
Supplementary material of the article
“Contribution of maternal gut carriage to neonatal acquisition of extended-spectrum beta-lactamase-producing Enterobacterales in Madagascar and Cambodia.”

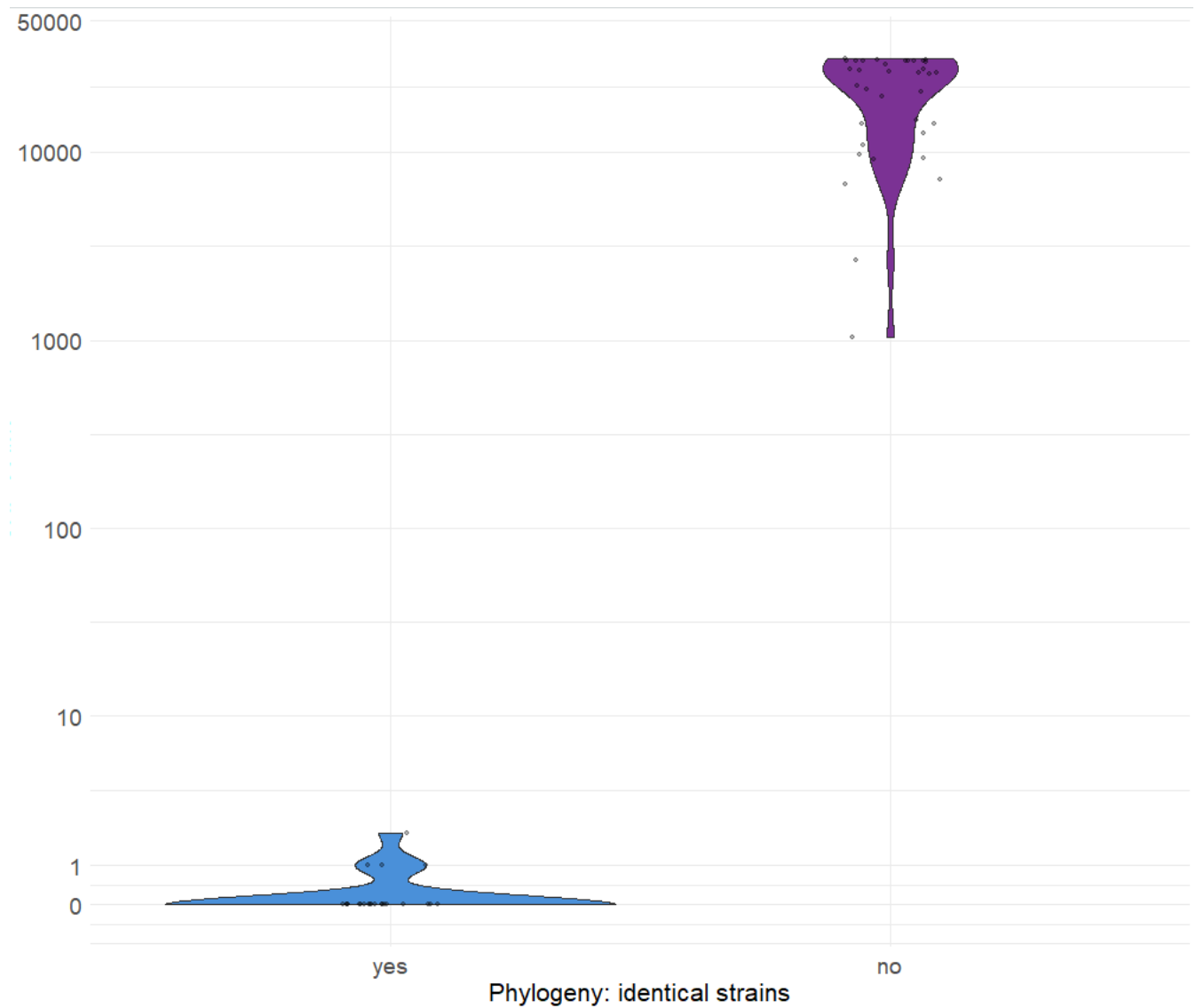
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Supplementary Figure 1. Violin plot showing the distribution of minimal SNP counts between maternal and neonatal isolates in mother–neonate pairs sharing at least one bacterial species.

Pairs identified as identical by phylogenetic inference are shown in blue; pairs identified as different by phylogenetic inference are shown in purple. Individual data points are overlaid. The y-axis is pseudo-log transformed for clarity. Source data are provided as a Source Data file.

SNP : Single Nucleotid Polymorphism.



Supplementary Figure 2. Antibiotic susceptibility testing (AST) results (disc diffusion method), divided in four categories by country and origin.

Antibiotic resistance profiles were established for 513 isolates. For quinolone, CIPROFLOXACIN has been tested for all subcategories except for Cambodian neonates, for which a MOXIFLOXACIN disc was used, due to technical constraints. Source data are provided as a Source Data file.

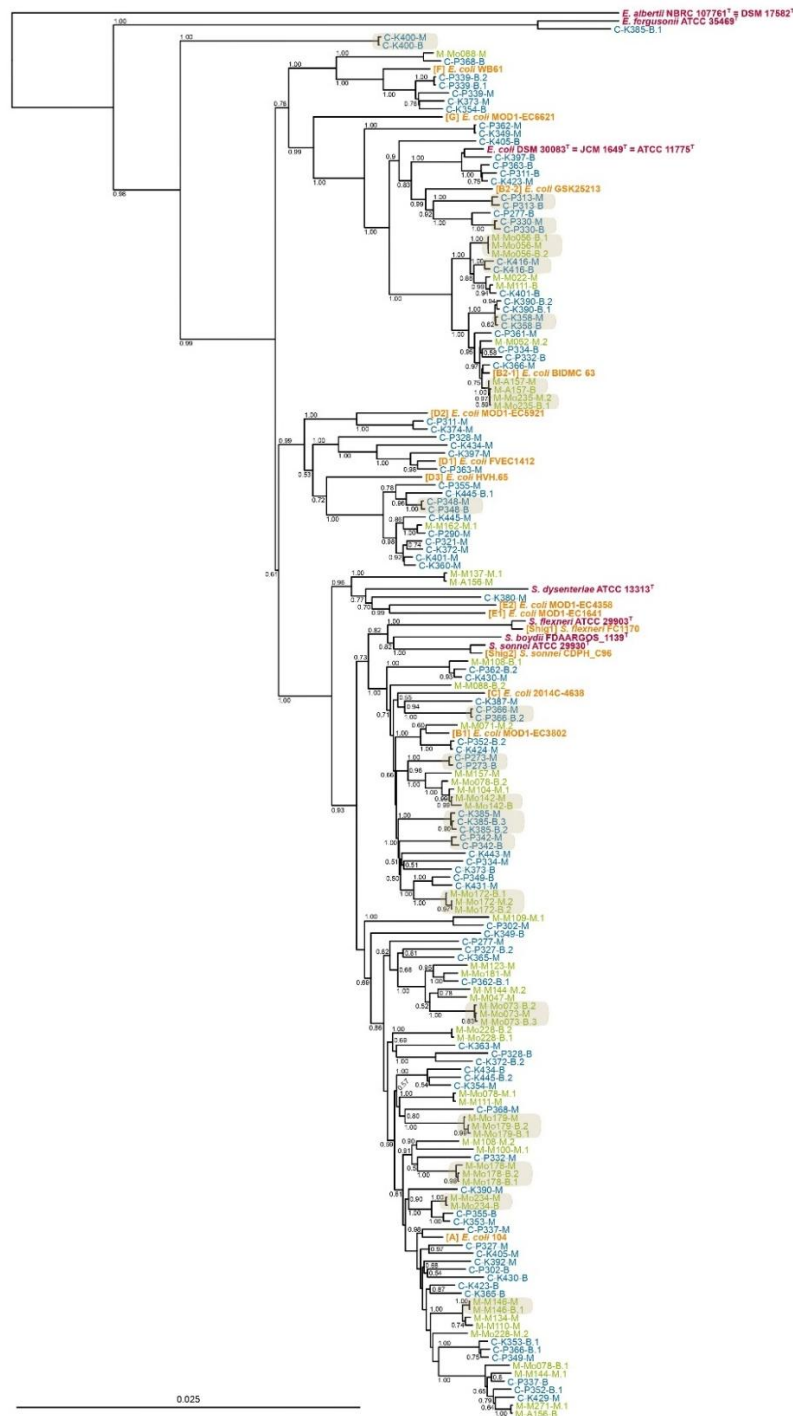
Acronyms: S: susceptible, I: intermediate, R: resistant according to EUCAST 2018 cut-offs. NA: missing data.

Camb: Cambodia, Mada: Madagascar.



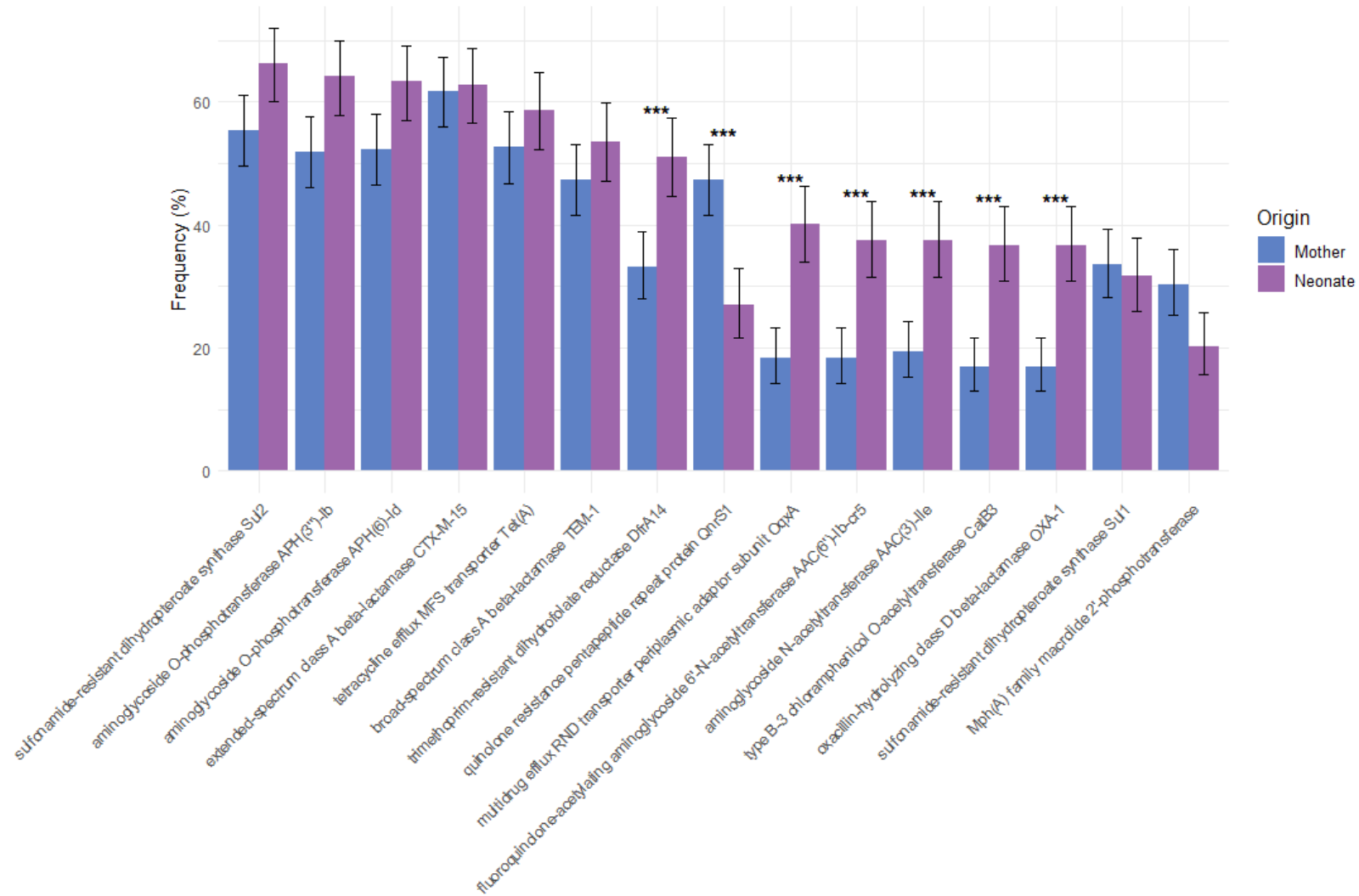
Supplementary Figure 3. Genome-based phylogenetic classification of the isolates belonging to the genus *Escherichia*.

Minimum evolution midpoint-rooted phylogenetic tree displaying all the isolates belonging to a mother-neonate pair both colonized by an *Escherichia* species. Cambodian (blue) and Madagasy (green) isolate names start with C and M prefixes, respectively, whereas M and B suffixes correspond to mother and neonate, respectively. Three *Escherichia* and four *Shigella* type strain genomes (red) were added to identify the species of the different ESBL-PE strains, as well as the genomes of the representative strains (orange) of the 14 *E. coli* phylogroups (Abram et al. 2021, doi:10.1038/s42003-020-01626-5) in order to illustrate the diversity of the sampled ESBL-PE isolates within the *E. coli* population structure. Neonatal acquisitions from maternal source (highlighted in sand color) are assessed by a mother-neonate cherry. Branch supports (only supports >0.5 were specified) were assessed by the rate of elementary quartets, as estimated by JolyTree. Bar, 0.025 nucleotide substitutions per character.



Supplementary Figure 4. Histogram of the frequency of the 15 most common resistance genes identified by AMRFinder, stratified by maternal and neonatal strains.

Error bars indicate 95% confidence intervals for proportions. Statistical significance between maternal and neonatal strains was assessed using a two-sided chi-squared test with Bonferroni correction for multiple comparisons (p-value cutoff: < 0.003), with stars indicating significant differences. Source data are provided as a Source Data file.

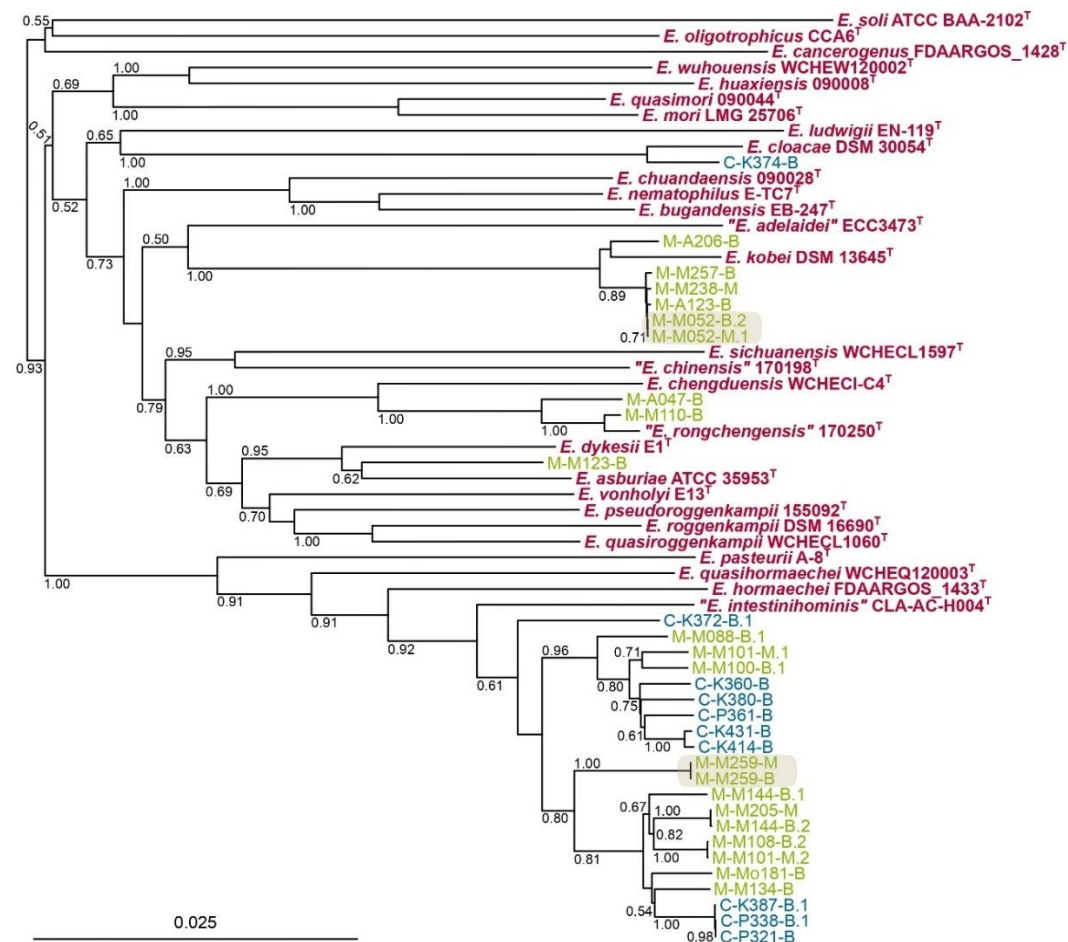


Supplementary Figure 5. Genome-based phylogenetic classification of the isolates belonging to the genus *Klebsiella*.

Minimum evolution midpoint-rooted phylogenetic tree displaying all the isolates belonging to a mother-neonate pair both colonized by a *Klebsiella* species. Cambodian (blue) and Madagasy (green) isolate names start with C and M prefixes, respectively, whereas M and B suffixes correspond to mother and neonate, respectively. A total of 16 *Klebsiella* type strain genomes (red) were added to identify the species of the different ESBL-PE isolates. The type strains belonging to the *Klebsiella pneumoniae* complex are also labeled using their Kp1-Kp7 standard phylogroups identifiers (e.g. Rodrigues et al. 2019, doi:10.1016/j.resmic.2019.02.003). Neonatal acquisitions from maternal source (highlighted in sand) are assessed by a mother-neonate cherry. Branch supports (only supports >0.5 were specified) were assessed by the rate of elementary quartets, as estimated by JolyTree. Bar, 0.025 nucleotide substitutions per character.



Minimum evolution midpoint-rooted phylogenetic tree displaying all the isolates belonging to a mother-neonate pair both colonized by an *Enterobacter* species. Cambodian (blue) and Malagasy (green) isolate names start with C and M prefixes, respectively, whereas M and B suffixes correspond to mother and neonate, respectively. A total of 28 *Enterobacter* type strain genomes (red) were added to identify the species of the different ESBL-PE isolates. Neonatal acquisitions from maternal source (highlighted in sand color) are assessed by a mother-neonate cherry. Branch supports (only supports >0.5 were specified) were assessed by the rate of elementary quartets, as estimated by JolyTree. Bar, 0.025 nucleotide substitutions per character.



Supplementary Table 1. Cross-table of neonatal sampling day vs ESBL-E colonization status, acquisition from maternal origin, and acquisition from other sources.

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

	Day of neonatal sampling			p-value*
	Day 1	Day 2	Day 3	
N	397	28	74	
Neonatal ESBL-PE colonization (n, %)	143 (36%)	6 (21.4%)	21 (28.4%)	0.15
ESBL-PE acquisition from the mother	24 (6.0%)	1 (3.6%)	3 (4.1%)	0.92
ESBL-PE acquisition from another source	119 (30.0%)	5 (17.9%)	18 (24.3%)	0.27

*Two-sided Pearson's Chi-squared test; Two-sided Fisher test

Supplementary Table 2. Population characteristics, stratified by ESBL-PE colonization status.

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.*

		ESBL-PE colonization status		p-value ²
		Not colonized ¹	Colonized ¹	
- MOTHERS -	N	254	243	
Study project	497			3.189e-17
<i>Cambodia (2016-17)</i>		27 (11%)	104 (43%)	
<i>Madagascar (BIRDY 2019-20)</i>		197 (78%)	102 (42%)	
<i>Madagascar (NeoLIC 2021-22)</i>		30 (12%)	37 (15%)	
Setting	497			0.6719
<i>Rural</i>		179 (70%)	167 (69%)	
<i>Urban</i>		75 (30%)	76 (31%)	
Age of the mother at inclusion	497	25.5 (20.7, 29.8)	26.0 (22.0, 30.6)	0.1692
Latrine localization	489			0.0101
<i>inside</i>		37 (15%)	59 (24%)	
<i>outside</i>		209 (85%)	184 (76%)	
Number of household members	497	4 (3, 5)	4 (3, 5)	0.0110
Housing with electricity	497	197 (78%)	204 (84%)	0.0712
Education of the mother	497			1.134e-04
<i>primary or no formal education</i>		57 (22%)	90 (37%)	
<i>secondary</i>		174 (69%)	121 (50%)	
<i>higher</i>		23 (9.1%)	32 (13%)	
Profession of the mother	497			0.3923
<i>paid job</i>		123 (48%)	127 (52%)	
<i>unemployed or student</i>		131 (52%)	116 (48%)	
Primigravidae	497	96 (38%)	87 (36%)	0.6452
Maternal antibiotic intake during pregnancy	497	32 (13%)	19 (7.8%)	0.0792
Delivery location	495			1.078e-09
<i>home</i>		98 (39%)	45 (19%)	
<i>primary healthcare center</i>		108 (43%)	94 (39%)	
<i>hospital/clinic</i>		47 (19%)	103 (43%)	
Caesarean section	497	24 (9.4%)	29 (12%)	0.3695
Peripartum antibiotic administration	485	46 (19%)	62 (26%)	0.0440
- NEONATES -	499	329	170	
Sex of the baby	499			0.1756
<i>Female</i>		172 (52%)	78 (46%)	
<i>Male</i>		157 (48%)	92 (54%)	
Low-birth weight (<2500g)	492	17 (5.2%)	7 (4.2%)	0.5979
Neonatal resuscitation	498	33 (10%)	29 (17%)	0.0249
Neonatal systemic antibiotic administration	499	11 (3.3%)	12 (7.1%)	0.0607

¹n (column %); Median (Q1, Q3)

²Two-sided pearson's Chi-squared test; Two-sided wilcoxon rank sum test.

In the first column, bold text indicates the variable name, while italic text indicates different values of the same variable.

Supplementary Table 3. Results of multinomial regression models from imputed datasets.

This table presents the adjusted odds ratios from multinomial regression models computed on 10 imputed datasets, where missing data were handled using the Predictive Mean Matching (PMM) method from the mice package. *p*-values were obtained from Wald tests on regression coefficients, are two-sided, and no adjustment for multiple comparisons was applied. Source data are provided as a Source Data file, containing exact *p*-values.

Variable	Model Estimates									
	Model m1	Model m2	Model m3	Model m4	Model m5	Model m6	Model m7	Model m8	Model m9	Model m10
Outcome Level: ESBL-PE acquisition from maternal source										
(Intercept)	0.06 [0.02-0.18], p=0	0.06 [0.02-0.19], p=0	0.06 [0.02-0.18], p=0	0.06 [0.02-0.18], p=0	0.06 [0.02-0.18], p=0	0.06 [0.02-0.19], p=0	0.06 [0.02-0.18], p=0	0.06 [0.02-0.18], p=0	0.06 [0.02-0.19], p=0	0.06 [0.02-0.19], p=0
Study site: Madagascar (BIRDY 2019-20)	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0	0.16 [0.06-0.47], p=0
Study site: Madagascar (NeOLIC 2021-22)	1.28 [0.45-3.63], p=0.64	1.29 [0.45-3.64], p=0.64	1.28 [0.45-3.63], p=0.64	1.28 [0.45-3.63], p=0.64	1.28 [0.45-3.63], p=0.64	1.29 [0.45-3.64], p=0.64	1.28 [0.45-3.63], p=0.64	1.28 [0.45-3.63], p=0.64	1.29 [0.45-3.64], p=0.64	1.29 [0.45-3.64], p=0.64
Primigravidae	2.51 [1.1-5.74], p=0.03	2.52 [1.1-5.76], p=0.03	2.51 [1.1-5.74], p=0.03	2.51 [1.1-5.74], p=0.03	2.51 [1.1-5.74], p=0.03	2.52 [1.1-5.76], p=0.03	2.51 [1.1-5.74], p=0.03	2.51 [1.1-5.74], p=0.03	2.52 [1.1-5.76], p=0.03	2.52 [1.1-5.76], p=0.03
Cesarean section	2.12 [0.54-8.28], p=0.28	2.1 [0.54-8.21], p=0.29	2.12 [0.54-8.28], p=0.28	2.12 [0.54-8.28], p=0.28	2.12 [0.54-8.28], p=0.28	2.1 [0.54-8.21], p=0.29	2.12 [0.54-8.28], p=0.28	2.12 [0.54-8.28], p=0.28	2.1 [0.54-8.21], p=0.29	2.1 [0.54-8.21], p=0.29
Neonatal resuscitation	1.92 [0.62-5.93], p=0.25	1.84 [0.6-5.65], p=0.29	1.92 [0.62-5.93], p=0.25	1.92 [0.62-5.93], p=0.25	1.92 [0.62-5.93], p=0.25	1.84 [0.6-5.65], p=0.29	1.92 [0.62-5.93], p=0.25	1.92 [0.62-5.93], p=0.25	1.84 [0.6-5.65], p=0.29	1.84 [0.6-5.65], p=0.29
Season: Wet	2.31 [0.94-5.68], p=0.07	2.3 [0.94-5.64], p=0.07	2.31 [0.94-5.68], p=0.07	2.31 [0.94-5.68], p=0.07	2.31 [0.94-5.68], p=0.07	2.3 [0.94-5.64], p=0.07	2.31 [0.94-5.68], p=0.07	2.31 [0.94-5.68], p=0.07	2.3 [0.94-5.64], p=0.07	2.3 [0.94-5.64], p=0.07
Outcome Level: ESBL-PE acquisition from other sources										
(Intercept)	0.55 [0.32-0.92], p=0.02	0.55 [0.33-0.93], p=0.03	0.55 [0.32-0.92], p=0.02	0.55 [0.32-0.92], p=0.02	0.55 [0.32-0.92], p=0.02	0.55 [0.33-0.93], p=0.03	0.55 [0.32-0.92], p=0.02	0.55 [0.32-0.92], p=0.02	0.55 [0.33-0.93], p=0.03	0.55 [0.33-0.93], p=0.03
Study site: Madagascar (BIRDY 2019-20)	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0	0.38 [0.23-0.62], p=0
Study site: Madagascar (NeOLIC 2021-22)	0.36 [0.17-0.78], p=0.01	0.37 [0.17-0.79], p=0.01	0.36 [0.17-0.78], p=0.01	0.36 [0.17-0.78], p=0.01	0.36 [0.17-0.78], p=0.01	0.37 [0.17-0.79], p=0.01	0.36 [0.17-0.78], p=0.01	0.36 [0.17-0.78], p=0.01	0.37 [0.17-0.79], p=0.01	0.37 [0.17-0.79], p=0.01
Primigravidae	0.84 [0.54-1.32], p=0.46	0.85 [0.54-1.32], p=0.46	0.84 [0.54-1.32], p=0.46	0.84 [0.54-1.32], p=0.46	0.84 [0.54-1.32], p=0.46	0.85 [0.54-1.32], p=0.46	0.84 [0.54-1.32], p=0.46	0.84 [0.54-1.32], p=0.46	0.85 [0.54-1.32], p=0.46	0.85 [0.54-1.32], p=0.46
Cesarean section	3.64 [1.92-6.89], p=0	3.6 [1.9-6.82], p=0	3.64 [1.92-6.89], p=0	3.64 [1.92-6.89], p=0	3.64 [1.92-6.89], p=0	3.6 [1.9-6.82], p=0	3.64 [1.92-6.89], p=0	3.64 [1.92-6.89], p=0	3.6 [1.9-6.82], p=0	3.6 [1.9-6.82], p=0
Neonatal resuscitation	2.23 [1.2-4.13], p=0.01	2.09 [1.13-3.87], p=0.02	2.23 [1.2-4.13], p=0.01	2.23 [1.2-4.13], p=0.01	2.23 [1.2-4.13], p=0.01	2.09 [1.13-3.87], p=0.02	2.23 [1.2-4.13], p=0.01	2.23 [1.2-4.13], p=0.01	2.09 [1.13-3.87], p=0.02	2.09 [1.13-3.87], p=0.02
Season: Wet	1.68 [1.08-2.63], p=0.02	1.67 [1.07-2.6], p=0.02	1.68 [1.08-2.63], p=0.02	1.68 [1.08-2.63], p=0.02	1.68 [1.08-2.63], p=0.02	1.67 [1.07-2.6], p=0.02	1.68 [1.08-2.63], p=0.02	1.68 [1.08-2.63], p=0.02	1.67 [1.07-2.6], p=0.02	1.67 [1.07-2.6], p=0.02