively. In univariate Cox analysis (Table 2), cesarean-born babies (Hazard-ratio (HR) = 3.5, 95% CI [1.7; 6.8]) or newborn from a mother who received an antibiotic during delivery (HR = 2.1, 95% CI [1.0; 4.2]) were at higher risk of ESBL-PE acquisition than vaginally born babies or newborn from a mother who did not receive an antibiotic during delivery, respectively. Compared to babies born from a mother not colonized with ESBL-PE, children born from an ESBL-PE colonized mother had an increased risk of acquiring ESBL-PE

Table 1. Characteristics of the mothers and newborns.

	Total	Urban area	Semi-urban area	P ^a
Mothers	340 (100)	103 (30.3)	237 (69.7)	
Age	n (%)	n (%)	n (%)	
Mean (SD ^b)	27.5 (6.5)	26.7 (5.8)	27.9 (6.7)	0.5
Median (IQR ^c)	26 (23; 32)	25 (23; 29)	26 (23; 32)	
Marital status				
Single or divorced	18 (5.3)	2 (1.9)	16 (6.8)	0.1
Married or consensual union	322 (94.7)	101 (98.1)	222 (93.3)	
ESBL colonisation	275 (100)	77 (28)	198 (72)	0.7
Yes	54 (19.6)	16 (20.8)	38 (19.2)	
No	221 (80.4)	61 (79.2)	160 (80.8)	
Education				
No education or primary	77 (22.7)	18 (17.5)	59 (24.9)	0.1
Partial secondary	186 (54.7)	56 (54.4)	130 (54.9)	
Complete secondary or University	77 (22.6)	29 (28.1)	48 (20.2)	
Parity				
Primigravidae	99 (29.1)	33 (32.0)	66 (27.9)	0.4
Multigravidae	241 (70.9)	70 (68.0)	171 (72.1)	
Toilets facilities				
Inside access	24 (7.1)	13 (12.6)	11 (4.6)	0.008
Outside access	316 (92.9)	90 (87.4)	226 (95.4)	
Electricity access				
Yes	253 (74.4)	89 (86.4)	164 (69.2)	0.001
No	87 (25.6)	14 (13.6)	73 (30.8)	
Profession				
Unemployed	229 (67.4)	63 (61.2)	166 (70.0)	0.06
Manual employment	101 (29.7)	34 (33.0)	67 (28.3)	
Office jobs	10 (2.9)	6 (5.8)	4 (1.7)	
Person who followed pregnancy				
No follow-up	10 (2.9)	2 (1.9)	8 (3.4)	0.9
Traditional birth attendant	7 (2.1)	2 (1.9)	5 (2.1)	
Health care worker ^d	323 (95.0)	99 (96.2)	224 (94.5)	
Newborns	343 (100)	105 (30.6)	238 (69.4)	
Sex				
Male	165 (48.1)	52 (49.5)	113 (47.5)	0.7
Place of delivery				
Health care center	190 (55.4)	74 (70.5)	116 (48.7)	>0.001
Home	153 (44.6)	31 (29.5)	122 (51.3)	
Cesarean delivery				
Yes	29 (8.5)	17 (16.2)	12 (5.0)	0.001
No	314 (91.5)	88 (83.8)	226 (95.0)	
Difficult birth				
Yes	25 (7.3)	13 (12.4)	12 (5.0)	0.016
No	318 (92.7)	92 (87.6)	226 (95.0)	
Weight at delivery (gr)	329 (100)	101 (30.7)	228 (69.3)	
< 2500	24 (7.3)	15 (14.9)	9 (3.9)	>0.001
> = 2500	305 (92.7)	86 (85.1)	219 (96.1)	
Hospitalization during the first month of life	308 (100)	89 (28.9)	219 (71.1)	

(Continued)

Table 1. (Continued)

	Total	Urban area	Semi-urban area	p ^a
No	297 (96.4)	82 (92.1)	215 (98.2)	0.01
Yes	11 (3.6)	7 (7.9)	4 (1.8)	
Antibiotics intake during the first month of life	308 (100)	89 (28.9)	219 (71.1)	
No	303 (98.4)	88 (98.9)	215 (98.2)	1
Yes	5 (1.6)	1 (1.1)	4 (1.8)	

^ap, p-value

^bSD, Standard deviation

^cIQR, Interquartile range.

^dHealth care worker: a doctor, a midwife or a nurse.

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(HR = 1.7, 95% CI [1.0; 3.0]). Low birth weight was significantly associated with a higher risk of ESBL-PE acquisition (HR = 2.6, 95% CI [1.2; 5.5]) compared to normal weight babies at birth.

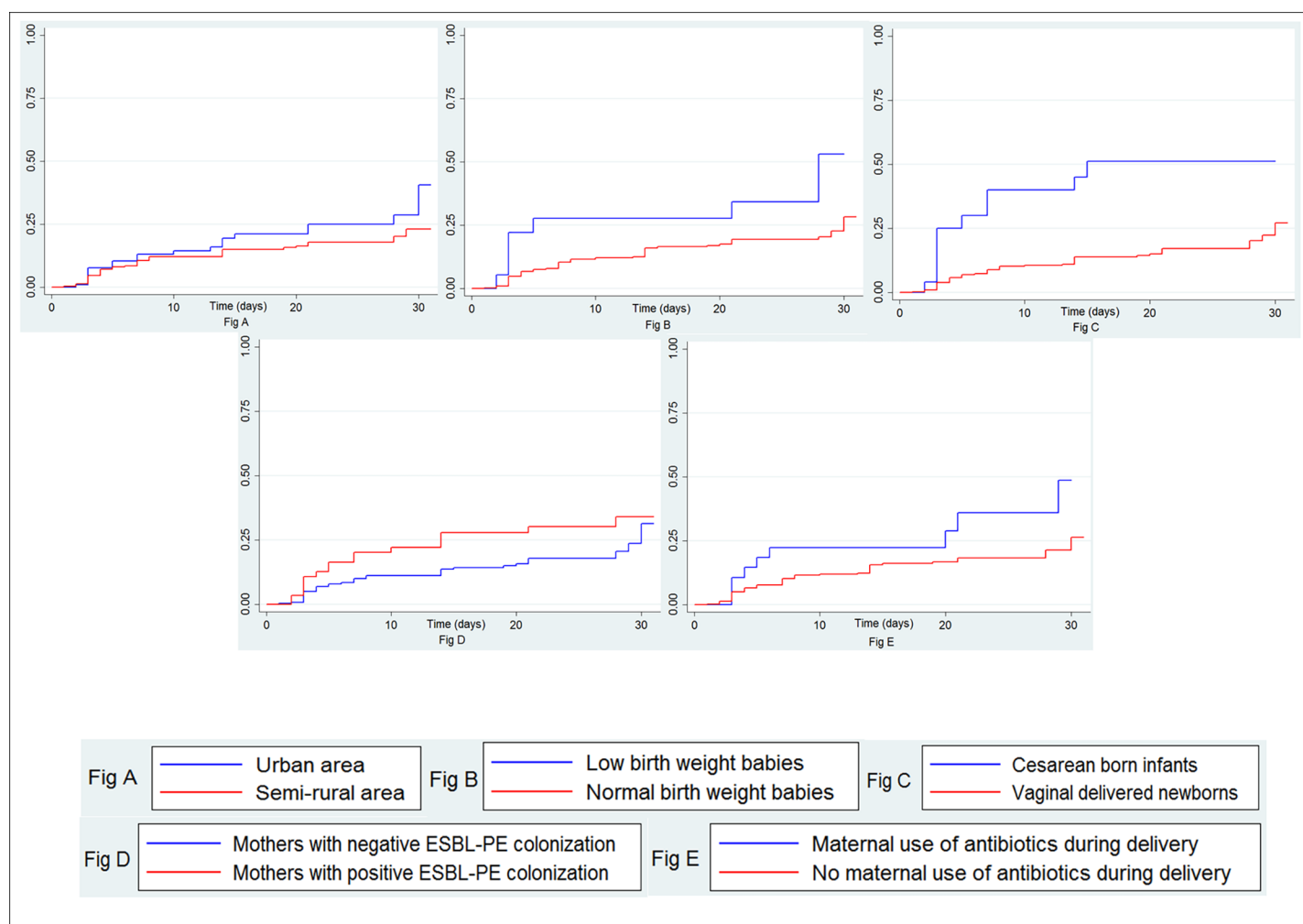


Fig 3. Cumulative incidences according to study areas (Fig 3A), newborn's weight at birth (Fig 3B), mode of delivery (Fig 3C), maternal ESBL-PE colonization status (Fig 3D) and antibiotics use during delivery (Fig 3E).

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Table 2. Cox proportional hazard analysis of ESBL-PE acquisition.

Variable	Person-days	Univariate analysis		Multivariate analysis	
		Crude HR ^a [95% CI ^b]	P ^c	Adjusted HR [95% CI]	p
Low birth weight			0.01		0.01
No (> = 2500gr)	5300	Ref. ^d		Ref.	
Yes (<2500gr)	324	2.6 [1.2; 5.5]		2.7 [1.2 ; 5.9]	
Maternal job			0.21		
No Job	3900	Ref.			
Manual job	1500	1.0 [0.6; 1.9]			
Office job	199	2.5 [1.0; 6.5]			
Latrine type			0.41		
Outside without flush	5100	Ref.			
Outside with flush	137	0.7 [0.1; 5.4]			
Inside without flush	176	0.5 [0.1; 3.8]			
Inside with flush	232	2.1 [0.9; 5.3]			
Habitat type			0.46		
Room shared with other household	4389	Ref.			
House shared with other household	500	0.8 [0.4; 1.4]			
Individual house	410	1.3 [0.2; 2.5]			
Number of rooms			0.63		
1 room		Ref.			
2 rooms and more		1.1 [0.6; 1.9]			
Number of household members			0.16		
Less than 5 members	4500	Ref.			
5 members and more	1097	1.01 [0.5; 1.7]			
Animal ownership			0.32		
No	5370	Ref.			
Yes	214	1.7 [0.2; 10.6]			
Site			0.19		0.96
Semi-rural site	4100	Ref.		Ref.	
Urban site	1500	1.4 [0.8; 2.5]		1.0 [0.5; 1.8]	
Mode of delivery			<0.001		0.001
Vaginal delivery	5300	Ref.		Ref.	
Cesarean	320	3.5 [1.8; 6.8]		3.4 [1.7 ; 7.1]	
Supervising person at delivery			0.14		
Non medical staff	1700	Ref.			
Medical staff	3800	1.6 [0.8 ; 2.9]			
Maternal ESBL-carriage			0.06		0.09
No	4000	Ref.		Ref.	
Yes	1100	1.7 [1.0 ; 3.0]		1.6 [0.9 ; 2.9]	
Antibiotic during delivery			0.04		0.04
No	5200	Ref.		Ref.	
Yes	423	2.1 [1.0; 4.2]		2.2 [1.1; 4.5]	
Place of delivery			0.09		
Home	2600	Ref.			
Health-care facilities	3000	1.6 [0.9 ; 2.7]			
Hospitalization during the first month of life			0.23		
No	5500	Ref.			
Yes	84	2.7 [0.62; 11.2]			

(Continued)

Table 2. (Continued)

Variable		Univariate analysis		Multivariate analysis	
	Person-days	Crude HR ^a [95% CI ^b]	P ^c	Adjusted HR [95% CI]	p
Antibiotics intake during the first month of life			0.54		
No	5500	Ref.			
Yes	58	2.0 [0.27;14.7]			

^aHR, Hazard Ratio

^b95% CI, 95% Confidence Interval

^cp, p-value

^dRef., reference.

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In multivariate analysis (Table 2), factors independently associated with a higher risk of first ESBL-PE acquisition included low birth weight (adjusted Hazard-ratio (aHR) = 2.7, 95% CI [1.2; 5.9]), cesarean delivery (aHR = 3.4, 95% CI [1.7; 7.1]) and maternal use of antibiotics at delivery (aHR = 2.2, 95% CI [1.1; 4.5]). Maternal ESBL-PE colonization status was no longer statistically significant after adjustment. We found no statistically significant interactions.

Discussion

This study showed an overall incidence of 10.4 ESBL-PE acquisitions per 1000 newborn-days within a cohort of newborns in the community in Madagascar. Also, we found that low birth weight, cesarean delivery and maternal use of antibiotic at delivery were major risk factors of acquiring ESBL-PE during the first month of life.

To our best knowledge, this is the first community-based estimate of the incidence of ESBL-PE acquisition in newborns in a LMIC. In these settings, there are very few data from the community and the majority of published studies estimated a prevalence (ranging from 10.0% to 46.0%) which is less accurate than an incidence [12,23,26,32–36]. In addition, most studies [37–40] on the acquisition of ESBL-PE were conducted in neonatal intensive care units where ESBL-PE are more likely to be hospital-acquired and where neonates have serious illness, are more exposed to broad-spectrum antibiotics and are thus not representative of the general population. In these studies, two swabs were usually performed: the first one at admission and the second one at discharge which does not allow an accurate incidence estimation. Our estimate is just below the lower limit of monthly incidence of ESBL-PE acquisition [12 and 53 cases per 1000 patient-days] estimated by Mammina et al. in a neonatal intensive care unit in Italy [31]. Thus, this finding highlights that, even in neonates not particularly exposed to hospital environment, ESBL-PE spread is significant and very fast from the very beginning of life.

We found that cesarean delivery is a risk factor of ESBL-PE acquisition which is concordant with others studies on ESBL-PE infection or carriage [32,41]. Babies delivered by cesarean section are more at risk of handling by health personnel and have generally longer stay in hospital than vaginally delivered newborns. These two factors might increase the risk of acquiring ESBL-PE. Moreover, birth by cesarean section deprives newborns from maternal vaginal and gut flora exposure and may influence the newborn microbiome development [42,43]. Recent studies show that the microbiota play a key role in the protection against infectious diseases [44]. One plausible explanation to our finding is that the maternal microbiota might also play a role in the ESBL-PE acquisition during the first month of life. Concordantly, it has been shown that infants delivered by cesarean section have longer ESBL-PE carriage compared to those vaginally delivered [45].

The rate of cesarean section in low-income countries remains low compared to those observed in high- and middle-income countries [46–49]. However, in LMICs also, cesarean section might be performed while it is not medically indicated, with negative consequences [50]. Our result provides an additional argument to avoid unnecessary cesarean sections.

In the present study, pregnant women were included in their third trimester of pregnancy, which rendered difficult an accurate measurement of gestational age, as none of the included women had a first-trimester ultrasound. Therefore, in our analysis, we used LBW instead, which can be either the consequence of prematurity or intra-uterine growth retardation, or both. We showed that low birth weight babies were at increased risk of acquiring ESBL-PE whatever the ESBL-PE status of the mother. Among the 18 LBW babies with collected stool samples, seven were colonized by ESBL-PE, and only one had been hospitalized among the seven colonized babies, suggesting that the colonization was likely not hospital-acquired. Prematurity has been shown to be a risk factor for acquisition of ESBL-PE [51], whereas to our knowledge the role of intra-uterine growth retardation has never been studied. More studies are then needed to understand which is the underlying pathway of ESBL-PE acquisition in LBW babies, who are consequently more at risk to develop a potentially fatal drug-resistant infection.

Antibiotics during delivery are administered for various reasons [52], including for preventing the transmission of maternal group B *streptococcus* (GBS) to newborns. However, in our study, all mothers (n = 28) who received antibiotics during delivery had fetid amniotic fluid and/or premature rupture of the membrane and/or fever during delivery. Although these factors did not indicate that the mother was definitively infected, the delivery of these women might occurred in a septic context, which might increases the risk of an earlier ESBL-PE acquisition in newborns.

It is the first time, to our knowledge, that maternal use of antibiotics at delivery has been shown as a risk factor of ESBL-PE acquisition in newborns. Penicillin A, the most common antibiotics used [53], is able to cross the placenta and thus to be transmitted to the fetus. Consequently, it is likely that the newborn also received a part of maternal antibiotic and may explain why infants born from mothers who had antibiotics during delivery might be at increased risk of an earlier ESBL-PE acquisition in newborns.

It is well known that unnecessary use of antibiotics has subsequent impact on drug resistance and cost of healthcare in both LMICs and high-resource countries [54–58]. Measures to prevent it are currently under development (rapid diagnostic test) or have already been implemented, for example: through integrated community case management [59]. Thus, our findings support that reinforcing antibiotic stewardship is of the utmost importance. We did not find that hospitalization or antibiotic intake was associated with an increased risk of the first acquisition of ESBL-PE in newborns. As our study was community-based, few newborns were hospitalized (3.6%) or took antibiotics during their first month of life (1.6%) and therefore less exposed compared to neonates of the studies which are for the majority hospital-based [51,60–63]. In community-settings, risk factors might therefore be different and the mother and more specifically pregnancy conditions are predominant for ESBL-PE acquisition in neonates.

Although marginally significant in the univariate analysis, we did not found that maternal ESBL-PE colonization significantly increases the risk of ESBL-PE carriage for the newborns in the multivariate model. When we considered the acquisition of ESBL-PE during the first week of life, only 3 pairs of mother/infant carried the same pathogen. Although other studies found that maternal ESBL-PE was associated with ESBL-PE colonization in newborns [64,65], this finding suggests that mother to child transmission during delivery might not play such a significant role in the acquisition of colonization in the first week of life.

However, we cannot exclude that the none statistically significant associations between hospitalization, use of antibiotics during the first month of life, maternal ESBL-PE carriage and ESBL-PE acquisition in neonates, may be due to a lack of statistical power. Therefore, the interpretation of these results must be cautious.

Our study had some limitations. Six anal swabs were planned during the follow-up. For ethical reasons, it was not possible to perform more frequent swabs. Therefore, the time of acquisition of ESBL-PE might have occurred earlier than at the time of the positive ESBL-PE swab, within the span-time of two consecutive swabs. However, this inaccurate estimation of the acquisition date concerns all included newborns in the same way and is not likely to have had an impact on the risk factors we found associated with a higher risk of ESBL-acquisition. Also, the exposures to LBW, cesarean section and maternal carriage do not change over time and occur before and at the time of birth.

A great majority of the newborns (75.6%) had their first swab within the 3 first days. However, due to logistical issues, 75 newborns could not have their sample taken during this period. Among them, seven had only one sample during their whole follow-up (missed visits or lost-to follow-up). In the 68 remaining newborns, all subsequent samples were negative, suggesting that the missing sample within the three first days of life was likely negative. We are then confident that these limitations may not impact the associations we found.

Eleven Gram negative bacteria (GNB) in newborns stool samples and 14 GNB in mothers stool samples could not be identified by the MALDI-TOF SM. They were all Gram-negative, oxidase-negative, aerobic rods. The scores provided by the MALDI-TOF mass spectrometer were too low (1.2–1.7) and did not allow us to identify the species but gave us an idea about the bacteria genus (Enterobacteriaceae: *Acinetobacter* sp., *Enterobacter* sp., *Kluyvera* sp., *Raoultella* sp. and other Gram negative rods such as *Stenotrophomonas* sp.).

We did not have collect data on ESBL-PE colonization in household members. However, characteristics reflecting a potential transmission from the household members (habitat type, number of room, number of household members) were not significantly associated with ESBL-PE acquisition in newborns.

Conclusions

To our knowledge, we provide the first community-based estimate of the incidence of ESBL-PE acquisition in newborn in LMICs. This incidence is substantial; one other striking result of our study is the key role played by the mother in the acquisition of ESBL-PE by the neonates. We highlight that the course of pregnancy, including delivery and the previous months before birth, may influence the ESBL-PE acquisition and consequently potential subsequent neonatal infection. Our results reinforce that enhancing public-health interventions to promote antibiotic stewardship and to better monitor pregnancy to avoid unnecessary caesarean section, unnecessary antibiotic use at delivery and low birth weight newborns is of utmost importance.

Supporting information

S1 File. BIRDY study group.

(DOCX)

S1 Table. Pathogens isolated from maternal stool samples.

(PDF)

S2 Table. Pathogens isolated from neonatal stool samples during the follow-up.

(PDF)

S3 Table. Pathogens isolated from neonatal stool sample at the first acquisition.
(PDF)

S4 Table. Antibiotic susceptibility in ESBL strains isolated from neonates.
(PDF)

S5 Table. Antibiotic susceptibility in ESBL strains isolated from mothers.
(PDF)

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References

1. Liu L, Hill K, Oza S, Hogan D, Chu Y, et al. (2016) Levels and Causes of Mortality under Age Five Years. In: Black RE, Laxminarayan R, Temmerman M, Walker N, editors. Reproductive, Maternal, New-born, and Child Health: Disease Control Priorities, Third Edition (Volume 2). Washington (DC).
2. UNICEF (New York 2015) Levels & Trends in Child Mortality, Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation, Report 2015.
3. Liu L, Oza S, Hogan D, Chu Y, Perin J, et al. (2016) Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 388: 3027–3035. [https://doi.org/10.1016/S0140-6736\(16\)31593-8](https://doi.org/10.1016/S0140-6736(16)31593-8) PMID: 27839855

4. Oza S, Lawn JE, Hogan DR, Mathers C, Cousens SN (2015) Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000–2013. *Bull World Health Organ* 93: 19–28. <https://doi.org/10.2471/BLT.14.139790> PMID: 25558104
5. Seale AC, Blencowe H, Manu AA, Nair H, Bahl R, et al. (2014) Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. *Lancet Infect Dis* 14: 731–741. [https://doi.org/10.1016/S1473-3099\(14\)70804-7](https://doi.org/10.1016/S1473-3099(14)70804-7) PMID: 24974250
6. Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, et al. (2005) Hospital-acquired neonatal infections in developing countries. *Lancet* 365: 1175–1188. [https://doi.org/10.1016/S0140-6736\(05\)71881-X](https://doi.org/10.1016/S0140-6736(05)71881-X) PMID: 15794973
7. Huynh BT, Padget M, Garin B, Herindrainy P, Kermorvant-Duchemin E, et al. (2015) Burden of bacterial resistance among neonatal infections in low income countries: how convincing is the epidemiological evidence? *BMC Infect Dis* 15: 127. <https://doi.org/10.1186/s12879-015-0843-x> PMID: 25888320
8. Cosgrove SE (2006) The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin Infect Dis* 42 Suppl 2: S82–89.
9. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, et al. (2012) High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Res Notes* 5: 38. <https://doi.org/10.1186/1756-0500-5-38> PMID: 22260454
10. Basu S (2015) Neonatal sepsis: the gut connection. *Eur J Clin Microbiol Infect Dis* 34: 215–222. <https://doi.org/10.1007/s10096-014-2232-6> PMID: 25213719
11. Bhattacharya S (2013) Early diagnosis of resistant pathogens: how can it improve antimicrobial treatment? *Virulence* 4: 172–184. <https://doi.org/10.4161/viru.23326> PMID: 23302786
12. Woerther PL, Burdet C, Chachaty E, Andremont A (2013) Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 26: 744–758. <https://doi.org/10.1128/CMR.00023-13> PMID: 24092853
13. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 18: 657–686. <https://doi.org/10.1128/CMR.18.4.657-686.2005> PMID: 16223952
14. Vardakas KZ, Tansarli GS, Rafailidis PI, Falagas ME (2012) Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum beta-lactamases: a systematic review and meta-analysis. *J Antimicrob Chemother* 67: 2793–2803. <https://doi.org/10.1093/jac/dks301> PMID: 22915465
15. Hsu AJ, Tamma PD (2014) Treatment of multidrug-resistant Gram-negative infections in children. *Clin Infect Dis* 58: 1439–1448. <https://doi.org/10.1093/cid/ciu069> PMID: 24501388
16. Stoesser N, Xayaheuang S, Vongsouvath M, Phommason K, Elliott I, et al. (2015) Colonization with Enterobacteriaceae producing ESBLs in children attending pre-school childcare facilities in the Lao People's Democratic Republic. *J Antimicrob Chemother*.
17. Koleva PT, Kim JS, Scott JA, Kozyrskyj AL (2015) Microbial programming of health and disease starts during fetal life. *Birth Defects Res C Embryo Today* 105: 265–277. <https://doi.org/10.1002/bdrc.21117> PMID: 26663884
18. Bourlioux P, Koletzko B, Guarner F, Braesco V (2003) The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium "The Intelligent Intestine," held in Paris, June 14, 2002. *Am J Clin Nutr* 78: 675–683. PMID: 14522724
19. Carl MA, Ndao IM, Springman AC, Manning SD, Johnson JR, et al. (2014) Sepsis from the gut: the enteric habitat of bacteria that cause late-onset neonatal bloodstream infections. *Clin Infect Dis* 58: 1211–1218. <https://doi.org/10.1093/cid/ciu084> PMID: 24647013
20. Nordberg V, Quizhpe Peralta A, Galindo T, Turlej-Rogacka A, Iversen A, et al. (2013) High proportion of intestinal colonization with successful epidemic clones of ESBL-producing Enterobacteriaceae in a neonatal intensive care unit in Ecuador. *PLoS One* 8: e76597. <https://doi.org/10.1371/journal.pone.0076597> PMID: 24146896
21. Collado MC, Cernada M, Bauerl C, Vento M, Perez-Martinez G (2012) Microbial ecology and host-microbiota interactions during early life stages. *Gut Microbes* 3: 352–365. <https://doi.org/10.4161/gmic.21215> PMID: 22743759
22. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE (2013) Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. *PLoS Med* 10: e1001502. <https://doi.org/10.1371/journal.pmed.1001502> PMID: 23976885
23. Chereau F, Herindrainy P, Garin B, Huynh BT, Randrianirina F, et al. (2015) Colonization of extended-spectrum-beta-lactamase- and NDM-1-producing Enterobacteriaceae among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant

- Enterobacteriaceae to neonates. *Antimicrob Agents Chemother* 59: 3652–3655. <https://doi.org/10.1128/AAC.00029-15> PMID: 25845871
24. Huynh B-T, Kermorant-Duchemin E, Herindrainy P, Rakotoarimanana FMJ, Herisoa F, et al. (2017 (In Press)) Antibiotic resistance, early detection, and treatment of neonatal infections in a community-based cohort, Madagascar. *Emerg Infect Dis*.
25. Beukes LS, King TLB, Schmidt S (2017) Assessment of pit latrines in a peri-urban community in Kwa-Zulu-Natal (South Africa) as a source of antibiotic resistant *E. coli* strains. *Int J Hyg Environ Health* 220: 1279–1284. <https://doi.org/10.1016/j.ijheh.2017.08.002> PMID: 28867170
26. Herindrainy P, Randrianirina F, Ratovoson R, Ratsima Hariniana E, Buisson Y, et al. (2011) Rectal carriage of extended-spectrum beta-lactamase-producing gram-negative bacilli in community settings in Madagascar. *PLoS One* 6: e22738. <https://doi.org/10.1371/journal.pone.0022738> PMID: 21829498
27. Ewers C, Grobbel M, Stamm I, Kopp PA, Diehl I, et al. (2010) Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-beta-lactamase-producing *Escherichia coli* among companion animals. *J Antimicrob Chemother* 65: 651–660. <https://doi.org/10.1093/jac/dkq004> PMID: 20118165
28. Platell JL, Cobbold RN, Johnson JR, Heisig A, Heisig P, et al. (2011) Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. *Antimicrob Agents Chemother* 55: 3782–3787. <https://doi.org/10.1128/AAC.00306-11> PMID: 21646486
29. Comité d'Antibiogramme de la Société Française de Microbiologie (CA-SFM) European Committee on antimicrobial Susceptibility testing (EUCAST). Recommendations Janvier 2015;V1.
30. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, et al. (2006) Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 118: 511–521. <https://doi.org/10.1542/peds.2005-2824> PMID: 16882802
31. Mammina C, Di Carlo P, Cipolla D, Giuffrè M, Casuccio A, et al. (2007) Surveillance of multidrug-resistant gram-negative bacilli in a neonatal intensive care unit: prominent role of cross transmission. *Am J Infect Control* 35: 222–230. <https://doi.org/10.1016/j.ajic.2006.04.210> PMID: 17482993
32. Hijazi SM, Fawzi MA, Ali FM, Abd El Galil KH (2016) Multidrug-resistant ESBL-producing Enterobacteriaceae and associated risk factors in community infants in Lebanon. *J Infect Dev Ctries* 10: 947–955. <https://doi.org/10.3855/jidc.7593> PMID: 27694727
33. Kothari C, Gaiind R, Singh LC, Sinha A, Kumari V, et al. (2013) Community acquisition of beta-lactamase producing Enterobacteriaceae in neonatal gut. *BMC Microbiol* 13: 136. <https://doi.org/10.1186/1471-2180-13-136> PMID: 23773627
34. Ruppe E, Woerther PL, Diop A, Sene AM, Da Costa A, et al. (2009) Carriage of CTX-M-15-producing *Escherichia coli* isolates among children living in a remote village in Senegal. *Antimicrob Agents Chemother* 53: 3135–3137. <https://doi.org/10.1128/AAC.00139-09> PMID: 19364858
35. Tellevik MG, Blomberg B, Kommedal O, Maselle SY, Langeland N, et al. (2016) High Prevalence of Faecal Carriage of ESBL-Producing Enterobacteriaceae among Children in Dar es Salaam, Tanzania. *PLoS One* 11: e0168024. <https://doi.org/10.1371/journal.pone.0168024> PMID: 27936054
36. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E (2016) Fecal Colonization with Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae and Risk Factors among Healthy Individuals: A Systematic Review and Meta-Analysis. *Clin Infect Dis*.
37. Woerther PL, Angebault C, Jacquier H, Hugede HC, Janssens AC, et al. (2011) Massive increase, spread, and exchange of extended spectrum beta-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. *Clin Infect Dis* 53: 677–685. <https://doi.org/10.1093/cid/cir522> PMID: 21890771
38. Andriatahina T, Randrianirina F, Hariniana ER, Talarmin A, Raobijaona H, et al. (2010) High prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a pediatric unit in Madagascar. *BMC Infect Dis* 10: 204. <https://doi.org/10.1186/1471-2334-10-204> PMID: 20624313
39. Das P, Singh AK, Pal T, Dasgupta S, Ramamurthy T, et al. (2011) Colonization of the gut with Gram-negative bacilli, its association with neonatal sepsis and its clinical relevance in a developing country. *J Med Microbiol* 60: 1651–1660. <https://doi.org/10.1099/jmm.0.033803-0> PMID: 21719575
40. Peretz A, Skuratovsky A, Khabra E, Adler A, Pastukh N, et al. (2016) Peripartum maternal transmission of extended-spectrum beta-lactamase organism to newborn infants. *Diagn Microbiol Infect Dis*.
41. T GE, Moges F, Eshetie S, Yeshitela B, Abate E (2017) Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia. *BMC Pediatr* 17: 137. <https://doi.org/10.1186/s12887-017-0892-y> PMID: 28587631

42. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG (2015) The infant microbiome development: mom matters. *Trends Mol Med* 21: 109–117. <https://doi.org/10.1016/j.molmed.2014.12.002> PMID: 25578246
43. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, et al. (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 107: 11971–11975. <https://doi.org/10.1073/pnas.1002601107> PMID: 20566857
44. Blaser MJ, Falkow S (2009) What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol* 7: 887–894. <https://doi.org/10.1038/nrmicro2245> PMID: 19898491
45. Lohr IH, Rettedal S, Natas OB, Naseer U, Oymar K, et al. (2013) Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing *Klebsiella pneumoniae* following a nosocomial outbreak. *J Antimicrob Chemother* 68: 1043–1048. <https://doi.org/10.1093/jac/dks502> PMID: 23288401
46. Althabe F, Sosa C, Belizan JM, Gibbons L, Jacquerioz F, et al. (2006) Cesarean section rates and maternal and neonatal mortality in low-, medium-, and high-income countries: an ecological study. *Birth* 33: 270–277. <https://doi.org/10.1111/j.1523-536X.2006.00118.x> PMID: 17150064
47. Betran AP, Merialdi M, Lauer JA, Bing-Shun W, Thomas J, et al. (2007) Rates of caesarean section: analysis of global, regional and national estimates. *Paediatr Perinat Epidemiol* 21: 98–113. <https://doi.org/10.1111/j.1365-3016.2007.00786.x> PMID: 17302638
48. Stanton CK, Holtz SA (2006) Levels and trends in cesarean birth in the developing world. *Stud Fam Plann* 37: 41–48. PMID: 16570729
49. Buekens P, Curtis S, Alayon S (2003) Demographic and Health Surveys: caesarean section rates in sub-Saharan Africa. *BMJ* 326: 136. PMID: 12531845
50. Nelson JP (2017) Indications and appropriateness of caesarean sections performed in a tertiary referral centre in Uganda: a retrospective descriptive study. *Pan Afr Med J* 26: 64. <https://doi.org/10.11604/pamj.2017.26.64.9555> PMID: 28451041
51. Rettedal S, Hoyland Lohr I, Natas O, Sundsfjord A, Oymar K (2013) Risk factors for acquisition of CTX-M-15 extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* during an outbreak in a neonatal intensive care unit in Norway. *Scand J Infect Dis* 45: 54–58. <https://doi.org/10.3109/00365548.2012.713116> PMID: 22991960
52. Persaud RR, Azad MB, Chari RS, Sears MR, Becker AB, et al. (2015) Perinatal antibiotic exposure of neonates in Canada and associated risk factors: a population-based study. *J Matern Fetal Neonatal Med* 28: 1190–1195. <https://doi.org/10.3109/14767058.2014.947578> PMID: 25053193
53. Mylonas I (2011) Antibiotic chemotherapy during pregnancy and lactation period: aspects for consideration. *Arch Gynecol Obstet* 283: 7–18. <https://doi.org/10.1007/s00404-010-1646-3> PMID: 20814687
54. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, et al. (2005) Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect Dis* 5: 481–493. [https://doi.org/10.1016/S1473-3099\(05\)70189-4](https://doi.org/10.1016/S1473-3099(05)70189-4) PMID: 16048717
55. Organization WH. Antimicrobial resistance: global report on surveillance. Geneva: WHO Press; 2014.
56. Bebell LM, Muir AN (2014) Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? *Glob Heart* 9: 347–358. <https://doi.org/10.1016/j.gheart.2014.08.009> PMID: 25667187
57. Goossens H (2009) Antibiotic consumption and link to resistance. *Clin Microbiol Infect* 15 Suppl 3: 12–15.
58. Shallcross LJ, Davies DS (2014) Antibiotic overuse: a key driver of antimicrobial resistance. *Br J Gen Pract* 64: 604–605. <https://doi.org/10.3399/bjgp14X682561> PMID: 25452508
59. Graham K, Sinyangwe C, Nicholas S, King R, Mukupa S, et al. (2016) Rational use of antibiotics by community health workers and caregivers for children with suspected pneumonia in Zambia: a cross-sectional mixed methods study. *BMC Public Health* 16: 897. <https://doi.org/10.1186/s12889-016-3541-8> PMID: 27567604
60. Abdel-Hady H, Hawas S, El-Daker M, El-Kady R (2008) Extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in neonatal intensive care unit. *J Perinatol* 28: 685–690. <https://doi.org/10.1038/jp.2008.73> PMID: 18580881
61. Crivaro V, Bagattini M, Salza MF, Raimondi F, Rossano F, et al. (2007) Risk factors for extended-spectrum beta-lactamase-producing *Serratia marcescens* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. *J Hosp Infect* 67: 135–141. <https://doi.org/10.1016/j.jhin.2007.07.026> PMID: 17884248
62. Li X, Xu X, Yang X, Luo M, Liu P, et al. (2017) Risk factors for the infection and/or colonization of extended-spectrum beta-lactamase-producing bacteria in a neonatal intensive care unit: a meta-analysis. *Int J Antimicrob Agents*.

63. Shakil S, Ali SZ, Akram M, Ali SM, Khan AU (2010) Risk factors for extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. *J Trop Pediatr* 56: 90–96. <https://doi.org/10.1093/tropej/fmp060> PMID: 19608665
64. Denkel LA, Schwab F, Kola A, Leistner R, Garten L, et al. (2014) The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E). *J Antimicrob Chemother* 69: 2230–2237. <https://doi.org/10.1093/jac/dku097> PMID: 24729603
65. Rettedal S, Lohr IH, Bernhoff E, Natas OB, Sundsfjord A, et al. (2015) Extended-spectrum beta-lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal-neonatal transmission. *J Perinatol* 35: 907–912. <https://doi.org/10.1038/jp.2015.82> PMID: 26507147