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## Transmission Routes of Extended-Spectrum Beta-Lactamase–Producing Enterobacteriaceae in a Neonatology Ward in Madagascar

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**Abstract.** The diffusion of extended-spectrum beta-lactamase (E-ESBL)–producing Enterobacteriaceae is a major concern worldwide, especially in low-income countries, where they may lead to therapeutic failures. In hospitals, where colonization is the highest, E-ESBL transmission is poorly understood, limiting the possibility of establishing effective control measures. We assessed E-ESBL–acquisition routes in a neonatology ward in Madagascar. Individuals from a neonatology ward were longitudinally followed-up (August 2014–March 2015). Newborns’ family members’ and health-care workers (HCWs) were stool-sampled and tested for E-ESBL colonization weekly. Several hypothetical acquisition routes of newborns—e.g. direct contact with family members and HCWs and indirect contact with other newborns through environmental contamination, colonization pressure, or transient hand carriage—were examined and compared using mathematical modeling and Bayesian inference. In our results, high E-ESBL acquisition rates were found, reaching > 70% for newborns, > 55% for family members, and > 75% for HCWs. Modeling analyses indicated transmission sources for newborn colonization to be species dependent. Health-care workers’ route were selected for *Klebsiella pneumoniae* and *Escherichia coli*, with respective estimated transmission strengths of 0.05 (0.008; 0.14) and 0.008 (0.001; 0.021) ind<sup>-1</sup> day<sup>-1</sup>. Indirect transmissions associated with ward prevalence, e.g. through hand carriage or environment, were selected for *Enterobacter cloacae*, *E. coli*, and *K. pneumoniae* (range 0.27–0.41 ind<sup>-1</sup> day<sup>-1</sup>). Importantly, family members were not identified as transmission source. To conclude, E-ESBL acquisition sources are strongly species dependent. *Escherichia coli* and *E. cloacae* involve more indirect contamination, whereas *K. pneumoniae* also spreads through contact with colonized HCWs. These findings should help improve control measures to reduce in-hospital transmission.

### INTRODUCTION

Bacterial diseases are a major cause of neonatal mortality in low-income countries.<sup>1,2</sup> The dissemination of antibiotic-resistant bacteria (ARB), particularly extended-spectrum beta-lactamase–producing Enterobacteriaceae (E-ESBL), is of major concern. Extended-spectrum beta-lactamase–producing Enterobacteriaceae, specifically *Klebsiella pneumoniae* and *Escherichia coli*, can cause severe infections, including urinary tract infections, gastroenteritis (for *E. coli*), intra-abdominal infections and bacteremia.<sup>3</sup> These infections frequently lead to therapeutic failures with third-generation cephalosporins. The drugs required to treat them are expensive and largely unaffordable in low-income countries.

In hospitals, ARB spread can have major consequences on patients’ prognoses and increase treatment costs.<sup>4,5</sup> Newborns in the neonatal intensive care unit are at particularly high risk of acquiring resistant infection due to the immaturity of their immune systems, frequent antibiotic treatment, and frequent use of central or peripheral venous lines or intubation.<sup>6</sup> Controlling nosocomial E-ESBL spread<sup>4,5</sup> requires efficient prevention strategies. However, in low-income countries, very little is known about prevalence and hospital transmission of E-ESBL, and ARB more generally.<sup>4,7</sup> This knowledge gap

impedes reliable estimation of infection burden estimates and consequently, optimal infection prevention strategies.

Mathematical models are useful tools to analyze data and gain insight into the dynamics of ARB acquisition in hospitals.<sup>8,9</sup> They have been widely used to assess in-hospital *Staphylococcus aureus* transmission.<sup>7,8,10</sup> Fewer studies have explored nosocomial E-ESBL spread, limiting our understanding of in-hospital transmission routes.

In this study, we examined E-ESBL acquisition routes in a neonatology ward by analyzing data from a longitudinal Madagascan study using mathematical modeling and statistical inference.

### METHODS

**Study design.** The longitudinal study was conducted in the neonatal unit in CENHOSOA in Antananarivo, Madagascar (August 27, 2014–March 06, 2015). The ward has two monitoring rooms and two bedrooms shared by mothers and their newborns. During the study period, 21 health-care workers (HCWs) worked in the unit sharing day and night shifts. Details about ward organization are provided in Supplemental Material 1.

Among the 36 neonates admitted to monitoring rooms during the study period, 14 were not included because of Christmas interruption of inclusion or parental refusal (see Supplemental Material 2 for details). The remaining 22 were included in the cohort and were followed-up until discharge or death. Average stay in the unit was 18 days. All HCWs and newborns’ accompanying family members were also followed-up. Mothers represented most included family

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members, who were involved in basic infant care, except for one child who had four distinct accompanying family members, including her mother (details about newborns in Supplemental Table 1).

At enrollment, a rectal swab was obtained from the newborn and a stool sample from the family member to detect E-ESBL colonization. Weekly rectal swabs were systematically obtained from newborns over the duration of their stay. For stays < 7 days in the unit, a stool sample was obtained the day of discharge. Stool and hand-carriage samples were also collected from the family member (every week) and from the HCWs (every week for stools and every other day for hand-carriage). In total, 22 newborns, 21 HCWs, and 24 family members were included in the study.

An additional 98 samples were taken from 23 environmental locations over the study period, including surfaces of incubators, door handles, baby-scales, benches, infant radiant warmers, taps, and toilets.

The study was approved by the Madagascar Public Health Ministry Ethics Committee (Reference number: 040-MSANP/CE).

**Microbiology.** All samples were transported for analysis to the Pasteur Institute in Madagascar. Bacteria from samples were identified by culture on CHROMagar ESBL (CHROMagar, Paris, France),<sup>11</sup> a medium-selecting bacteria resistant to third-generation cephalosporins. The plates were incubated at 37°C for 24 hours. For each positive sample, all morphotypes on the selective medium were isolated. The isolated species were identified by Gram staining and mass spectrometry Matrix Assisted Laser Desorption Ionisation - Time of Flight. Susceptibility testing for 10 antibiotics (amoxicillin-clavulanic acid, aztreonam, ceftazidime, cefalotin, cefotaxime, cefepime, cefoxitin, imipenem, ciprofloxacin, and gentamicin) used the disk-diffusion method on Mueller-Hinton agar (BioRad, Marnes-la-Coquette, France) according to the guidelines of the *Comité de l'Antibiogramme de la Société Française de Microbiologie*.<sup>12</sup> Extended-spectrum beta-lactamase-producing Enterobacteriaceae production was confirmed by double-disk synergy testing between amoxicillin-clavulanic acid and ceftazidime and/or cefotaxime and/or cefepime. Among all identified Enterobacteriaceae, we focus here only on the three most common species: *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae*.

**Mathematical modeling.** To assess the sources of transmission to newborns, we developed a stochastic, discrete-time dynamic model.

**Extended spectrum beta-lactamases acquisition.** Acquisition of a new bacterial species was defined as a positive test sample following previous negative results. To account for imperfect sensitivity of bacterial detection in stool samples (which may not reflect the colonization of the whole intestine<sup>13</sup>), sample results were smoothed based on the strain's phenotypic antibiotic resistance profile. When up to two successive samples were negative between two positive samples (+ - + or + - - +) with the same antibiotic resistance profile, the negative result(s) (-) was (were) considered false-negatives (+) (Supplemental Material 3).

**Transmission model.** We developed a model of bacterial acquisition for newborns in the ward. We assumed three possible sources of E-ESBL acquisition in newborns (Figure 1): 1) through contact with bacteria present in the ward environment (e.g. contaminated surfaces, incubator etc. or

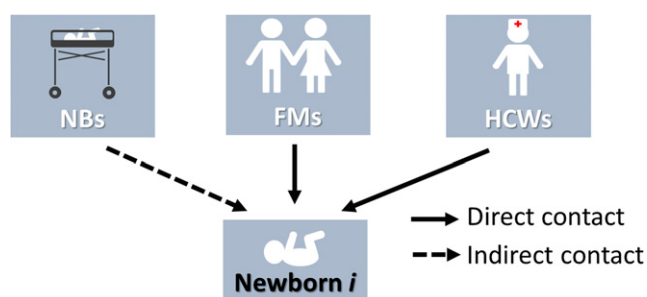


FIGURE 1. Hypothetical extended-spectrum beta-lactamase acquisition sources for newborns (NBs). Three acquisition routes were assumed in the mathematical model (from left to right): environmental contamination or undetectable transient hand carriage by adults, considered to be proportional to the colonization pressure from NBs on the ward, direct contacts with family members (FMs) and direct contacts with health-care workers (HCWs). The shape of the line defines type of contact (straight line: direct inter-individual contact; and dotted line: indirect contact through environmental contamination). This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

undetetectable transient hand carriage by adults), 2) infectious contacts with newborn's colonized family members, and/or 3) infectious contacts with colonized HCWs. Environmental contamination was considered to be proportional to colonization pressure from newborns on the ward, assuming that a high ward frequency of colonized newborns would represent a higher risk of environmental contamination. For a given E-ESBL species, the probability of acquisition in non-colonized newborn  $k$  on day  $t$  is defined by the force of infection,  $\lambda_k$  as follows<sup>14</sup>:

$$\lambda_k(t) = \beta_1 y_{\text{NB}}(t) + \beta_2 y_{\text{FM},k}(t) + \beta_3 y_{\text{HCW}}(t),$$

where  $\lambda_k$  is the daily acquisition risk for newborn  $k$ ,  $\beta_1$  is the daily acquisition rate from the environment for each additional ward newborn colonized,  $\beta_2$  is the daily transmission risk from each colonized family member, and  $\beta_3$  is the daily transmission risk from each colonized HCW.  $y_{\text{NB}}(t)$ ,  $y_{\text{FM},k}(t)$ , and  $y_{\text{HCW}}(t)$  define, respectively, the numbers of colonized newborns, family member(s) of the newborn  $k$ , and HCWs on day  $t$ . Colonized HCWs were only included in the model during their working days.

**Parameter estimations.** Data were analyzed by fitting the model to the observed newborn E-ESBL acquisitions. Acquisition likelihood was defined assuming a hierarchical model with three components: observation model, transmission model, and the prior model. Because exact acquisition dates are unknown, we used data-augmentation techniques and uniformly sampled among all possible acquisition dates, between the last negative and the first positive samples. Details about model definitions, likelihood calculations and data augmentation are provided in Supplemental Materials 4 and 5.

Infectious contact rates ( $\beta$ s) of the different acquisition routes were estimated, based on the observed data, using Markov chain Monte Carlo with the Metropolis-Hastings algorithm.

**Model comparison.** For each bacterial species (*E. coli*, *K. pneumoniae* and *E. cloacae*), this modeling framework was used to compare four hypothetical models describing distinct hypotheses regarding transmission routes to newborns. For each model, the force of infection was defined as a linear combination of forces of infections for each assumed source (Table 1). The four hypothetical models were independently

TABLE 1

List of hypothetical models with different complexity levels concerning extended-spectrum beta-lactamase-producing Enterobacteriaceae transmission routes to newborns

Acquisition source(s), <i>n</i>	Model*		Estimated parameters, <i>n</i> (number(s) of $\beta$ s; number(s) of augmented dates)		
	Name	Equation	<i>E. coli</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>
1	M1 <sub>NB</sub>	$\lambda_i(t) = \beta_1 Y_{NB}(t)$	1; 8	1; 12	1; 13
2	M2 <sub>NB,FM</sub>	$\lambda_i(t) = \beta_1 Y_{NB}(t) + \beta_2 Y_{FM,i}(t)$	2; 17	2; 22	2; 22
	M2 <sub>NB,HCW</sub>	$\lambda_i(t) = \beta_1 Y_{NB}(t) + \beta_3 Y_{HCW}(t)$	2; 40	2; 13	2; 22
3	M3	$\lambda_i(t) = \beta_1 Y_{NB}(t) + \beta_2 Y_{FM,i}(t) + \beta_3 Y_{HCW}(t)$	3; 49	3; 23	3; 31

\* From top to bottom, the first model is characterized by one exclusive acquisition source, named environmental contamination, for which transmission is assumed to be proportional to the colonization pressure in the ward (M1<sub>NB</sub>). The two next models assume two acquisition sources: M2<sub>NB,FM</sub>, for environmental contamination and contacts with family members and M2<sub>NB,HCW</sub> for environmental and HCW contacts. The last model allows for the three distinct transmission sources.

fitted to the observed data and posterior distributions were drawn for each parameter. We compared the different models based on an adapted version of the deviance information criterion (DIC) for the augmented data<sup>15</sup> (Supplemental Material 6), the best model being the one minimizing the DIC.

**Transmission tree.** Based on parameter estimation, model-selection results, and selected-acquisition dates, potential transmission networks via the different colonization routes for each bacterial species were constructed. The most probable colonization date for each newborn *k* was selected from the posterior distribution of the augmented dates, and potential transmitters were selected by searching among all potential acquisition sources around the newborn on that date.

## RESULTS

**Epidemiological results.** Of 68 rectal swabs collected from newborns, 50 (73.5%) were E-ESBL positive. Among family members, 27 (56.3%) of the 48 collected stool samples were E-ESBL positive. Among HCWs, 50 of the 105 (47.6%) stool samples were E-ESBL positive. Notably, 72.7% of newborns, 62.5% of family members, and 76.2% of HCWs had at least one positive sample to E-ESBL during the study period. It represented 15, 27, and 59 ESBL *E. coli*; 62, 19, and 19 ESBL *K. pneumoniae*; and 16, 11, and 1 ESBL *E. cloacae* isolated, respectively, from newborns, family members, and HCWs (Supplemental Table 2, Supplemental Figures 2–4). More than 1 distinct studied ESBL-producing species were carried by 35.8% of individuals over the study period, not necessarily simultaneously (Supplemental Table 3). For family members, only 3/62 (4.84%) hand-carriage samples were positive for *K. pneumoniae*, much lower than for rectal swabs (39.6%). Similarly, for HCWs, among 268 hand carriage samples performed, only four ESBL *E. coli* (1.49%), 13 ESBL *K. pneumoniae* (4.85%), and three ESBL *E. cloacae* (1.12%) were isolated. Owing to their scarcity, positive hand-carriage samples were not included in the modeling analysis to keep the simplest model possible.

Of 98 environmental samples, only four were found to be E-ESBL positive. Because these samples were not available all through the study period, this variable was not included in the model either.

**Acquisition events.** Only individuals who were not positive to a given species at inclusion were assumed to be at risk of acquisition for this particular species. After smoothing of the individual data, E-ESBL *E. coli*, *K. pneumoniae*, and *E. cloacae* acquisitions were reported, respectively, in five, seven, and six newborns (Figure 2). Extended-spectrum beta-lactamase-producing Enterobacteriaceae acquisitions were also observed

in family members with one *E. cloacae*, one *E. coli*, and four *K. pneumoniae* acquisitions. Health-care workers had 13 E-ESBL acquisition events: 10 *E. coli* and three *K. pneumoniae*.

**Model comparison.** For each studied species, the four hypothetical models were compared to identify the most parsimonious model that best reproduced the newborns' E-ESBL acquisition throughout the study (Table 2 and detail in Supplemental Tables 4–6 in 7.2). For *E. coli* and *K. pneumoniae*, the selected model was M2<sub>NB,HCW</sub>, which includes acquisition through indirect contacts with other newborns (including potential environmental contamination of surfaces, or transient hand carriage by surrounding adults) and contacts with colonized HCWs. For *E. cloacae*, the best model was M1<sub>NB</sub>, which includes only acquisition via indirect contacts with other newborns. Interestingly, none of the selected models included acquisition from family members.

**Transmission rates.** Figure 3 depicts the estimated transmission rates for each selected model and bacterial species. Similar median daily transmission rates through other newborns' colonization pressure in the ward were found,  $\beta_{NB}$ , for the three bacterial species: *E. coli* (M2<sub>NB,HCW</sub>), 0.027 ind<sup>-1</sup> day<sup>-1</sup> (0.0035; 0.072) (median value [95% credible interval]); *E. cloacae* (M1<sub>NB</sub>), 0.041 ind<sup>-1</sup> day<sup>-1</sup> (0.012; 0.094); and for *K. pneumoniae*, 0.041 ind<sup>-1</sup> day<sup>-1</sup> (0.011; 0.088). We also found similar HCW-to-newborn transmission rates for *K. pneumoniae*,  $\beta_{HCW}$  0.049 ind<sup>-1</sup> day<sup>-1</sup> (0.008; 0.145), M2<sub>NB,HCW</sub>. This parameter was roughly 6.4 times smaller for *E. coli* (0.0076 ind<sup>-1</sup> day<sup>-1</sup> [0.001; 0.021]), than for *K. pneumoniae*. For each selected model, posterior distributions of estimated transmission parameters, correlation plot, and posterior distributions of newborns' acquisition dates are detailed in Supplemental Figures 5–7 (in 7.3).

**Transmission trees.** The reconstructed networks from the model selected for each bacterium, as shown in Figure 4, illustrate the importance of HCWs and colonization pressure by other newborns in the selected transmission routes. The transmission trees differ markedly for the three species of interest. Notably, although the same models were selected for *E. coli* and *K. pneumoniae*, the colonization pattern of HCWs strongly differed between the two species. For ESBL *E. coli*, six colonized HCWs were highlighted as potential colonizers for five distinct newborn acquisitions. For ESBL *K. pneumoniae*, three colonized HCWs were highlighted as potential colonizers for seven newborn acquisitions. These identified HCWs represented 38% (6/16) of all ESBL *E. coli* colonized HCWs and 50% (3/6) of all ESBL *K. pneumoniae* colonized HCWs. Regarding the role of newborn colonization pressure in the ward, a smaller number of newborns seeded

TABLE 2  
Monte Carlo results for all hypothetical models applied to the extended-spectrum beta-lactamase *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* acquisition data

Model	<i>E. coli</i>			<i>E. cloacae</i>			<i>K. pneumoniae</i>		
	Log-likelihood*	DIC	† f	Log-likelihood <sup>a</sup>	DIC	† f	Log-likelihood <sup>a</sup>	DIC	† f
M1NB	-1,503.35 (-1,505.94; -1,502.96)	3,031.76	2	<b>-2,245.74 (-2,248.24; -2,245.02)</b>	<b>4,516.39</b>	<b>3</b>	-1,507.86 (-1,510.32; -1,506.98)	3,040.68	2
M2NB-FM	-764.12 (-767.59; -763.22)	1,577.59	1	-2,246.71 (-2,250.57; -2,245.37)	4,543.84	3	-1,507.13 (-1,510.60; -1,504.98)	3,064.58	2
M2NB-HCW	<b>-25.27 (-28.72; -23.74)</b>	<b>100.66</b>	<b>0</b>	-2,246.75 (-2,250.23; -2,245.39)	4,543.44	3	<b>-768.35 (-771.62; -766.93)</b>	<b>1,586.03</b>	<b>1</b>
M3	-26.50 (-30.48; -24.93)	149.97	0	-2,247.69 (-2,252.02; -2,245.83)	4,570.24	3	-767.83 (-772.08; -765.13)	1,611.15	1

CI = credible interval; DIC = deviance information criterion. For each bacterial species (in columns), we provide the resulting numerical approximation of the log-likelihood, as characterized by its median and 95% CrI and DIC for the seven hypothetical models (in rows). For each bacterial species, the selected mode(s) is (are) highlighted in bold.

\* Expressed as median (95% CrI).

† f: number of not explained acquisition(s) for each model, each unexplained acquisition adds -7.44 to the numerical approximation of the log-likelihood.

to be involved in the transmission of ESBL *E. coli* and ESBL *E. cloacae* (respectively, three and four colonized newborns identified as potential sources for five and three newborn acquisitions) compared with ESBL *K. pneumoniae* (seven colonized newborns identified as potential sources for six newborn acquisitions). Notably, the assessed models could not explain three acquisitions of ESBL *E. cloacae* (NB 11 on day 88; NB 13 on day 105; one among NBs 18, 20, or 21 over days 147–160) and one acquisition of ESBL *K. pneumoniae* (newborns 10 on day 110).

Bacterial phenotypic resistance profiles were used to assess the predicted transmission paths. For all new acquisitions with at least one potential colonizer found, one transmission path at least involved  $\leq 2$  differences between the phenotypic resistance profiles over the 10 tested antibiotics of potential donor and acquirer. The trees and distances are provided in detail in Supplemental Tables 7–9, Supplemental Figure 8 in 7.4.

## DISCUSSION

Based on longitudinal follow-up of newborns in a Madagascan neonatology ward, we used mathematical models to estimate the most relevant E-ESBL bacterial transmission routes in newborns. In *E. coli*, indirect contamination dominated, i.e. contamination not mediated by contact with colonized adults, with a particularly low estimated transmission rate from colonized HCWs. In *K. pneumoniae*, the same transmission routes as *E. coli* were selected but a higher transmission rate from colonized HCWs was estimated. For *E. cloacae*, only indirect transmission from other newborns from the ward was retained as relevant for transmission.

Data on E-ESBL colonization in newborns in low-income countries are very limited. A few studies have described high ARB-colonization rates in Africa.<sup>1,16–23</sup> Thirty-one percent fecal E-ESBL carriage was found at hospital admission in Niger,<sup>20</sup> and 94% at discharge in admitted non carriers.<sup>21</sup> In another Madagascan study, ~21% of newborns were colonized at admission and 57% at discharge.<sup>22</sup> Herein, among the 15 newborns hospitalized for > 5 days in the unit, all acquired at least one E-ESBL during their stay.

Most previous modeling studies of E-ESBL transmission have analyzed multiple E-ESBL species as one group,<sup>24–26</sup> as after sampling, the first step of microbiological analysis is the characterization of E-ESBL. To our knowledge, this is the first mathematical modeling study to assess acquisition routes according to specific species. After E-ESBL characterization, the identification of the strain is the next step, in which, because of technical constraints, it might be possible that some isolate remains unidentified. Therefore, working at the species level, as we did in our study, may lead to under-detection of the presence of a specific bacteria, compared with the others studies which considered global E-ESBL in their analyses.

To avoid over-identification of new acquisitions, given the imperfect sensitivity of bacterial detection in stool samples, the data underwent preliminary smoothing. Others have proposed estimation of swab sensitivity and use of data augmentation to reconstruct full swab sequences.<sup>14</sup> The smoothing choice made here was more conservative, and may have led to missing a few re-acquisitions. Nevertheless, analysis without data smoothing resulted in similar findings (details in Supplemental Material 8.1). An important novelty of

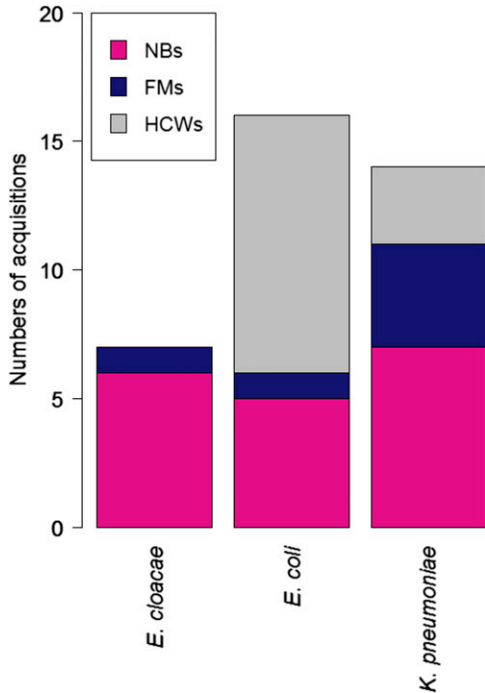


FIGURE 2. Total number of acquisitions observed during the study for the three bacterial species (extended spectrum beta-lactamases producing Enterobacteriaceae *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae*) for each type of individual (FMs = family members; HCWs = health-care workers; NBs = newborns). Smoothing was preliminarily carried out (see methods for details).

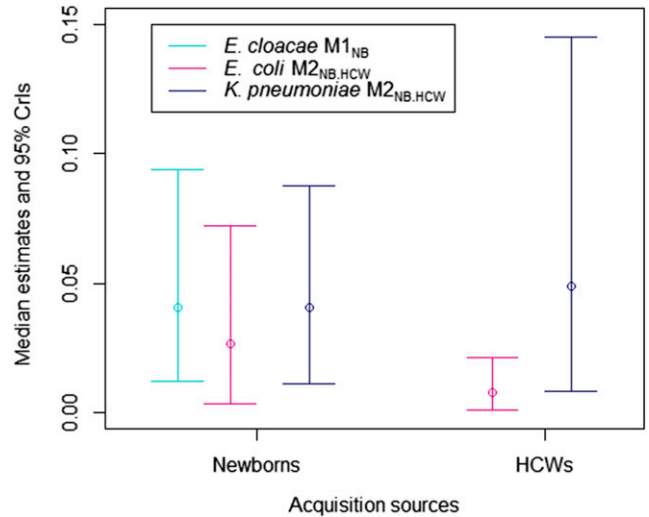


FIGURE 3. Median estimates and credible intervals (CrIs) for transmission parameters of the selected models. For each of the three bacterial species studied, the posterior-estimate median ( $\text{ind}^{-1} \text{day}^{-1}$ ) and its 95% CrI are shown. Left side: posterior estimates of the daily acquisition rate per colonized individual in the newborn's (NB's) environment ( $\beta_{\text{NB}}$ ) for the three studied species. Right side: health-care workers (HCWs) indicates posterior estimated of  $\beta_{\text{HCW}}$  for *Escherichia coli* and *Klebsiella pneumoniae*'s selected models.

our study stands in precise knowledge of HCWs' and family members' colonization status making direct estimation of their respective transmission strengths possible.

The median estimated daily acquisition rate ranged 0.0076–0.049  $\text{patient}^{-1} \text{day}^{-1}$  for contacts with HCWs and 0.027–0.041  $\text{patient}^{-1} \text{day}^{-1}$  from other in-unit newborns.

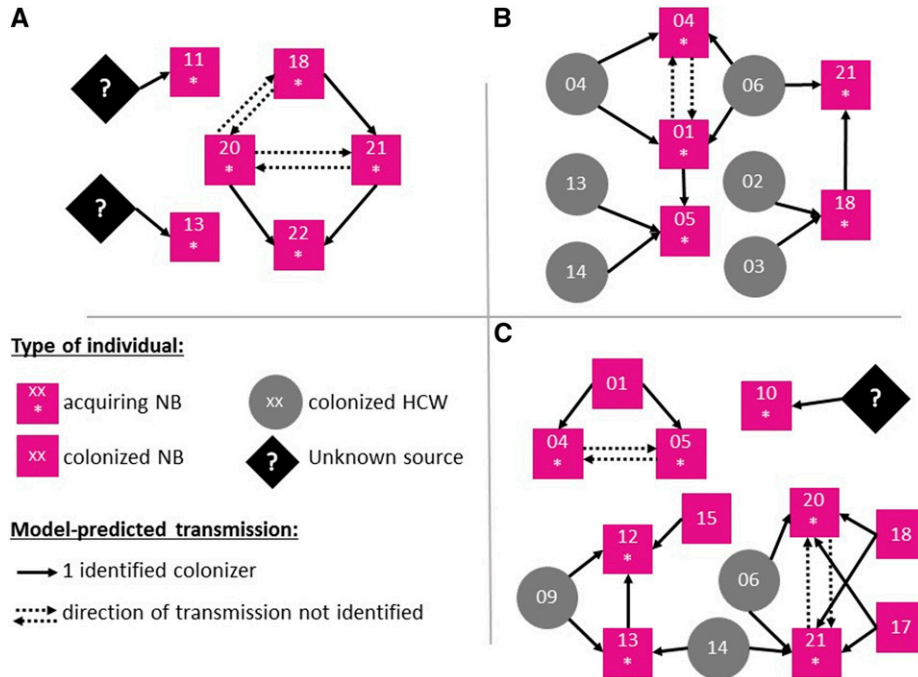


FIGURE 4. Transmission trees for the three species and their associated selected model. (A) Estimated posterior transmission tree for *Enterobacter cloacae* when considering other newborns (NBs) as the only transmission source. (B and C) Estimated posterior transmission tree for, respectively, *Escherichia coli* (B) and *Klebsiella pneumoniae* (C) when considering NB and health-care worker (HCW) as potential acquisition sources. Nodes shape and color represent a transmission source: magenta square, environmental contamination following in-ward colonization of another NB; grey plain circle, contact with HCW; and black diamond shape with question mark, no identified source. Numbers specified in each node design individuals' identification numbers. Newborn acquisitions are identified by a star.

Surprisingly, family members were not found to play an important role in newborns' acquisition of E-ESBL bacteria. Our estimates are consistent with estimates reported in other studies. From pulse-field gel electrophoresis analysis, a patient-to-patient E-ESBL transmission rate of 0.028 patient<sup>-1</sup> day<sup>-1</sup> was estimated for E-ESBL in an acute care facility<sup>27</sup>; and an average rate of 0.0056 patient<sup>-1</sup> day<sup>-1</sup> for *E. coli* and 0.0138 patient<sup>-1</sup> day<sup>-1</sup> for *K. pneumoniae* in a hospital.<sup>28</sup> Our estimates are also consistent with, although slightly higher than, estimates from previous modeling studies conducted in different settings with major model differences. One study in an intensive care unit in the Netherlands and another one in a neonatal unit in France estimated transmission rates ranging 0.006–0.02 patient<sup>-1</sup> day<sup>-1</sup>.<sup>24,25</sup> Most of those models defined the force of infection as a combination of an endogenous term and a patient's cross-transmission term.

Here, a human source was found for 14 of the 18 newborns acquisitions. Among those unresolved episodes, some newborns were colonized by more than one strain. For example, before the *E. cloacae* acquisition, newborn 13 was colonized with an E-ESBL *K. pneumoniae* strain. The phenotypic resistance profiles of the two strains were closely related (Supplemental Tables 8 and 9), suggesting possible horizontal genetic transfer within the gut.

Better understanding of acquisition routes of resistant bacteria for newborns is critical to establish effective control measures to limit their spread. Because prevalence of ESBL is high in the community in such settings,<sup>16,17,29</sup> an initial hypothesis was that family members might contribute strongly to neonatal colonization and infection risk in the ward. The results of our model comparison, which never selected a model including the family member transmission hypothesis, do not suggest that this is the case.

Larger studies should allow better characterization of ARB transmission routes. By contrast, colonized HCWs were selected as a significant transmission route for two of the three bacterial species, with a potentially lower role for *E. coli*. These findings are consistent with previous studies showing that inter-human transmission is much weaker for *E. coli* than for *K. pneumoniae*. In 13 European intensive Care Units, Gurieva et al.<sup>30</sup> found that *K. pneumoniae* were implicated in 3.7 times more transmission than *E. coli*. Smit et al.<sup>31</sup> identified that 3rd generation cephalosporin-resistant *K. pneumoniae* were mainly inter-human transmitted and only 2/9 newborn's acquisitions could be due to environmental transmission. Harris et al.<sup>26,32</sup> also found that patient-to-patient transmission represented 52% of E-ESBL *K. pneumoniae* infections but only 13% of E-ESBL *E. coli*. These results show that *E. coli* patient-to-patient transmission is weak and it could be mostly transmitted through the environment.<sup>26</sup> Because in all the studied acquisition episodes, newborn's gut was initially free of ESBL *E. coli*, and because direct newborn–newborn contacts do not occur, contamination is likely to be acquired, in part, by transient hand carriage by surrounding adults (family members or HCWs) and by newborn–environment contacts through surfaces (e.g., changing table and incubator).

Environment and hand carriage was not considered in the model. The samples undertaken over the study resulted in very low positive rates of E-ESBL. Results for hand carriage are consistent with others studies ranging from 0% to 7% in USA, Egypt, and Turkey,<sup>33–35</sup> despite high E-ESBL prevalences in

their respective units. Similarly, Thurlow et al.<sup>36</sup> reported that just 0.5% of environmental swabs were positive for *K. pneumoniae* Carbapenemase–Producing Enterobacteriaceae across six long-term acute hospitals.

Our study has several limitations. First, follow-up was only available in 22 newborns, resulting in 68 swabs from newborns, 48 samples from family members, and 105 samples from HCWs. Despite this small number of patients, the longitudinal aspect of the data combined with the modeling analysis enabled discriminating between models and highlighting specific features of species. Second, mode and context of delivery have been shown to influence ESBL acquisition in newborns. In case of C-section for example, the newborn is not exposed to maternal microbiota that may confer protection against ESBL colonization.<sup>37,38</sup> In addition, delivery in a potentially septic context (maternal fever, prolonged rupture of membranes etc.) has been shown to increase infant ESBL colonization risk, consistent with potential vertical transmission from mother to child.<sup>39</sup> To keep the simplest possible model and because our main objective was to study the transmission in the ward, we did not take into account the mode of delivery and context of septic delivery. However, the data related to newborns' mode of delivery is provided in Supplemental Material 2.3. Third, molecular typing information was not available, impeding the full comparison of sequences among transmission paths. However, mathematical modeling techniques facilitated integration of all available microbiological and epidemiological data to assess hypotheses and compare their likelihoods. It is important to highlight that identified transmissions are only hypothetical and should be validated in future work using molecular data. To provide a first validation of the predicted transmission paths, the phenotypic resistance profiles against 10 antibiotic drugs were compared between strains detected in identified transmitters and acquirers. Although most transmission paths were validated using phenotypic resistance profiles, in some acquisition cases no potential donor with identical resistance profiles could be found. Several hypotheses may explain such observation, including combined transmission of a species and within-host evolution process. In future transmission models, the inclusion in the likelihood of the differences of phenotypic resistance profiles as weight or even more specifically the incorporation of full genome sequences should sharpen disentanglement of the full transmission paths. Because phenotypic or molecular information improves the discriminatory power between strains, incorporating such data here would certainly lead to less identified transmissions and therefore lower estimated transmission rates. However, our conclusions concerning the minor role of family members in newborns acquisition would not be affected.

It is known that Enterobacteriaceae, especially *E. coli*, can persist for weeks on surfaces.<sup>32,40</sup> In our study, a 5 days strain persistence in the ward after patients' discharge did not result in any difference regarding the modeling selected routes and transmission-rate estimates (details on the sensitivity analysis are provided in Supplemental Material 8.2). Future studies, including extensive ARB-contamination tests in the ward environment and transient carriage on the skin and clothes should be undertaken to identify the role of environmental bacterial persistence in patients' acquisition.

In this study, all newborns received at least two different antibiotic classes during their stays, mainly  $\beta$ -lactams,

aminoglycosides, and carbapenems. We tested the introduction of antibiotic exposure as a potential acquisition amplifier (or reducer) in our model but surprisingly did not find any significant effect, possibly because of a lack of power and a very high rate of antibiotic exposure in newborns in the ward over the study period (see Supplemental Material 8.3).

Finally, our results could have several public health implications. In low-income countries, where budgets for hospital infection surveillance and control programs are limited, implementing high-cost control programs from high-income countries is often unrealistic. Targeting cost-effective measures is, therefore, of utmost importance. Our results suggest that, in the studied setting, interventions decreasing environmental contamination, such as efficient and frequent disinfection of surfaces shared by newborns (e.g., baby scales, benches for formula, and bottle preparation), should be prioritized.

Our results further suggest that sources of transmission vary according to the tracked species. This finding should help in implementing adequate interventions in case of specific outbreaks. For example, measures targeting HCWs rather than family members should be re-enforced in case of an *E. coli* outbreak. Consistent with our findings, it has previously been suggested that decreases in neonatal nosocomial infection may be achieved through initiatives to increase mothers' involvement (e.g. monitoring vital signs by trained mothers instead of HCWs, or co-bedding of mother and infant instead of using heated cots of incubators).<sup>41</sup>

In conclusion, in this first attempt to assess ARB-acquisition routes in neonatology wards in a low-income country, our results demonstrated that transmission routes differ among E-ESBL species. Although *E. coli* and *E. cloacae* may involve more indirect environmental or transient contamination, *K. pneumoniae* transmission was mostly attributable to direct contacts. Our results highlight the importance of increasing our knowledge about in-hospital ARB transmission and suggest that control measures based on disinfection and better hygiene may effectively prevent transmission.

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