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# Early neonatal acquisition of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales in Madagascar and Cambodia: clonal and plasmid-level contributions of maternal gut carriage

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# Abstract

Early neonatal infections caused by extended-spectrum-beta-lactamase-producing *Enterobacterales* (ESBL-PE) are prevalent in low- and middle-income countries, posing significant treatment challenges.

This study investigated ESBL-PE colonization in 499 mother-neonate pairs from Madagascar and Cambodia, with stool samples collected at delivery and from neonates before day three. Using short- and long-read sequencing, identical isolates or plasmids were identified within each pair to assess neonatal acquisition from maternal source. Multinomial regression identified associated risk factors.

Maternal gut carriage accounted for only 16.5% of neonatal ESBL-PE acquisition, suggesting that most cases originated from other sources. Primiparity and wet season were associated with an increased risk of acquisition from the mother, while cesarean delivery, neonatal resuscitation, and wet season were associated with acquisition from other sources.

These findings challenge the paradigm that maternal carriage is the primary source of early neonatal ESBL-PE colonization, highlighting the need for further research into alternative sources to inform targeted interventions.

## Introduction

Neonatal bacterial sepsis represents a critical health challenge, particularly in low- and middle-income countries (LMICs)<sup>1,2</sup>. Most of these infections occur during the first week of life, particularly within the first three days<sup>3</sup>, the timeframe defined as early-onset neonatal sepsis<sup>4</sup>.

In LMICs, *Enterobacterales* are the primary bacteria driving neonatal sepsis<sup>5-7</sup>, with a significant proportion producing extended-spectrum beta-lactamases (ESBL-PE)<sup>8,9</sup>. Consequently, antibiotic treatment options are limited to molecules such as carbapenems<sup>10</sup>, which are either unavailable or prohibitively expensive in many LMICs. This contributes to an estimated 214,000 neonatal deaths annually linked to antimicrobial resistance<sup>11</sup>.

Early-onset neonatal sepsis is primarily attributed to the maternal transmission of pathogens during pregnancy, labor, or delivery<sup>12,13</sup>. In the case of ESBL-PE infections, this assumption is supported by the high prevalence of ESBL-PE gut colonization among pregnant women in LMICs, reaching up to 80% in Cambodia<sup>14</sup> and 18.5% in Madagascar<sup>15</sup>. While studies on hospitalized neonates have documented instances of ESBL-PE acquisition from maternal gut strains<sup>16</sup>, the overall predominance of this source, particularly in community settings, has yet to be confirmed. Newborns are also exposed to *Enterobacterales* from other sources, including surfaces, or caregiver's hands. Distinguishing and quantifying the maternal contribution to acquisition is critical to design prevention strategies, but molecular studies tracking ESBL-PE transmission remain limited<sup>17</sup>. Most rely on comparing maternal

and neonatal strains using low-resolution techniques, such as pulsed-field gel electrophoresis (PFGE)<sup>18,19</sup>, which may overestimate the role of maternal source.

The source of bacterial acquisition is most often inferred from the detection of identical isolates, indicating clonal transmission. However, if the focus is on resistance acquisition, the identification of shared plasmids carrying resistance genes, even among genetically distinct strains, is equally important. Analyzing plasmid content is beyond the scope of traditional typing methods and short-read sequencing, as these approaches lack the required resolution to accurately reconstruct plasmids from fragmented data<sup>20</sup>. In contrast, long-read sequencing enables complete plasmid sequence assembly and accurate comparison of plasmid contents<sup>21</sup>. However, to our knowledge, the detection of shared plasmids carrying ESBL-encoding genes has not yet been explored in studies investigating neonatal sources of ESBL-PE acquisition.

Community-level studies combining whole-genome sequencing with plasmid analysis to elucidate neonatal ESBL-PE transmission pathways are yet to be conducted in LMICs, limiting the development of effective prevention strategies. By identifying both identical isolates and plasmids, this study aims to quantify the contribution of the maternal gut to early ESBL-PE acquisition in newborns from two mother-newborn cohorts in Madagascar and Cambodia.

## Results

### Population characteristics and ESBL-PE colonization prevalence

Overall, 147 mother-neonate pairs (hereafter referred to as pairs) in Cambodia and 377 in Madagascar, were screened for this ancillary study of two community-based mother and child cohorts called BIRDY<sup>3</sup> (Bacterial Infections and antibiotic Resistant Diseases among Young children in low income countries) and NeoLIC<sup>22</sup> (Neonatal acquisition of ESBL-PE in the community of a Low-Income Country). A total of 131 pairs from Cambodia and 368 from Madagascar, including two twin pairs, were ultimately included (Fig. 1), resulting in a study population of 499 pairs (996 individuals). In Madagascar, 299 and 67 pairs were included through BIRDY and NeoLIC studies, respectively. Population characteristics are detailed in **Table 1**.

The overall prevalence of ESBL-PE colonization was 48.9% [95% CI: 44.4–53.4%] in mothers and 34.1% [29.9–38.4%] in neonates. Maternal colonization prevalence was 79.4% [71.3–85.8%] (104/131) in Cambodia compared to 38.0% [33.0–43.2%] (139/366) in Madagascar ( $p < 0.01$ ). Neonatal colonization prevalence was also higher in Cambodia, at 53.4% [44.5–62.1%] (70/131), versus 27.2% [22.8–32.1%] (100/368) in Madagascar ( $p < 0.01$ ). In colonized mothers, *Escherichia coli* was the most frequently identified species, detected in 91.3% (95/104) of cases in Cambodia and 75.5% (105/139) in Madagascar. *Klebsiella* spp. were the second most common, found in 8.7% (9/104) of colonized

mothers in Cambodia and 28.1% (39/139) in Madagascar, followed by *Enterobacter* spp., which were absent in Cambodia (0%) but present in 7.2% (10/139) of cases in Madagascar. Among colonized newborns, the species distribution differed significantly. *E. coli* prevalence was notably lower, detected in 64.3% (45/70) of cases in Cambodia and 33.0% (33/100) in Madagascar ( $p < 0.01$ ). In contrast, *Klebsiella* spp. were identified in 18.6% (13/70) of colonized neonates in Cambodia and 36.0% (36/100) in Madagascar, while *Enterobacter* spp. were found in 20.0% (14/70) and 30.0% (30/100), respectively.

## Contribution of maternal versus other sources to ESBL-PE acquisition

A total of 170 neonates were colonized, among whom 72 were born to non-colonized mothers, while 98 had mothers colonized with an ESBL-PE (Fig. 1). Among the 98 pairs where both the mother and the newborn were colonized, two pairs were excluded from the analysis because of the insufficient sequence quality of one of the isolates. Out of the 96 remaining pairs, 59 shared at least one isolate of the same bacterial species (i.e., *Escherichia coli*, *Klebsiella quasipneumoniae*, *Enterobacter kobei* and "*Enterobacter intestinhominis*"). Phylogenetic classifications showed 23 cases of identical isolates within pairs (Fig. 2): 10 in Cambodia (all *E. coli*) and 13 in Madagascar, comprising 10 *E. coli* pairs, one *Klebsiella quasipneumoniae* pair, one *Enterobacter kobei* pair, and one "*Enterobacter intestinhominis*" pair. Notably, fewer than 10 Single Nucleotide Polymorphisms (SNPs, **Supplementary Fig. 1**) were observed between genomes of mother-neonate pairs forming a cherry in the phylogenetic trees, therefore confirming the suitability of this cut-off to determine identical isolates<sup>23</sup>.

For the remaining 75 ESBL-PE positive pairs, 30 pairs exhibited at least one ESBL-encoding gene in common, including 17 with a common plasmid replicon. After plasmid reconstruction, five pairs with identical plasmids were identified (three in Cambodia and two in Madagascar). Aligned plasmid sequences are displayed in Fig. 3. A total of 28 neonatal acquisition cases were attributed to maternal gut carriage. Of these, 17.8% [3.7–32.0%] (5/28) of cases linked to maternal source were identified by plasmid content analysis.

Overall, combining cases with identical isolates or identical plasmids, 16.5% [11.4–23.1%] (28/170) of neonatal colonization cases were attributable to maternal source. As a result, the remaining 83.5% [76.9–88.6%] (142/170) were attributable to other sources. The proportion of cases attributed to maternal source rate was similar in Cambodia (18.5%, 13/70) and Madagascar (15%, 15/100,  $p = 0.68$ ).

In Madagascar, a significant difference was observed between the two studies through which mothers were included, with a lower proportion of acquisition attributed the mother in BIRDY (7.6%, 6/79) compared to NeoLIC (42.8%, 9/21,  $p < 0.01$ ). In the latter study, maternal colonization was more prevalent (34% versus 55%,  $p < 0.01$ ), antibiotic administration at delivery was significantly more frequent (10% versus 60%,  $p < 0.01$ ), and only vaginally delivered infants were included (Table 1).

## Risk factor analysis

After univariate analysis, a multinomial regression model (Fig. 4) was fitted to identify maternal, neonatal and delivery features associated with a three-level categorical variable: (1) ESBL-PE acquisition attributed to maternal source, (2) ESBL-PE acquisition attributed to other sources, and (3) no acquisition (used as the reference group). The model was applied on complete cases, including 472 pairs. With adjustment on the study project, factors significantly associated with acquisition from maternal source compared to no acquisition were primiparity (adjusted odds ratio aOR 2.76 [1.18–6.47]) and delivery during wet compared to dry season (aOR 2.59 [1.02–6.55]). Factors significantly associated with acquisition from other sources compared to no acquisition were cesarean section (aOR 3.71 [1.90–7.22]), neonatal resuscitation (aOR 2.32 [1.22–4.42]) and delivery during wet season (aOR 1.73 [1.08–2.77]). The multinomial regression models performed on imputed datasets yielded very similar results (**Supplementary table 1**).

## Phenotypic characteristics of isolates

A total of 515 ESBL-producing isolates were obtained. More than one colonizing isolate was identified in 17.9% (42/243) of ESBL-colonized mothers and 25.4% (43/170) of ESBL-colonized neonates.

Antibiotic resistance profiles were established for 513 isolates (**Supplementary Fig. 2**). Up to 44% (226/513) of isolates were resistant to both amoxicillin and gentamicin, which is the recommended combination by World Health Organization (WHO)<sup>24</sup> for neonatal sepsis. Up to 91.4% (469/513) of isolates were multidrug-resistant, defined as showing resistance to  $\geq 1$  agent(s) in  $\geq 3$  antimicrobial categories<sup>25</sup>. The majority of isolates remained susceptible to carbapenems, with only 2.1% (11/513) showing resistance to ertapenem and none resistant to imipenem.

## Genomic characteristics of isolates

Fourteen samples were excluded due to poor sequencing quality, leaving 501 sequences suitable for genotypic analysis (Fig. 5). Taxonomic distribution was diverse with 10 identified genera: *Atlantibacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Lelliottia*, *Morganella* and *Phytobacter* (**Supplementary Table 2**). The predominant species was *E. coli*, with isolates spread across its overall population structure (Fig. 3, **Supplementary Fig. 3**), though species distribution substantially differed between mothers and neonates, as previously described (Fig. 2). Diversity among species was high, with notably 105 distinct sequence types (ST) identified among 299 *Escherichia* isolates, and 53 distincts ST among 95 isolates belonging to the *Klebsiella pneumoniae* species complex (**Supplementary Table 2**).

The *bla*<sub>CTX-M</sub> family accounted for the majority of ESBL genes (89.3%, 469/525), with *bla*<sub>CTX-M-15</sub> being the most prevalent (62.1%, 326/525). It was more common in Madagascar (76.7%, 254/331) than in Cambodia (37.1%, 72/194,  $p < 0.001$ ), where *bla*<sub>CTX-M-55</sub> (18.6%, 36/194) and *bla*<sub>CTX-M-27</sub> (17.5%, 34/194) were also frequently detected. Genotypic co-resistance was found in 98.2% (492/501) of

isolates, mainly to sulfonamides (*sul2*, 62.6%, 314/501), aminoglycosides (*aph(3'')-Ib* and/or *aph(6)-Id*, 59.6%, 299/501), and tetracyclines (*tet(A)*, 57.4%, 288/501, **Supplementary Fig. 4**). Carbapenemase genes were detected in two isolates, both from Cambodian mothers (*bla<sub>NDM-5</sub>* and *bla<sub>NDM-1</sub>*). The number of identified resistance genes was significantly higher in isolates from neonates (median 11 [interquartile range IQR 8–15]) compared to isolates from mothers (median 9 [IQR6–12],  $p < 0.01$ ). Plasmid replicons were found in 90.6% (454/501) of isolates, with no statistical difference between isolates from mothers (90.9%, 251/276) and from neonates (90.2%, 203/225,  $p = 0.9$ ). The most frequent plasmids were from the IncF incompatibility group (63.7%, 319/501), followed by Col (47%, 239/501).

## Discussion

In a community-based, multicentric mother-child cohort with high rates of maternal and early neonatal ESBL-PE colonization, maternal gut carriage contributed for 16.5% of neonatal acquisition, even when both identical clones and plasmids were considered. Our findings challenge the prevailing assumption that the maternal gut is the primary source of neonatal ESBL-PE colonization. This study is the first to apply high-resolution genomic analyses (including plasmid reconstruction) in a community cohort to assess the role of maternal gut in early neonatal ESBL-PE acquisition. These findings highlight the critical role of other sources of acquisition, and are essential for developing more effective prevention strategies.

Our result underscore the need to reconsider existing paradigms in neonatal sepsis, especially in reexamining sources of acquisition of antibiotic-resistant bacteria. This aligns with a recent critical review that advocated for rethinking the traditional classification of neonatal sepsis into early- and late-onset categories<sup>26</sup>, highlighting in particular its limited utility for predicting the pathogen spectrum in LMICs. Several studies conducted in high-income countries attributed neonatal early ESBL-PE acquisition to maternal source. In Israel, studies have reported that between 72%<sup>27,28</sup> and 100%<sup>29</sup> of neonatal colonization cases were linked to maternal gut carriage, while a study in Germany estimated this proportion at 42%<sup>30</sup>. Other studies<sup>18,19</sup> could not quantify the role of different sources due to analyses limited to subsets of isolates. Additionally, these conclusions often rely on low-resolution comparison methods such as PFGE<sup>18,30</sup> or AST profiles<sup>27,31</sup>. Their relevance to LMICs is further limited by the difference in ESBL-E prevalence and epidemiology compared to HICs<sup>32</sup>.

An hospital-based Cambodian study<sup>16</sup> found that only eight out of 25 neonatal ESBL-PE colonization cases were attributable to maternal gut carriage, aligning with our findings. However, plasmid content comparison between different strains was not assessed. In the large-scale BARNARDS study<sup>33</sup> across seven LMICs, neonatal and maternal microbiota were screened for ESBL-encoding genes. While systematic mother-neonate pairwise analyses were absent, they identified one indistinguishable isolate between a mother and her neonate, but also between unrelated neonates at the same clinical site, pointing to acquisition from the hospital environment. Studies using other methodologies also reinforce this conclusion. In Gambia, Okomo et al.<sup>34</sup> compared blood culture isolates from hospitalized newborns

with sepsis alongside maternal rectovaginal samples. They identified identical isolates in five out of 36 cases (all *Staphylococcus aureus*), none for the 15 sepsis cases caused by *Enterobacterales*. This is further corroborated by studies on the neonatal resistome<sup>35</sup>. Metagenomic sequencing has revealed that neonatal microbiota harbor more antibiotic resistance genes and mobile genetic elements than the maternal ones<sup>36,37</sup>, even without antimicrobial exposure.

To the best of our knowledge, our study is the first to identify identical plasmids carrying ESBL genes between distinct maternal and neonatal bacterial isolates. In our cohort, approximately 20% of ESBL acquisitions from maternal source were detected through plasmid content analysis, emphasizing the need to explore resistance transmission mechanisms beyond clonal transfer to fully understand neonatal ESBL-PE acquisition. Plasmids play a major role in the spread of antimicrobial resistance<sup>38</sup>. The human microbiome is a hotspot for horizontal gene transfers (HGT), with rates of HGT between human-associated bacteria 25 times higher than in non-human isolates<sup>39</sup>. Childbirth represents a unique moment of microbiome interaction between mother and neonate. Although the newborn gut plasmidome remains underexplored, recent studies show that plasmids are disproportionately abundant in the infant gut early in life<sup>40</sup>. Our findings underscore the importance of tracking plasmids to understand neonatal ESBL-PE acquisition and stress the need to incorporate plasmid analysis in transmission studies more broadly<sup>41</sup>.

The limited role of maternal source was observed despite a notably high prevalence of ESBL-PE colonization, both among mothers and neonates. The prevalence rate aligns with previous studies on maternal colonization at delivery<sup>14,42</sup> and neonatal colonization<sup>43,44</sup>. Our results suggest that maternal ESBL-PE colonization does not necessarily lead to transmission to the newborn. Our multivariate analysis identified primiparity as a risk factor of acquisition from the mother. Longer labor duration in primiparas<sup>45</sup> may increase the time of exposure of the neonate to the maternal gut microbiota, potentially heightening the risk of transmission. Additionally, immunological variations between first and subsequent pregnancies<sup>46</sup> may also play a role. Microbiota composition of primiparous mothers differs from that of multiparous ones<sup>47-49</sup> and multiparous women typically show a gestational decline in inflammatory cytokines (TNF- $\alpha$ , IL-10, IFN- $\gamma$ ), absent in first pregnancies<sup>50</sup>. This heightened inflammatory state may promote transmission through mechanisms such as oxidative stress or dysbiosis.

Conversely, the limited role of maternal gut carriage in neonatal acquisition underscores the importance of exploring alternative transmission pathways. Although our study was not designed to identify these pathways, cesarean section and resuscitation were statistically associated with non-maternal sources of acquisition. Cesarean section delivery, previously reported as a risk factor for ESBL-PE acquisition<sup>44,51</sup>, may increase neonatal exposure to nosocomial ESBL-PE sources (e.g., healthcare workers' hands, prolonged hospitalization) and disrupt microbiome development, impairing colonization resistance<sup>52,53</sup>. Similarly, neonatal resuscitation may reflect higher exposure to invasive procedures or fetal distress during childbirth, which could increase vulnerability to colonization. The BARNARDS study also identified

similar influencing factors, with complicated births and cesarean sections significantly associated with the presence of ESBL genes in the neonatal gut<sup>33</sup>.

Being born during the wet season was also significantly associated with a higher risk of acquisition, either from maternal or from other sources. The association between the wet season and an increased prevalence of ESBL-*E. coli* colonization has been previously documented in pregnant women<sup>15,23</sup>. Conditions of higher humidity and temperature during the wet season may promote HGT<sup>54</sup> and bacterial growth<sup>55</sup>. Similarly, an increase in infection rates by Gram-negative bacteria during warmer months has been observed in neonatal intensive care units in Canada<sup>56</sup>. These findings highlight the importance of considering seasonality in surveillance and intervention studies on neonatal sepsis, notably when evaluating future vaccines targeting this condition. Stratification and/or adjustment for the season of birth should therefore be incorporated in statistical analyses.

Our study has several notable strengths. First, it was community-based, with recruitment including deliveries in various settings, such as home births, which helps reduce the potential for selection bias compared to previous hospital-based studies<sup>16</sup>. Second, samples were systematically collected from mothers at the time of delivery and from neonates within the first three days of life. This approach has rarely been implemented, especially in LMICs, where studies often collect samples only when neonates show signs of sepsis, and not necessarily just after birth<sup>16,33,34</sup>. In addition, we employed cutting-edge methodologies, combining short- and long-read sequencing, therefore providing a higher resolution compared to techniques used in the majority of previous studies<sup>18,19,27,29–31</sup>.

However, our study also has some limitations. First, by focusing on morphologically distinct colonies from each sample, we may have missed some clonally distinct isolates. These likely represent low-inoculum strains with lower transmission risk, minimizing bias; however, the role of the maternal source is probably still underestimated. Second, while we aimed to trace transmission, it remains impossible to definitively determine whether the observed events were due to direct maternal transmission or exposure to a third common source. This latter scenario appears nevertheless unlikely given the narrow timeframe during which the samples were collected. Furthermore, it is still unknown how often identical plasmids could occur without a transmission, as “core plasmids” of some lineages<sup>57</sup>. Finally, we observed variations in the proportion of neonatal acquisition cases attributable to maternal sources between the two studies through which mothers were recruited in Madagascar. One key difference between the two populations is that no cesarean sections were performed in mothers recruited through NeoLIC, whereas 12% of mothers in the BIRDY study underwent cesarean sections, which could have significantly influenced neonatal acquisition. This highlights the need for caution when extrapolating findings to other settings, as transmission pathways may vary depending on population characteristics, particularly the prevalence of cesarean deliveries.

Our study has significant implications for policy and future research. Non-maternal sources were the predominant in neonatal ESBL-PE acquisition, statistically associated to cesarean deliveries and birth

resuscitations in our setting. The neonatal period, even in community settings, involves substantial interactions with the healthcare environment. While nosocomial risks for hospitalized neonates are well-recognized, the role of routine hospital deliveries in neonatal ESBL-PE acquisition remains underexplored. Strengthening maternity ward hygiene is crucial, yet optimal interventions are unclear. Future studies should map transmission pathways across diverse sources, including, among others, baby wipes, healthcare workers and formula bottles. Alternative maternal sources like breastmilk, should also be investigated. While breastfeeding is known to offer protective effects against antimicrobial resistance<sup>58</sup>, it has also been shown to carry antimicrobial resistance genes (ARGs), with studies reporting that 70% of ARGs detected in breastmilk are also found in infant feces<sup>36</sup>. Such research could guide strategies to delay or reduce neonatal ESBL-PE colonization, a key goal given the heightened vulnerability in the first three days of life, when most severe sepsis cases occur. Reducing maternal colonization, for example by curbing antibiotic use in pregnancy (estimated at 45.3% in low-income countries<sup>59</sup>), could be an additional measure. However, evaluating the appropriateness of these prescriptions is challenging, and recent antibiotic use has not been consistently linked to colonization in several studies<sup>14,23</sup>. Further investigation is essential to better understand the contributing factors and to identify more targeted, effective interventions.

## Conclusion

Our study highlights the complexity of neonatal ESBL-PE acquisition in LMICs, showing that maternal gut carriage accounts for less than 20% of neonatal colonization cases, even when considering both identical bacterial isolates and plasmids. By using high-resolution genomic analyses, including plasmid reconstruction, in a community-based cohort, we provide new molecular insights that challenge the assumption that maternal carriage is the primary source of early neonatal ESBL-PE colonization.

## Methods

### *Definitions and conceptual framework*

'Acquisition' refers to the event in which a neonate was colonized by an ESBL-PE (**Supplementary Figure 5**). Two scenarios were considered to confirm the maternal gut as the source of acquisition: either sharing an identical isolate or sharing an identical plasmid between the maternal and neonatal gut.

### *Study design*

This prospective, cross-sectional study was nested within two multicenter, community-based mother and child cohorts: BIRDY (Bacterial Infections and antibiotic Resistant Diseases among Young children in low income countries) and NeOLIC (Neonatal acquisition of ESBL-PE in the community of a Low-Income Country), whose methodologies have already been described<sup>3,22</sup>. This ancillary study of BIRDY was conducted from 2019 to 2020 in Madagascar, and from 2016 to 2017 in Cambodia (under the name of PEECNIC study, for Producing Extended Spectrum Beta Lactamase Enterobacteria Carriage in Newborns

and Infants in Cambodia). Two sites were involved in each country: one rural (Kampong Speu and Moramanga, respectively) and one urban (Phnom Penh and Antananarivo, respectively). Mothers were recruited during pregnancy through collaboration with community health workers. This design allowed women to be enrolled regardless of their antenatal care status and enabled the inclusion of home births. NeoLIC project took place between 2021 and 2022 in the same rural site as BIRDY in Madagascar (Moramanga), where mothers were recruited at the primary healthcare center. Stool and/or rectal swabs were collected from mothers at delivery and from their respective neonates before 72 hours of life. Socioeconomic data and medical information at recruitment and after delivery were collected.

### ***Microbiological analysis***

After collection, samples were transported on the same day to the Institut Pasteur of the study site for immediate analysis or were stored frozen at  $-80^{\circ}\text{C}$  until further analyses. All samples were pre-enriched following the method described by Jazmati *et al*<sup>60</sup>. Subsequently, they were inoculated onto antibiotic-supplemented media and incubated overnight in an aerobic atmosphere at  $36.5\pm 2^{\circ}\text{C}$ . In Cambodia, Drigalski plates supplemented with 2 mg/L cefotaxime (Bio-Rad, Marnes-La-Coquette, France) were used, while in Madagascar, CHROMagar ESBL (CHROMagar, Paris, France) media were employed. Up to three colonies per plate were selected based on their color and morphological characteristics. Single bacterial colonies were cultured further, until pure isolates were obtained. Species identification was performed using MALDI-ToF (Madagascar) or API 20E system (Cambodia). The antimicrobial susceptibility (AST) of *Enterobacterales* was determined by disc-diffusion antibiotic susceptibility testing on Mueller-Hinton agar (Bio-Rad) using the Kirby-Bauer disc diffusion method with interpretation according to the European Committee on Antimicrobial Susceptibility Testing (2018; [www.eucast.org](http://www.eucast.org)). ESBL production was detected by the double-disc synergy test by placing the disk of cefotaxime (30  $\mu\text{g}$ ), ceftazidime (30  $\mu\text{g}$ ) and combination of amoxicillin/clavulanic acid (20  $\mu\text{g}$ /10  $\mu\text{g}$ ) on a lawn culture of bacteria on Muller-Hinton agar plate, with a 20 mm distance between each disk from center to center.

### ***Short-read sequencing***

Based on DNA extracts from each ESBL-PE, Illumina short-read sequencing of every ESBL-PE isolate was performed on NextSeq 500 instruments using Nextera XT libraries with a 2 $\times$ 150 bp paired-end protocol. Small subunit gene segments coding for 16S rRNA were assembled using ASSU v1.1 ([gitlab.pasteur.fr/GIPhy/ASSU](https://gitlab.pasteur.fr/GIPhy/ASSU)), and genome sequences were assembled using fq2dna v24.02 ([gitlab.pasteur.fr/GIPhy/fq2dna](https://gitlab.pasteur.fr/GIPhy/fq2dna)). Quality controls were performed using various metrics (e.g. coverage depth distribution, N50).

Taxonomic assignment of each isolate was performed in two steps: (i) the assembled 16S rRNA segment was used as a query to perform a BLASTn search against the RefSeq Targeted Loci Project sequences ([www.ncbi.nlm.nih.gov/refseq/targetedloci](http://www.ncbi.nlm.nih.gov/refseq/targetedloci)) to assess the genus (i.e. the one associated to the best BLASTn hit); (ii) the Average Nucleotide Identity (ANI) between the assembled genome and every type strain genome from its genus was computed using FastANI<sup>61</sup> v1.33 to assess the species (i.e.

the one of the closest type strain genome with ANI > 96%). Of note, species names between quotes were not yet validly published (March 2025) according to the International Code of Nomenclature of Prokaryotes.

Sequence types (ST) were determined using mlst v2.23.0 ([github.com/tseemann/mlst](https://github.com/tseemann/mlst)). Acquired resistance genes and plasmids were searched using AMRfinder<sup>62</sup> v3.12.8 and PlasmidFinder<sup>63</sup> v2.1.1.

### ***Phylogenetic analysis***

In pairs where both the mother and newborn were ESBL-PE carriers, insufficient quality of any sample led to an exclusion from analysis. At the genus level, genome-based phylogenetic trees were inferred using JolyTree<sup>64,65</sup> v2.1 (**Supplementary Figures 3, 6 and 7**). At the species level, phylogenetic trees were inferred using IQ-TREE<sup>66</sup> v2.3.4 from recombination-purged core genome alignments built using Parsnp<sup>67</sup> v1.6.2. Species trees were rooted according to the phylogenetic relationships supported by the genus trees. Neonatal bacterial isolate acquisition from a maternal source was determined by the presence of a "cherry", defined as a pair of adjacent tips on the phylogenetic tree representing isolates from the mother-neonate pair.

### ***Plasmid content analysis***

To identify putative neonatal acquisition of an ESBL-carrying plasmid from the mother, additional long-read sequencing was performed for selected pairs. Selection criteria included: (1) pairs both colonized by ESBL-PE, (2) no identified clonal transmission, (3) at least one common ESBL gene and plasmid replicon (same incompatibility group) between neonatal and maternal isolates. For one isolate per selected pair, DNA was extracted with Genomic-tip 100/G columns (Qiagen). DNA integrity and RNA absence were assessed via agarose gel electrophoresis and  $A_{260}/A_{230}$  and NanoDrop™ 2000 spectrophotometer ( $A_{260}/A_{230}$ ,  $A_{260}/A_{280}$  ratios). DNA concentrations were measured using Qubit and dsDNA BR Assay Kit (Invitrogen). Libraries were prepared with the SQK-RBK114.24 rapid sequencing kit (Oxford Nanopore Technologies, RBK\_9176\_v114\_revN\_2024) and sequenced on a MinION-Mk1C with R10.4.1 flow cells. Hybrid genome assemblies (combining long- and short-reads) were inferred using Unicycler<sup>68</sup> v0.4.8. Circularized plasmid sequences were analyzed using AMRfinder to select the potential transmitted plasmid. The *bla*-gene carrier served as reference for aligning short reads from the pair's other isolate using bwa-mem2<sup>69</sup> v2.2.1, with >99% sequence similarity as the cut-off.

### ***Statistical analysis***

#### ***Descriptive analysis***

The contribution of maternal versus other sources to ESBL-PE acquisition in newborns was estimated as  $(c+p)/n$  and  $1-(c+p)/n$ , respectively, where  $c$  is the number of pairs with identical isolates,  $p$  the number of pairs with identical plasmids, and  $n$  the total number of newborns colonized with ESBL-PE.

The wet season was defined as the period from May to October in Cambodia, and from November to April in Madagascar, with the remainder of the year classified as the dry season.

### *Risk factor analysis*

The outcome variable for risk factor analysis was early **neonatal ESBL-PE acquisition**, a three-level categorical variable: acquisition resulting from a maternal source, acquisition resulting from another source, and no acquisition of ESBL-PE (reference).

First, a univariate analysis was conducted to explore the associations between neonatal ESBL-PE acquisition and key exposures of interest, including potential confounding factors. Categorical variables were compared using Fisher's exact test or chi-squared tests, depending on the distribution of the data. For continuous variables, differences between groups were assessed using t-tests. Next, a multinomial logistic regression was performed to assess the relationship between exposures and the outcome variable. Variables that showed an association with the outcome at a significance level of  $p < 0.2$  in the univariate analysis were included in the multivariate model. The model was then refined through a backward selection procedure.

Analyses were conducted on complete cases. For validation, we also used a set of ten imputed datasets, where missing data were addressed via multiple imputation. All statistical analyses were conducted in RStudio v2024.04.2+764 with the packages nnet v7.3.20 and mice v3.17.0. Statistical significance was defined at  $p < 0.05$ .

## **Declarations**

### *Ethics*

BIRDY study was approved by the ethics committees of Madagascar (N° 068-MSANP/CE), Cambodia (N° 108-NECHR, N° 275-NECHR), and the Institutional Review Board of Institut Pasteur (N° IRB/2016/08/03), France. NeoLIC study was approved by the ethical committee in Madagascar (N° 190-MSANP/SG/AGMED/CERBM) and by the institutional review board (IRB2020-010) of Institut Pasteur, France. Written informed consent was given by every participant.

### *Funding and role of the funding source*

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The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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## Contributors

BTH supervised the overall project. BTH, DG, EKD, JMC, ADL and MV designed and implemented the study. PH, MANR, NFR, SB, LP, LB, ADL, ALB and TC took part in data collection, microbiological experiments planning, realization and analyses. LF performed long-read sequencing. AC, LF and ALB performed the bioinformatic analyses. ADL and ALB performed data management. ALB performed statistical analysis and drafted the first version of the manuscript. All authors contributed to the final version of the manuscript.

## Declaration of interests

We declare no competing interests.

## Data sharing statement

The raw sequencing reads and genome assemblies have been deposited in the European Nucleotide Archive (ENA) repository under the BioProject accession number PRJEB85868. Detailed run and GenBank/EMBL/DDBJ accession numbers are provided in **Supplementary Table 2**. Researchers wishing to ask further questions are invited to contact the corresponding author.

## References

1. Mahtab, S. *et al.* Causes of death identified in neonates enrolled through Child Health and Mortality Prevention Surveillance (CHAMPS), December 2016 –December 2021. *PLOS Glob. Public Health* **3**, e0001612 (2023).
2. Perin, J. *et al.* Global, regional, and national causes of under-5 mortality in 2000-19: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet Child Adolesc. Health* **6**, 106–115 (2022).
3. Huynh, B.-T. *et al.* Severe bacterial neonatal infections in Madagascar, Senegal, and Cambodia: A multicentric community-based cohort study. *PLOS Med.* **18**, e1003681 (2021).

4. Sands, K. *et al.* Early-Onset Neonatal Sepsis in Low- and Middle-Income Countries: Current Challenges and Future Opportunities. *Infect. Drug Resist.* **15**, 933–946 (2022).
5. Wen, S. C. H. *et al.* Gram-negative neonatal sepsis in low- And lower-middle-income countries and WHO empirical antibiotic recommendations: A systematic review and meta-analysis. *PLoS Med.* **18**, e1003787 (2021).
6. Zaidi, A. K. M. *et al.* Hospital-acquired neonatal infections in developing countries. *Lancet Lond. Engl.* **365**, 1175–1188 (2005).
7. Milton, R. *et al.* Neonatal sepsis and mortality in low-income and middle-income countries from a facility-based birth cohort: an international multisite prospective observational study. *Lancet Glob. Health* **10**, e661–e672 (2022).
8. Breurec, S. *et al.* High third-generation cephalosporin resistant Enterobacteriaceae prevalence rate among neonatal infections in Dakar, Senegal. *BMC Infect. Dis.* **16**, 587 (2016).
9. Huynh, B. *et al.* Bacterial Infections in Neonates, Madagascar, 2012-2014. *Emerg. Infect. Dis.* **24**, 710–717 (2018).
10. Vardakas, K. Z., Tansarli, G. S., Rafailidis, P. I. & Falagas, M. E. 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 (2012).
11. Laxminarayan, R. *et al.* Access to effective antimicrobials: a worldwide challenge. *Lancet Lond. Engl.* **387**, 168–175 (2016).
12. Gans, H. A. & Darmstadt, G. L. Bacterial Sepsis in the Neonate. in *Fetal and Neonatal Brain Injury* (eds. Stevenson, D. K., Druzin, M. L., Sunshine, P., Hintz, S. R. & Benitz, W. E.) 456–480 (Cambridge University Press, Cambridge, 2017). doi:10.1017/9781316275498.031.
13. Singh, M., Alsaleem, M. & Gray, C. P. Neonatal Sepsis. In *StatPearls* (StatPearls Publishing, Treasure Island (FL), 2025).
14. de Lauzanne, A. *et al.* Prevalence and factors associated with faecal carriage of extended-spectrum  $\beta$ -lactamase-producing Enterobacterales among peripartum women in the community in Cambodia. *J. Antimicrob. Chemother.* **77**, 2658–2666 (2022).
15. Chereau, F. *et al.* 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 (2015).
16. Chomkatekaw, C. *et al.* Detection of maternal transmission of resistant Gram-negative bacteria in a Cambodian hospital setting. *Front. Microbiol.* **14**, 1158056 (2023).
17. Patangia, D. V., Ryan, C. A., Dempsey, E., Stanton, C. & Ross, R. P. Vertical transfer of antibiotics and antibiotic resistant strains across the mother/baby axis. *Trends Microbiol.* **30**, 47–56 (2022).
18. Danino, D. *et al.* Mother-to-child transmission of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. *J. Hosp. Infect.* **100**, 40–46 (2018).

19. Rettedal, S. *et al.* Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal–neonatal transmission. *J. Perinatol.* **35**, 907–912 (2015).
20. Arredondo-Alonso, S., Willems, R. J., van Schaik, W. & Schürch, A. C. On the (im)possibility of reconstructing plasmids from whole-genome short-read sequencing data. *Microb. Genomics* **3**, e000128 (2017).
21. Koren, S. & Phillippy, A. M. One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly. *Curr. Opin. Microbiol.* **23**, 110–120 (2015).
22. Harimanana, A. *et al.* Neonatal acquisition of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the community of a low-income country (NeoLIC): protocol for a household cohort study in Moramanga, Madagascar. *BMJ Open* **12**, e061463 (2022).
23. Milenkov, M. *et al.* Prevalence, Risk Factors, and Genetic Characterization of Extended-Spectrum Beta-Lactamase Escherichia coli Isolated From Healthy Pregnant Women in Madagascar. *Front. Microbiol.* **12**, (2021).
24. World Health Organization. *WHO Recommendations for Management of Serious Bacterial Infections in Infants Aged 0-59 Days.* (World Health Organization, Geneva, 2024).
25. Magiorakos, A.-P. *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **18**, 268–281 (2012).
26. Russell, N. *et al.* Early-versus late-onset sepsis in neonates – time to shift the paradigm? *Clin. Microbiol. Infect.* **30**, 38–43 (2024).
27. Frank Wolf, M. *et al.* Vertical Transmission of Extended-Spectrum, Beta-Lactamase-Producing Enterobacteriaceae during Preterm Delivery: A Prospective Study. *Microorganisms* **9**, 506 (2021).
28. Sgayer, I. *et al.* Maternal colonization with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in term versus preterm pregnancies. *Int. J. Gynaecol. Obstet. Off. Organ Int. Fed. Gynaecol. Obstet.* **161**, 447–454 (2023).
29. Matok, L. A. *et al.* Mother-to-Neonate Transmission of Antibiotic-Resistant Bacteria: A Cross-Sectional Study. *Microorganisms* **9**, 1245 (2021).
30. Denkel, L. A. *et al.* The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum-lactamase-producing Enterobacteriaceae (ESBL-E). *J. Antimicrob. Chemother.* **69**, 2230–2237 (2014).
31. Sgayer, I. *et al.* Maternal colonization with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in term vs preterm pregnancies. *Int. J. Gynecol. Obstet.* **n/a**.
32. Bezabih, Y. M. *et al.* Comparison of the global prevalence and trend of human intestinal carriage of ESBL-producing *Escherichia coli* between healthcare and community settings: a systematic review and meta-analysis. *JAC-Antimicrob. Resist.* **4**, dlac048 (2022).
33. Carvalho, M. J. *et al.* Antibiotic resistance genes in the gut microbiota of mothers and linked neonates with or without sepsis from low- and middle-income countries. *Nat. Microbiol.* **7**, 1337–

- 1347 (2022).
34. Okomo, U. A. *et al.* Maternal colonization and early-onset neonatal bacterial sepsis in the Gambia, West Africa: a genomic analysis of vertical transmission. *Clin. Microbiol. Infect.* **29**, 386.e1-386.e9 (2023).
  35. Leo, S., Curtis, N. & Zimmermann, P. The neonatal intestinal resistome and factors that influence it—a systematic review. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **28**, 1539–1546 (2022).
  36. Pärnänen, K. *et al.* Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. *Nat. Commun.* **9**, 3891 (2018).
  37. Bargheet, A. *et al.* Development of early life gut resistome and mobilome across gestational ages and microbiota-modifying treatments. *eBioMedicine* **92**, 104613 (2023).
  38. Carattoli, A. Plasmids and the spread of resistance. *Int. J. Med. Microbiol.* **303**, 298–304 (2013).
  39. Smillie, C. S. *et al.* Ecology drives a global network of gene exchange connecting the human microbiome. *Nature* **480**, 241–244 (2011).
  40. He, W., Russel, J., Klincke, F., Nesme, J. & Sørensen, S. J. Insights into the ecology of the infant gut plasmidome. *Nat. Commun.* **15**, 6924 (2024).
  41. Akintayo, I. *et al.* Tracking clonal and plasmid transmission in colistin- and carbapenem-resistant *Klebsiella pneumoniae*. *mSystems* **10**, e01128-24 (2025).
  42. Milenkov, M. *et al.* Implementation of the WHO Tricycle protocol for surveillance of extended-spectrum  $\beta$ -lactamase producing *Escherichia coli* in humans, chickens, and the environment in Madagascar: a prospective genomic epidemiology study. *Lancet Microbe* **5**, 100850 (2024).
  43. Turner, P. *et al.* High Prevalence of Antimicrobial-resistant Gram-negative Colonization in Hospitalized Cambodian Infants. *Pediatr. Infect. Dis. J.* **35**, 856–861 (2016).
  44. Herindrainy, P. *et al.* Acquisition of extended spectrum beta-lactamase-producing enterobacteriaceae in neonates: A community based cohort in Madagascar. *PLOS ONE* **13**, e0193325 (2018).
  45. Albers, L. L. The duration of labor in healthy women. *J. Perinatol. Off. J. Calif. Perinat. Assoc.* **19**, 114–119 (1999).
  46. Boulanger, H. *et al.* Immunologic aspects of preeclampsia. *AJOG Glob. Rep.* **4**, 100321 (2024).
  47. Kennedy, K. M. *et al.* Parity modulates impact of BMI and gestational weight gain on gut microbiota in human pregnancy. *Gut Microbes* **15**, 2259316 (2023).
  48. Berry, A. S. F. *et al.* Remodeling of the maternal gut microbiome during pregnancy is shaped by parity. *Microbiome* **9**, 146 (2021).
  49. Jia, X. *et al.* Comparison of Fecal Microbiota Communities between Primiparous and Multiparous Cows during Non-Pregnancy and Pregnancy. *Anim. Open Access J. MDPI* **13**, 869 (2023).
  50. Mealy, G. *et al.* Impact of previous pregnancy and BMI on cellular and serum immune activity from early to late pregnancy. *Sci. Rep.* **14**, 16055 (2024).

51. Hijazi, S. M., Fawzi, M. A., Ali, F. M. & Galil, K. H. A. E. Multidrug-resistant ESBL-producing Enterobacteriaceae and associated risk factors in community infants in Lebanon. *J. Infect. Dev. Ctries.* **10**, 947–955 (2016).
52. Bäckhed, F. *et al.* Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* **17**, 690–703 (2015).
53. Dominguez-Bello, M. G. *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci.* **107**, 11971–11975 (2010).
54. Hashimoto, M., Hasegawa, H. & Maeda, S. High temperatures promote cell-to-cell plasmid transformation in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **515**, 196–200 (2019).
55. Ratkowsky, D. A., Olley, J., McMeekin, T. A. & Ball, A. Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.* **149**, 1–5 (1982).
56. Shah, P. S. *et al.* Seasonal variations in healthcare-associated infection in neonates in Canada. *Arch. Dis. Child. - Fetal Neonatal Ed.* **98**, F65–F69 (2013).
57. Scherff, N., Rothgänger, J., Weniger, T., Mellmann, A. & Harmsen, D. Real-time plasmid transmission detection pipeline. *Microbiol. Spectr.* **12**, e02100-24 (2024).
58. Nadimpalli, M. L. *et al.* Can breastfeeding protect against antimicrobial resistance? *BMC Med.* **18**, 392 (2020).
59. Orwa, S. A. *et al.* Global prevalence of antibiotic consumption during pregnancy: A systematic review and meta-analysis. *J. Infect.* **89**, 106189 (2024).
60. Jazmati, N., Jazmati, T. & Hamprecht, A. Importance of pre-enrichment for detection of third-generation cephalosporin-resistant Enterobacteriaceae (3GCREB) from rectal swabs. *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 1847–1851 (2017).
61. Jain, C., Rodriguez-R, L. M., Phillippy, A. M., Konstantinidis, K. T. & Aluru, S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat. Commun.* **9**, 5114 (2018).
62. Feldgarden, M. *et al.* AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci. Rep.* **11**, 12728 (2021).
63. Carattoli, A. *et al.* In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* **58**, 3895–3903 (2014).
64. Criscuolo, A. A fast alignment-free bioinformatics procedure to infer accurate distance-based phylogenetic trees from genome assemblies. *Res. Ideas Outcomes* **5**, e36178 (2019).
65. Criscuolo, A. On the transformation of MinHash-based uncorrected distances into proper evolutionary distances for phylogenetic inference. *F1000Research* **9**, 1309 (2020).
66. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).

67. Treangen, T. J., Ondov, B. D., Koren, S. & Phillippy, A. M. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol.* **15**, 524 (2014).
68. Wick, R. R., Judd, L. M., Gorrie, C. L. & Holt, K. E. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLOS Comput. Biol.* **13**, e1005595 (2017).
69. Vasimuddin, Md., Misra, S., Li, H. & Aluru, S. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. In *2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS)* 314–324 (2019). doi:10.1109/IPDPS.2019.00041.
70. Abram, K. *et al.* Mash-based analyses of *Escherichia coli* genomes reveal 14 distinct phylogroups. *Commun. Biol.* **4**, 1–12 (2021).

## Table

**Table 1. Population characteristics stratified by study project.**

*Notably, the study included two pairs of twins, leading to a total of two more neonates than mothers. The variable “neonatal systemic antibiotic administration” excludes antibiotics administered via the ophthalmic route.*

*ESBL-PE: Extended-Spectrum Beta-Lactamase-producing Enterobacterales.*

|                                                    |     | Madagascar                         |                                            |                                             |
|----------------------------------------------------|-----|------------------------------------|--------------------------------------------|---------------------------------------------|
|                                                    | N   | Cambodia<br>(2016-17) <sup>1</sup> | Madagascar<br>(BIRDY 2019-20) <sup>1</sup> | Madagascar<br>(NeoLIC 2021-22) <sup>1</sup> |
| <b>- MOTHERS / HOUSEHOLDS -</b>                    |     | <b>131</b>                         | <b>299</b>                                 | <b>67</b>                                   |
| <b>Setting</b>                                     | 497 |                                    |                                            |                                             |
| <i>Rural</i>                                       |     | 76 (58%)                           | 203 (68%)                                  | 67 (100%)                                   |
| <i>Urban</i>                                       |     | 55 (42%)                           | 96 (32%)                                   | 0 (0%)                                      |
| <b>Age of the mother at inclusion</b>              | 497 | 27.0 (24.0, 31.0)                  | 24.7 (20.4, 29.8)                          | 25.7 (22.0, 30.6)                           |
| <b>Latrine localisation</b>                        | 489 |                                    |                                            |                                             |
| <i>inside</i>                                      |     | 67 (51%)                           | 26 (8.9%)                                  | 3 (4.5%)                                    |
| <i>outside</i>                                     |     | 64 (49%)                           | 265 (91%)                                  | 64 (96%)                                    |
| <b>Number of household members</b>                 | 497 | 5 (3, 6)                           | 4 (3, 5)                                   | 3 (3, 4)                                    |
| <b>Housing with electricity</b>                    | 497 | 130 (99%)                          | 222 (74%)                                  | 49 (73%)                                    |
| <b>Education of the mother</b>                     | 497 |                                    |                                            |                                             |
| <i>primary or none</i>                             |     | 81 (62%)                           | 53 (18%)                                   | 13 (19%)                                    |
| <i>secondary</i>                                   |     | 35 (27%)                           | 219 (73%)                                  | 41 (61%)                                    |
| <i>higher</i>                                      |     | 15 (11%)                           | 27 (9.0%)                                  | 13 (19%)                                    |
| <b>Profession of the mother</b>                    | 497 |                                    |                                            |                                             |
| <i>paid job</i>                                    |     | 91 (69%)                           | 125 (42%)                                  | 34 (51%)                                    |
| <i>unemployed or student</i>                       |     | 40 (31%)                           | 174 (58%)                                  | 33 (49%)                                    |
| <b>Primigravidae</b>                               | 497 | 51 (39%)                           | 106 (35%)                                  | 26 (39%)                                    |
| <b>Maternal antibiotic intake during pregnancy</b> | 497 | 2 (1.5%)                           | 31 (10%)                                   | 18 (27%)                                    |
| <b>Delivery location</b>                           | 495 |                                    |                                            |                                             |
| <i>home</i>                                        |     | 3 (2.3%)                           | 140 (47%)                                  | 0 (0%)                                      |
| <i>primary healthcare center</i>                   |     | 21 (16%)                           | 116 (39%)                                  | 65 (100%)                                   |
| <i>hospital/clinic</i>                             |     | 107 (82%)                          | 43 (14%)                                   | 0 (0%)                                      |
| <b>Cesarean section</b>                            | 497 | 17 (13%)                           | 36 (12%)                                   | 0 (0%)                                      |

|                                                        |            | <b>Madagascar</b>                          |                                                         |                                                          |
|--------------------------------------------------------|------------|--------------------------------------------|---------------------------------------------------------|----------------------------------------------------------|
|                                                        | <b>N</b>   | <b>Cambodia<br/>(2016-17) <sup>1</sup></b> | <b>Madagascar<br/>(BIRDY 2019-<br/>20) <sup>1</sup></b> | <b>Madagascar<br/>(NeoLIC 2021-<br/>22) <sup>1</sup></b> |
| <b>- MOTHERS / HOUSEHOLDS -</b>                        |            | <b>131</b>                                 | <b>299</b>                                              | <b>67</b>                                                |
| <b>Perpartum antibiotic administration</b>             | 485        | 26 (20%)                                   | 42 (14%)                                                | 40 (60%)                                                 |
| <b>Maternal colonization with ESBL-<br/>PE</b>         | 497        | 104 (79%)                                  | 102 (34%)                                               | 37 (55%)                                                 |
| <b>- NEONATES -</b>                                    | <b>499</b> | <b>131</b>                                 | <b>301</b>                                              | <b>67</b>                                                |
| <b>Sex of the baby</b>                                 | 499        |                                            |                                                         |                                                          |
| <i>Female</i>                                          |            | 73 (56%)                                   | 143 (48%)                                               | 34 (51%)                                                 |
| <i>Male</i>                                            |            | 58 (44%)                                   | 158 (52%)                                               | 33 (49%)                                                 |
| <b>Low-birth weight (&lt;2500g)</b>                    | 492        | 4 (3.1%)                                   | 17 (5.8%)                                               | 3 (4.5%)                                                 |
| <b>Neonatal resuscitation</b>                          | 498        | 10 (7.6%)                                  | 39 (13%)                                                | 13 (19%)                                                 |
| <b>Neonatal systemic antibiotic<br/>administration</b> | 499        | 6 (4.6%)                                   | 12 (4.0%)                                               | 5 (7.5%)                                                 |
| <b>Neonatal colonization with ESBL-<br/>PE</b>         | 499        | 70 (53%)                                   | 79 (26%)                                                | 21 (31%)                                                 |

<sup>1</sup>n (%); Median (Q1, Q3)

## Figures

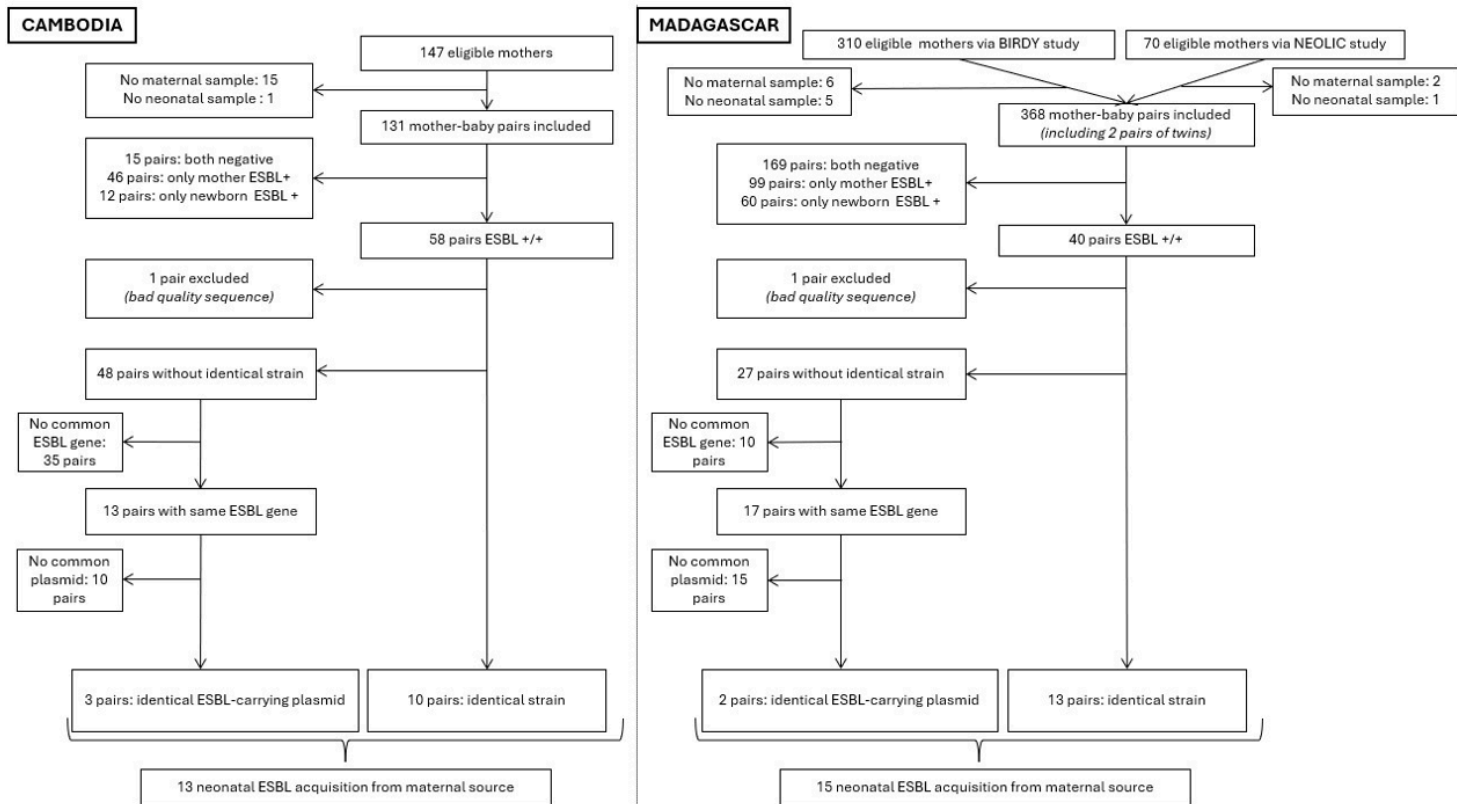


Figure 1

Study flow-chart.

*ESBL+:* colonized with extended-spectrum beta-lactamase (ESBL-)producing Enterobacterales.

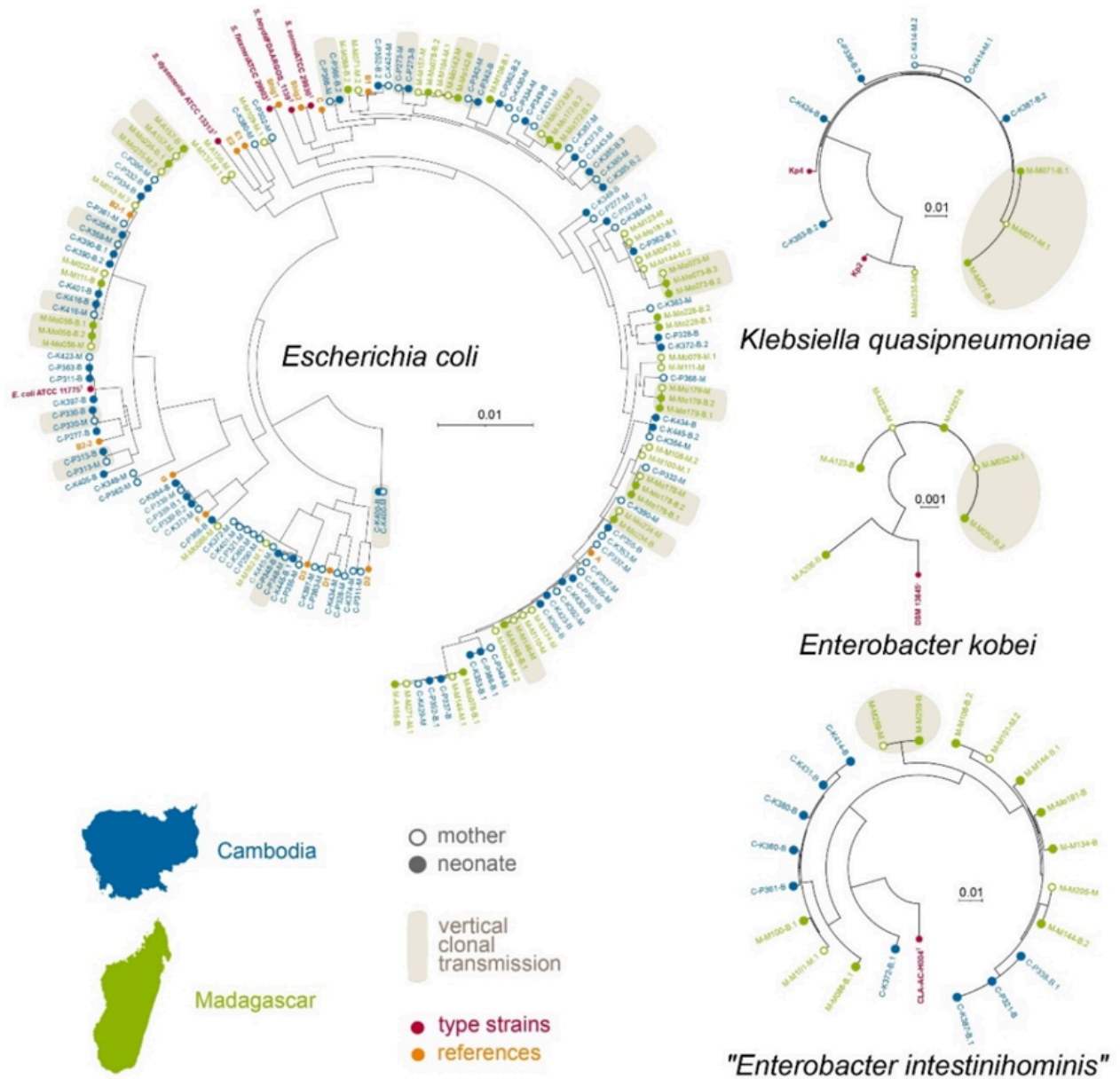
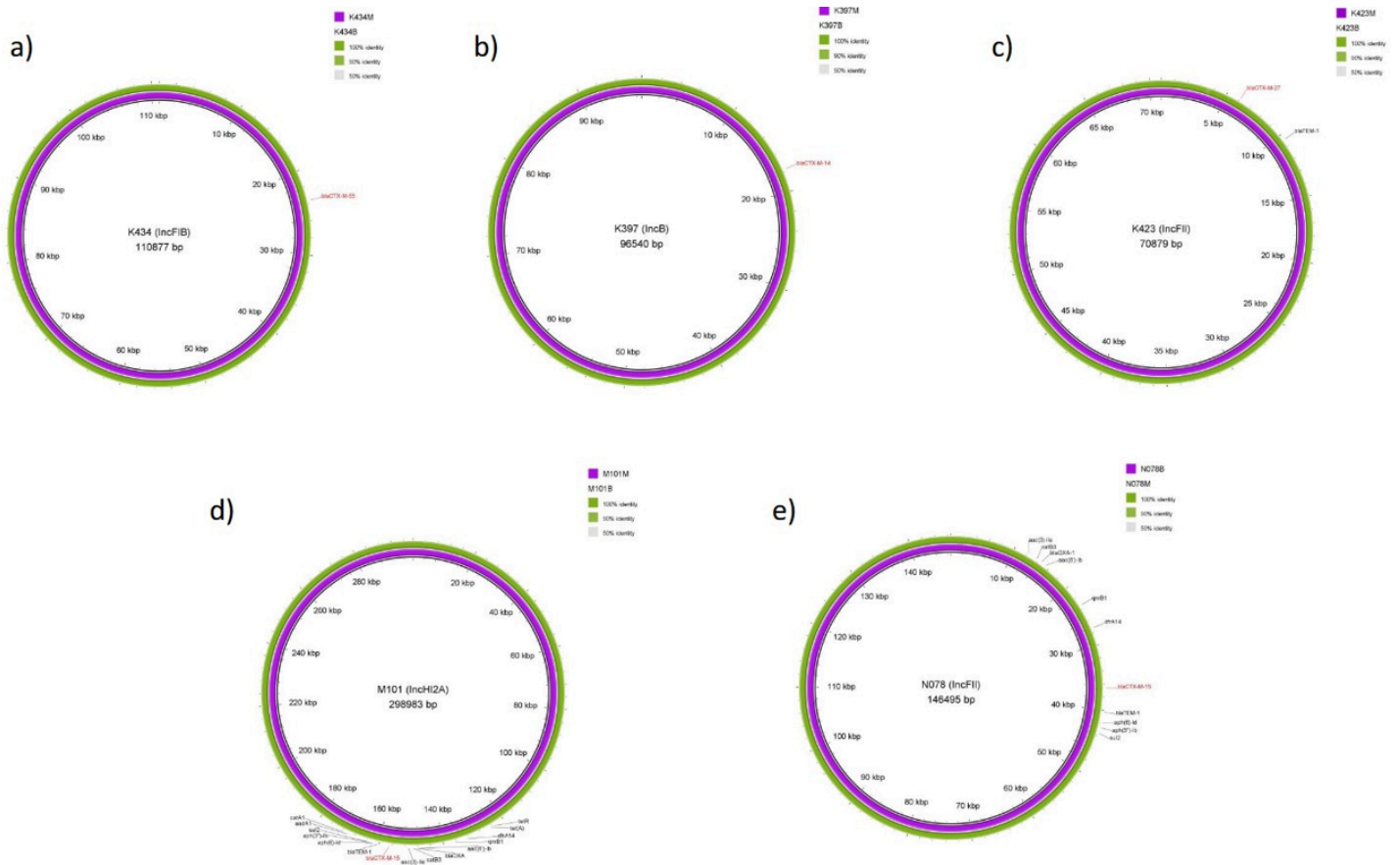


Figure 2

**Phylogenetic trees and identified clones within mother-neonate pairs.**

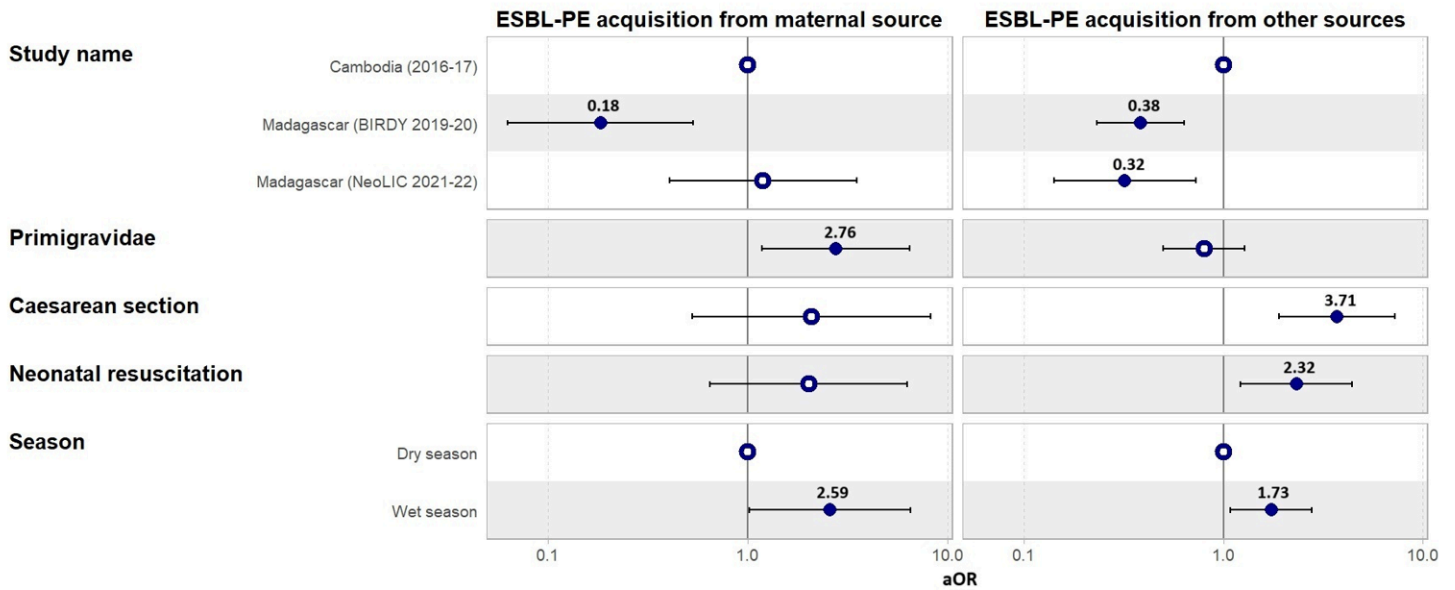
Maximum likelihood phylogenetic trees displaying all the isolates belonging to a mother-neonate pair both colonized by a common species, either *Escherichia coli*, *Klebsiella quasipneumoniae*, *Enterobacter kobei* or "*Enterobacter intestinhominis*". Blue and green leaves indicate Cambodia and Madagascar, respectively. Empty and filled circles correspond to mother and neonate, respectively. Red leaves indicate the type strain of the species, whereas orange leaves indicate the representative strains of the 14 *E. coli* phylogroups A, B1, B2-1, B2-2, C, D1, D2, D3, E1, E2, F, G, Shig1 and Shig2, as identified by Abram et al<sup>70</sup>. Genetically identical isolates within a pair are highlighted in sand color. Bars indicate the number of nucleotide substitutions per character.



**Figure 3**

**Alignment of maternal and neonatal plasmids in five pairs.**

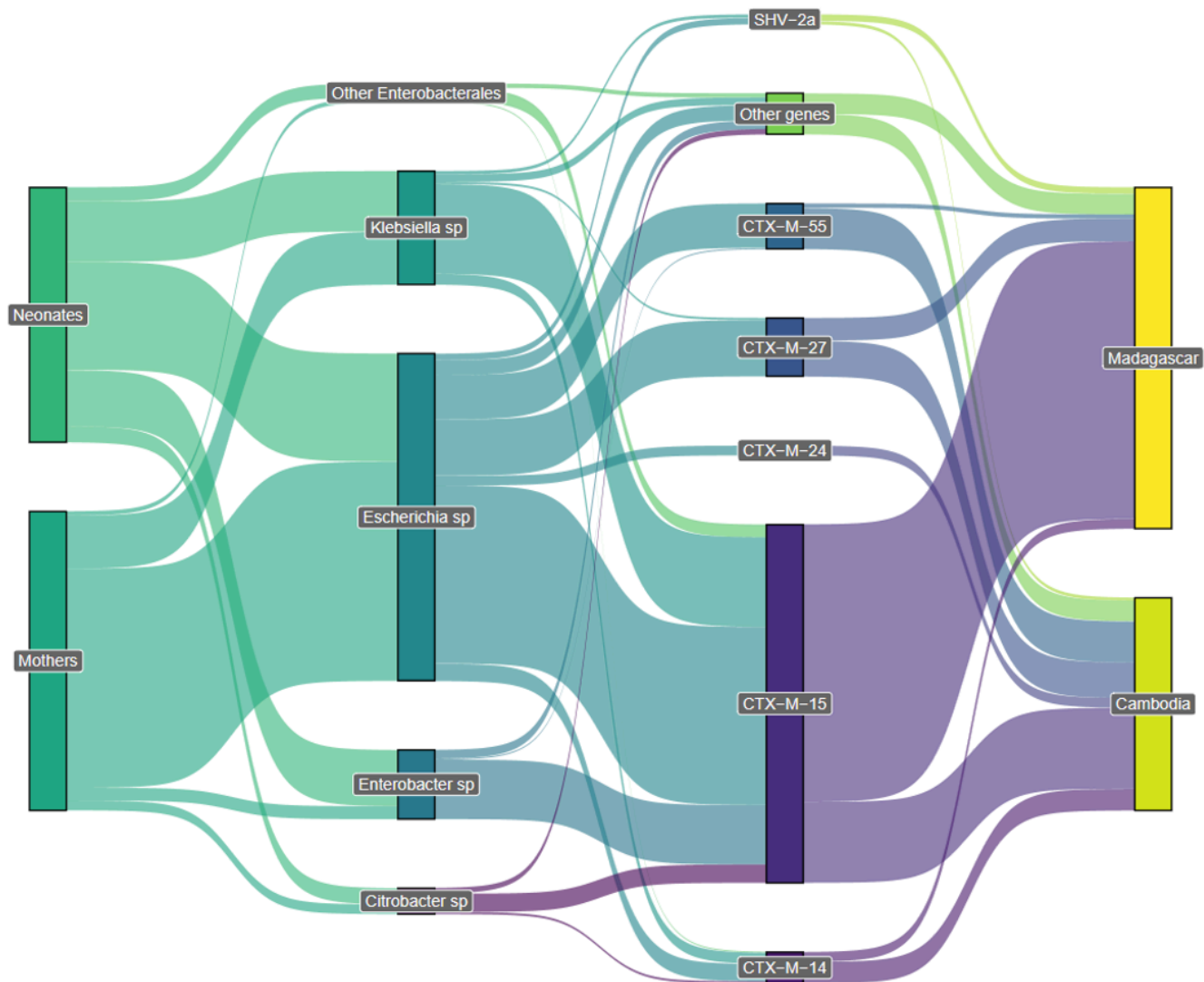
*Alignments of ESBL-encoding-genes-carrying plasmids in mother-neonate pairs where identical plasmids were observed. Cambodian and Malagasy plasmid pairs are displayed on the upper (a, b, c) and bottom (d, e) panels, respectively. For each pair, inner purple ring represents the fully circularized plasmid sequence of one mate, whereas outer green ring shows the shared nucleotide identity (according to BLASTn) with the assembly of the other mate. Nucleotide identity percentage is indicated at the upper right corner of plasmid pair. ESBL-encoding genes are indicated in red; other antibiotic resistance genes are indicated in black. The pair identification number, plasmid incompatibility group (Inc) and plasmid size (in bp) are indicated at the center of each alignment. The image was prepared using Blast Ring Image Generator ([sourceforge.net/projects/brig](https://sourceforge.net/projects/brig))*



**Figure 4**

**Multinomial regression models.**

*Forest plot displaying adjusted odds ratios (aOR) for ESBL-PE acquisition attributed to maternal source (left panel) or attributed to other sources (right panel) to no acquisition (reference group). Error bars represent 95% confidence intervals. The aOR is shown as a blue-filled dot when significantly different from 1 (with the estimated value displayed above the dot) and as a white-centered dot when not statistically significant. The absence of error bars indicates the reference category for a variable. The model was performed using complete cases, including 472 pairs.*



**Figure 5**

**Sankey diagram.**

*For the 501 bacterial isolates included in the genotypic analysis, this diagram represents the link between the origin of the sample (neonate or mother), the genus of the bacterial species identified in silico, the ESBL-encoding gene detected on the isolate, and the country. Of note, only one ESBL-encoding gene per isolate is displayed.*

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable2.xlsx](#)
- [SupplementaryMaterial.docx](#)