



Research Article

Concordance of Beta-Lactam Resistance Profiles in Mother-Placenta-Newborn Triads: Evidence for Vertical Transmission in Brazzaville, Congo

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Abstract

Mother-to-child transmission of beta-lactam-resistant Enterobacteriaceae is a growing public health concern in sub-Saharan Africa, yet few studies sample the mother, the placenta and the newborn simultaneously, and none in the region measures how often two unrelated isolates share a resistance profile by chance. This analytical cross-sectional study was conducted from October 2022 to July 2025 at the Marien Ngouabi Integrated Health Centre in Brazzaville. Ninety-one mother-newborn-placenta triads were included. Three specimens were collected per triad: a maternal rectal swab on the day of delivery, a neonatal rectal swab between the 1st hour of life and the 4th day, and a swab of the inner surface of the placenta after disinfection of the fetal side with 90% alcohol. Isolates were identified with API 20E strips and 16S rRNA gene sequencing; susceptibility to seven beta-lactams was determined by agar diffusion and ESBL production was sought by the double-disc synergy test. Observed concordance was compared with a null distribution generated by 20,000 species-stratified permutations. Enterobacteriaceae were isolated from 91 of 91 mothers, 66 of 91 newborns (73%) and 57 of 91 placentas (63%). Thirty-three triads were positive in all three compartments and formed the analysis population, yielding 267 identified isolates, of which 241 (90.3%) belonged to the genera *Escherichia*, *Enterobacter* and *Klebsiella*. Among these 241 isolates, 48.5% were resistant to at least one third-generation cephalosporin and 28.2% to at least one carbapenem. Seventy-nine triads contained an isolate of the same species in all three compartments, and 37 of them (47%) showed a strictly identical resistance profile in the mother, in the placenta and in the newborn. Fewer than one such triad would be expected under the hypothesis of independence, and across 20,000 random reallocations this number never exceeded seven. Among unrelated isolates, profile identity was only 1.9%. An extended-spectrum beta-lactamase was produced by 67 of the 111 isolates from concordant triads (60.4%). Concordance within triads therefore far exceeds chance and supports peripartum mother-to-child transmission of resistant Enterobacteriaceae; molecular typing remains necessary to establish clonal identity.

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Keywords

Enterobacteriaceae, Extended-Spectrum Beta-Lactamase, Vertical Transmission, Placenta, Newborn, Beta-Lactam Resistance, Intestinal Carriage

1. Introduction

Extended-spectrum beta-lactamase (ESBL) production is one of the main mechanisms by which Enterobacteriaceae resist penicillins and cephalosporins [1]. Community intestinal carriage of ESBL-producing strains has spread worldwide over the past two decades, driven by the diffusion of CTX-M-type enzymes [2]. This expansion is of particular concern in the neonatal period, since the immune immaturity of the newborn favours the acquisition and persistence of resistant strains [3].

Intestinal colonisation of the newborn begins in the first hours of life and draws on maternal and environmental sources [4, 5]. Maternal carriage is a documented risk factor for early-onset neonatal infection [6, 7], and a recent meta-analysis establishes that transmission of multidrug-resistant Gram-negative bacilli from a colonised mother to her newborn is frequent in resource-limited settings [8]. The infant intestinal resistome is closely linked to maternal strains [9, 10].

The role of the placental compartment remains debated. The hypothesis of a resident placental microbiome [11] has been strongly challenged by work showing that most detected bacterial signals arise from reagent contamination or from intrapartum acquisition [12]. Sampling the three compartments together nonetheless documents the circulation of strains at the maternal-fetal interface, independently of the existence of a resident placental microbiome.

In sub-Saharan Africa, the burden of resistance among neonatal Gram-negative bacilli is high [13, 14], and genomic analyses have established the reality of vertical transmission in this setting [15]. Congolese data remain scarce. Moyon et al. [16] documented the transmission of resistance between mothers and children in Brazzaville without sampling the placenta, and Dangui Nieko et al. [17] described the circulation of ESBL- and metallo-beta-lactamase-producing strains at the National Public Health Laboratory.

The main methodological obstacle is that a resistance profile shared by a mother and her child may reflect transmission, but equally a coincidence, since antibiograms are few in number and widely distributed. No study available in the region explicitly quantifies this chance component.

The present study pursued two objectives: to describe Enterobacteriaceae and their beta-lactam resistance profiles in the three compartments of the triad, and to determine whether concordance of profiles within a triad significantly exceeds what the distribution of antibiograms in the study population would produce on its own.

2. Materials and Methods

2.1. Study Design, Setting and Population

An analytical cross-sectional study was conducted from October 2022 to July 2025 in the maternity and neonatology wards of the Marien Ngouabi Integrated Health Centre, Brazzaville. Parturients who delivered vaginally and gave written informed consent were included. Caesarean delivery, an ongoing maternal condition or hospitalisation at the time of delivery, and refusal to participate were criteria for non-inclusion.

Ninety-one triads were recruited. Because the research question concerns the circulation of a single strain between the three compartments, the full microbiological workup was restricted, by prior decision, to triads in which all three specimens yielded a culture positive for Enterobacteriaceae, that is 33 triads (36%). Carriage prevalences are reported on the recruited sample ($n = 91$); all other results relate to the analysis population ($n = 33$).

2.2. Specimen Collection

Three specimens were collected per triad. A maternal rectal swab was taken on the day of delivery. A neonatal rectal swab was taken between the first hour of life and the fourth day, according to the mother's consent. The placental specimen was collected in the delivery room immediately after expulsion: the outer surface of the fetal side was first disinfected with 90% alcohol so that surface flora would not be carried towards the sampled area, then a sterile swab was applied to the fluid present on the inner surface. No tissue fragment was excised and no histological examination was performed. A sterile placenta processed in the same way served as a negative handling control.

Specimens were kept in a cool box with ice packs, transported the same day to the LUIZ Laboratory in Brazzaville and processed within 24 hours. Isolates were then stored and transferred to the Molecular Biology Laboratory of IRSN.

2.3. Isolation and Identification

Specimens were plated on eosin methylene blue agar and on CHROMagar Orientation, then incubated at 37 °C for 18 to 24 hours. Identification was based on colony morphology, Gram staining and the API 20E biochemical strip.

Fifty-two 16S rRNA gene sequences, from 18 triads and chosen to represent each morphotype and each compartment, were obtained by Sanger sequencing. After editing in BioEdit 7.0.9, each sequence was compared by BLASTn with the reference sequences of the NCBI GenBank database, the retained species being that of the best alignment. For isolates that were not sequenced, identification rests on the API 20E strip and is reported only at genus level.

Alignment was performed with MAFFT v7 [18] and then trimmed of columns carrying more than 30% gaps and of divergent ends, leaving 1,410 positions. The HKY+F+R2 model, selected by ModelFinder under the Bayesian information criterion [19], was used for maximum-likelihood reconstruction in IQ-TREE 2.0.7 [20], carried out in parallel with a neighbour-joining reconstruction on Kimura two-parameter distances [21, 22]. Each method was assessed with 1,000 bootstrap replicates [23], supplemented for maximum likelihood by an SH-aLRT test; only nodes supported at 70% or above are shown. Both trees were midpoint-rooted. Sequences were also grouped into haplotypes defined by strict identity over at least 400 comparable positions, whose relationships were examined with a median-joining network [24].

2.4. Beta-Lactam Susceptibility

Antibiotic susceptibility was determined by the disc diffusion method on agar medium (Mueller-Hinton agar), from an inoculum adjusted to 0.5 McFarland; the reading of inhibition diameters and their interpretation followed the CLSI M100 breakpoints [25]. Seven molecules were tested: piperacillin (PRL, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), aztreonam (ATM, 30 µg), imipenem (IMP, 10 µg) and ertapenem (ETP, 10 µg). Extended beta-lactam resistance was defined, in this work, as resistance to at least three of the seven molecules of the panel.

2.5. Detection of Extended-Spectrum Beta-Lactamases

ESBL production was sought by the double-disc synergy method on the 111 isolates of the 37 triads whose resistance profiles were concordant. One hundred and eleven tests were performed in total, positive and negative results combined, which unambiguously defines the denominator of the rates reported below. A clavulanic acid disc was placed 15 mm from the cefotaxime and ceftazidime discs. The appearance of a champagne-cork inhibition zone between the clavulanic acid disc and the cephalosporin discs, after 24 hours at 37 °C, indicated probable ESBL production [17]. Isolates from non-concordant triads were not tested, so that the association between ESBL production and antibiotic concordance cannot be assessed.

2.6. Statistical Analysis

Qualitative variables are presented as counts and percentages, with 95% confidence intervals (CI) calculated by the Wilson method. The antibiotic type of an isolate is the set of panel molecules to which it is categorised resistant. An isolate triad, defined as the three isolates of a single species from the three compartments of one parturient, is termed concordant when these three isolates share exactly the same antibiotic type, and discordant as soon as a single molecule separates one compartment from the other two. The size of the gap is then measured by the maximum symmetric difference between two profiles of the triad.

Whether concordance was fortuitous was assessed by a permutation test: neonatal and placental profiles were reallocated 20,000 times among triads of the same species, and *p* corresponds to the proportion of permutations producing a number of concordances at least equal to that observed. The discriminating power of the antibiotic type was estimated from the identity rate observed across all pairs of maternal isolates of the same species from different triads, and the analysis was repeated after removing each of the seven molecules in turn. Within the sequenced subset, a triad was judged concordant at the 16S level when its three compartments carried the same species with at least 99.4% identity across the three pairs, and partially concordant when a single pair reached that threshold. Calculations were performed in Python 3. Paired proportions were compared with the exact McNemar test, whose significance threshold was set at 0.05, two-sided.

2.7. Ethical Considerations

The study was approved by the Health Sciences Research Ethics Committee (CERSSA), reference N083/MESRST/CERSSA/-25. Written informed consent was obtained from each participant.

3. Results

3.1. Study Population

Ninety-one triads were recruited, corresponding to 273 specimens. Enterobacteriaceae were carried by all mothers (91/91; 100%; 95% CI: 96-100), by 66 of 91 newborns (73%; 95% CI: 63-81) and by 57 of 91 placentas (63%; 95% CI: 52-72). In total, 478 isolates were obtained.

Thirty-three triads (36%; 95% CI: 27-47) were positive in all three compartments and formed the analysis population. They yielded 267 identified isolates, of which 241 (90.3%) belonged to the three dominant genera and 26 (9.7%) to other genera (*Serratia*, *Proteus*, *Citrobacter*, *Salmonella*). Susceptibility testing was performed on the 241 isolates of the three dominant genera (Figure 1).

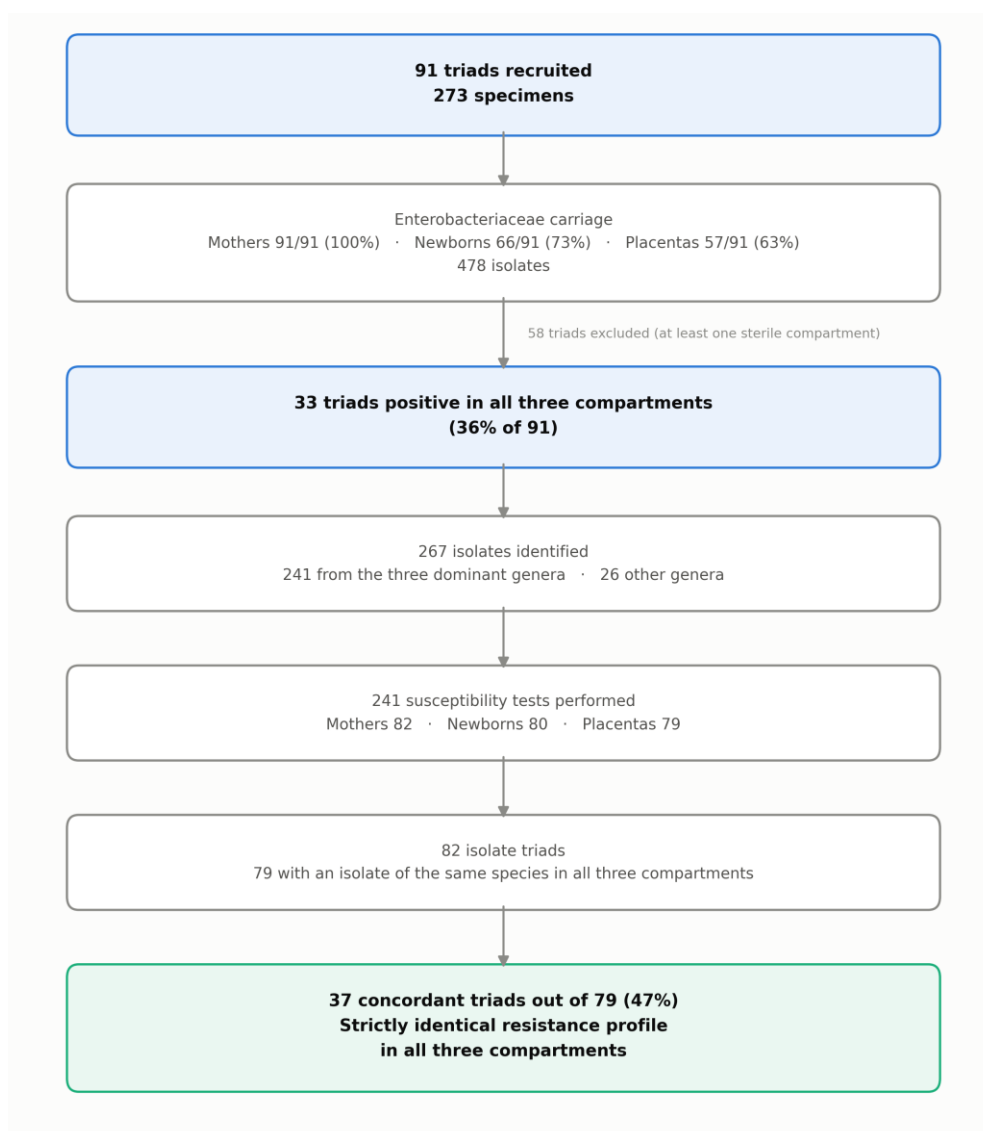


Figure 1. Flow of the study, from recruitment of the 91 triads to identification of the 37 concordant triads.

3.2. Distribution of Genera and Species

Three genera dominated the 241 isolates: *Escherichia* (97; 36.3% of all identified isolates), *Enterobacter* (85; 31.8%) and

Klebsiella (59; 22.1%). Species distribution is given in [Table 1](#). The genera were evenly distributed across the three compartments, with 82 maternal, 80 neonatal and 79 placental isolates, *Escherichia* remaining the most frequent genus in each.

Table 1. Distribution of the identified Enterobacteriaceae species (n = 241).

Genus	Species	n	% of genus	% of 241
<i>Escherichia</i>	<i>E. coli</i>	72	74	29.9
	<i>Escherichia</i> spp.	25	26	10.4
	Total	97	100	40.2
<i>Enterobacter</i>	<i>E. cloacae</i>	48	56	19.9
	<i>E. hormaechei</i>	37	44	15.4
	Total	85	100	35.3

Genus	Species	n	% of genus	% of 241
<i>Klebsiella</i>	<i>K. aerogenes</i>	26	44	10.8
	<i>K. oxytoca</i>	21	36	8.7
	<i>K. pneumoniae</i>	12	20	5.0
	Total	59	100	24.5

3.3. Beta-Lactam Resistance Profiles

Twenty-two isolates (9.1%) were susceptible to the seven

molecules of the panel; the other 219 carried at least one resistance (Table 2, Figure 2). Profiles were superimposable across the three compartments, maternal rates being consistently the highest and neonatal rates the lowest, without the differences reaching the significance threshold.

Table 2. Beta-lactam resistance of the 241 isolates, overall and by compartment.

Antibiotic	All (n = 241)	Mothers (n = 82)	Newborns (n = 80)	Placentas (n = 79)
Piperacillin	135 (56.0%)	52 (63%)	41 (51%)	42 (53%)
Cefotaxime	60 (24.9%)	26 (32%)	16 (20%)	18 (23%)
Ceftazidime	78 (32.4%)	32 (39%)	20 (25%)	26 (33%)
Cefepime	74 (30.7%)	31 (38%)	19 (24%)	24 (30%)
Aztreonam	36 (14.9%)	16 (20%)	8 (10%)	12 (15%)
Imipenem	44 (18.3%)	19 (23%)	12 (15%)	13 (16%)
Ertapenem	35 (14.5%)	14 (17%)	9 (11%)	12 (15%)

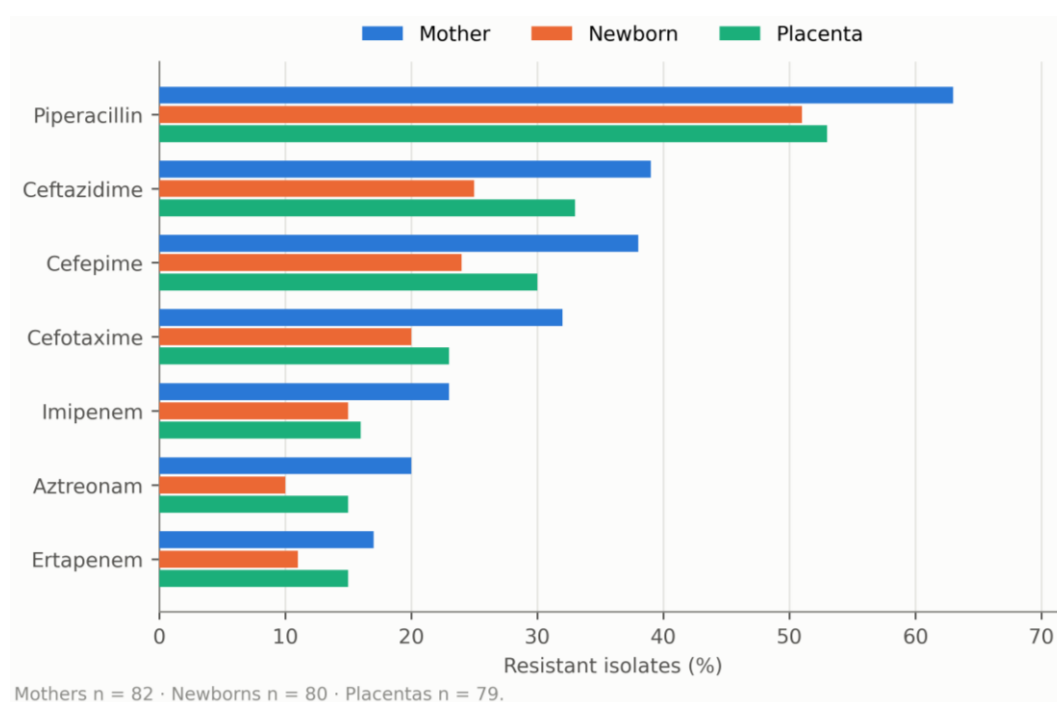


Figure 2. Beta-lactam resistance by compartment (n = 241 isolates).

Seventy-four isolates (30.7%) were resistant to at least three of the seven molecules, 117 (48.5%) to at least one third-generation cephalosporin and 68 (28.2%) to at least one carbapenem. The median number of molecules per profile was 2, with a range of 0 to 5.

Enterobacter showed the highest resistance to ceftazidime (52%), consistent with the expression of its chromosomal AmpC cephalosporinase, and *Klebsiella* the highest carbapenem resistance, with 25% for imipenem (Table 3).

Table 3. Beta-lactam resistance by genus.

Antibiotic	<i>Escherichia</i> (n = 97)	<i>Enterobacter</i> (n = 85)	<i>Klebsiella</i> (n = 59)
Piperacillin	65 (67%)	45 (53%)	25 (42%)
Cefotaxime	27 (28%)	19 (22%)	14 (24%)
Ceftazidime	26 (27%)	44 (52%)	8 (14%)
Cefepime	27 (28%)	34 (40%)	13 (22%)
Aztreonam	14 (14%)	10 (12%)	12 (20%)
Imipenem	16 (16%)	13 (15%)	15 (25%)
Ertapenem	15 (15%)	9 (11%)	11 (19%)

3.4. Concordance of Profiles Between Compartments

The 33 triads of the analysis population yielded 82 isolate triads, one per species and per parturient. Seventy-nine of them had an isolate of the same species in all three compartments and constitute the unit of the concordance analysis; the other three are incomplete, one result not having been recorded.

Thirty-seven of these 79 triads (47%; 95% CI: 36-58) showed a strictly identical resistance profile in the mother, in the placenta and in the newborn, that is the same response for each of the seven molecules in all three compartments at once. None of these concordances results from general susceptibility: all thirty-seven carry a resistance profile. Concordance was observed in the seven species without exception, with a maximum in *E. coli* (58%) and a minimum in *K. oxytoca* (29%) (Table 4).

Table 4. Concordance and discordance of resistance profiles, by species.

Species	Complete triads	Concordant	Discordant	Concordance (%)
<i>E. coli</i>	24	14	10	58
<i>Escherichia</i> spp.	8	4	4	50
<i>E. cloacae</i>	16	7	9	44
<i>E. hormaechei</i>	12	5	7	42
<i>K. pneumoniae</i>	4	2	2	50
<i>K. oxytoca</i>	7	2	5	29
<i>K. aerogenes</i>	8	3	5	38
Total	79	37	42	47

Examined by pairs of compartments, concordance reached 41/79 (52%; 95% CI: 41-63) between the mother and the

newborn, 42/79 (53%; 95% CI: 42-64) between the mother and the placenta, and 45/79 (57%; 95% CI: 46-67) between

the newborn and the placenta. These three proportions do not differ significantly from one another (exact McNemar test on paired data, $p \geq 0.39$).

Concordance can be read directly from the phenotypes. In triad 7, carrying an *E. coli*, the three compartments show the

same profile: mother 7, PRL CTX IMP ETP; newborn 7, PRL CTX IMP ETP; placenta 7, PRL CTX IMP ETP. Figure 3 gives one concordant and one discordant example for each of the seven species.

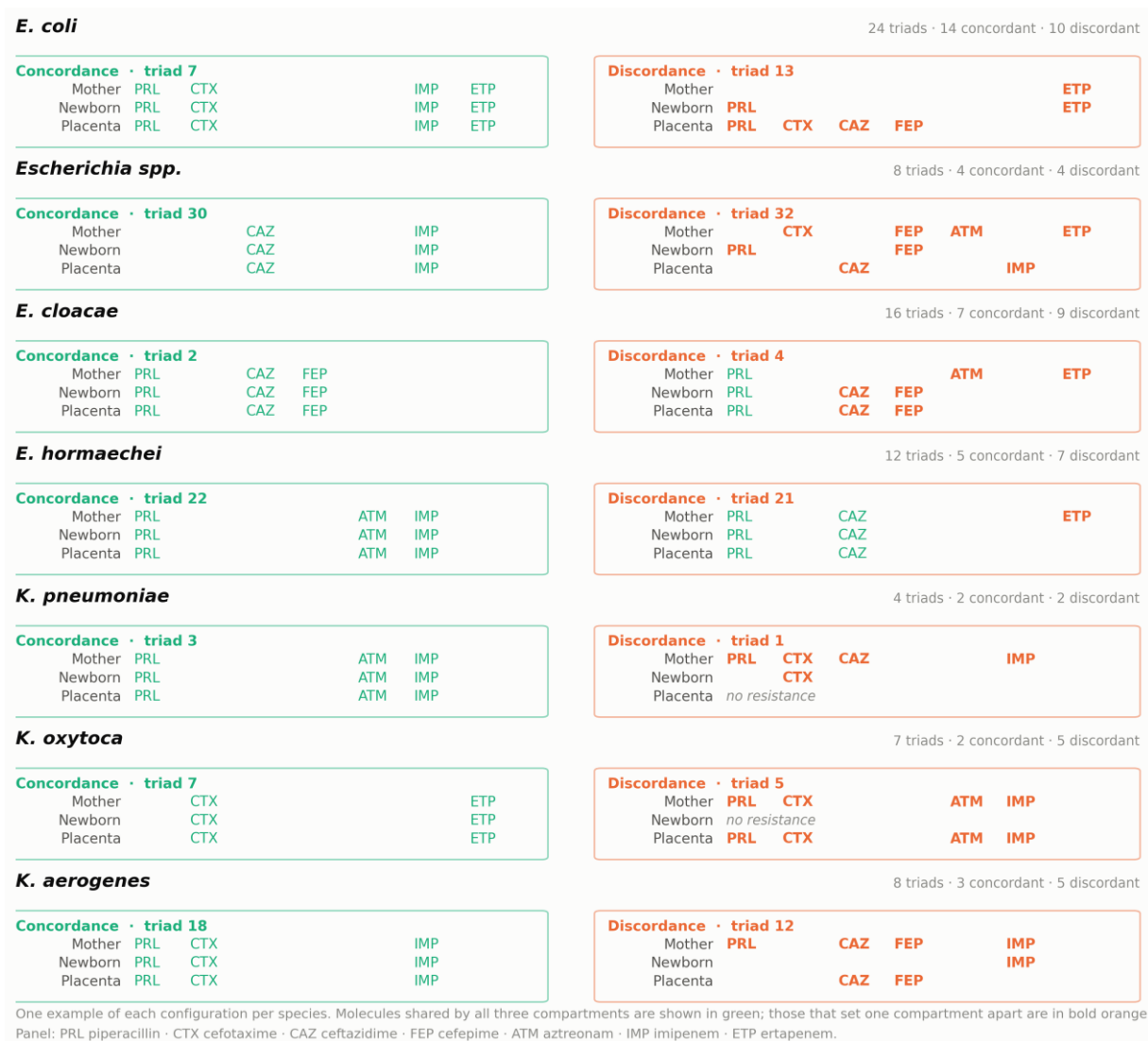


Figure 3. Concordance and discordance of resistance phenotypes, species by species. For each of the seven species, a concordant triad on the left and a discordant triad of the same species on the right, with the detail of the molecules to which each isolate is resistant. In green: the molecules shared by the three compartments. In orange: those that set one compartment apart from the others.

Among the 42 discordant triads, 25 of them (60%) show three profiles that differ from one another in each compartment. In the remaining 17, two compartments stay identical and only one departs; it is then most often the mother, in 8 of 17 triads, the newborn and the placenta keeping a common profile.

3.5. Concordance Within Triads Greatly Exceeds Chance

The object of this analysis is the circulation of a strain

within a single triad. Comparisons between different triads are not intended to describe transmission from one family to another; they serve only as a reference, by establishing how often two epidemiologically unrelated isolates share the same profile by coincidence.

The 82 maternal isolates form, within each species, 573 pairs of isolates belonging to different triads and therefore free of any epidemiological link. Only eleven of these pairs showed an identical antibiotype, that is 1.9%. The contrast is twenty-five to one: 1.9% identity between isolates of different triads, against 47% within a single triad.

Neonatal and placental profiles were then reallocated at random among triads of the same species, 20,000 times, and the number of concordant triads recounted at each draw (Table 5). Fewer than one concordance would be expected if profiles

were distributed independently; across 20,000 reallocations, the number of concordant triads never exceeded seven, whereas 37 are observed.

Table 5. Comparison of the observed concordance with the null distribution.

Analysis	Observed concordances	Expected under independence	Maximum obtained by chance	P
Species-stratified permutation, 20,000 replicates	37 / 79 (47%)	0.76	7	$< 10^{-4}$

Removing each molecule in turn leaves the number of concordant triads between 37 and 39: the result does not depend on any particular molecule of the panel.

3.6. Extended-Spectrum Beta-Lactamases

The 111 isolates of the 37 concordant triads, all carrying at

least one resistance, were submitted to the double-disc synergy test. ESBL production was confirmed in 67 of them (60.4%; 95% CI: 51.1-69.0), positivity showing as a characteristic distortion of the inhibition zones towards the clavulanic acid disc (Figure 4). Production decreases steadily from the mother to the newborn and then to the placenta, and concerns the three genera (Table 6, Figure 5).

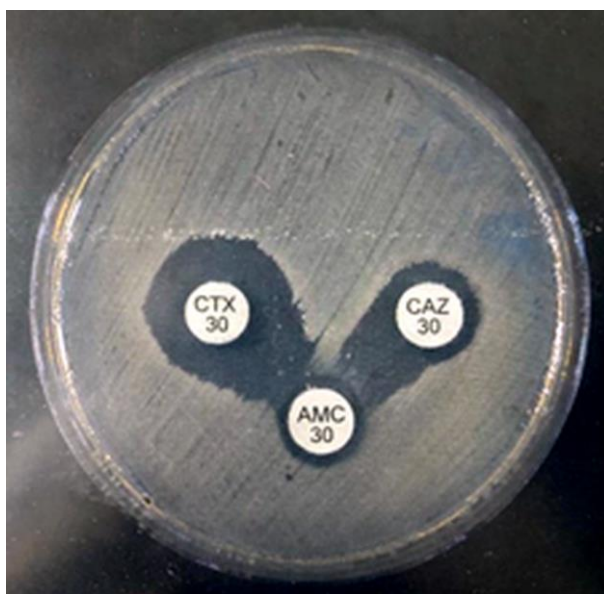


Figure 4. Positive double-disc synergy test. The inhibition zones of cefotaxime (CTX 30) and ceftazidime (CAZ 30) are distorted towards the amoxicillin-clavulanic acid disc (AMC 30), producing the champagne-cork image that indicates extended-spectrum beta-lactamase production. The photograph was only cropped and adjusted globally for white balance and contrast, applied uniformly to the whole image.

Table 6. ESBL-producing strains by compartment and by genus (37 concordant triads).

Group	ESBL positive	Isolates tested	%	95% CI
Mothers	26	37	70	54-83
Newborns	24	37	65	49-78
Placentas	17	37	46	31-62

Group	ESBL positive	Isolates tested	%	95% CI
<i>Escherichia</i>	28	54	52	-
<i>Enterobacter</i>	24	36	67	-
<i>Klebsiella</i>	15	21	71	-
All	67	111	60.4	51.1-69.0

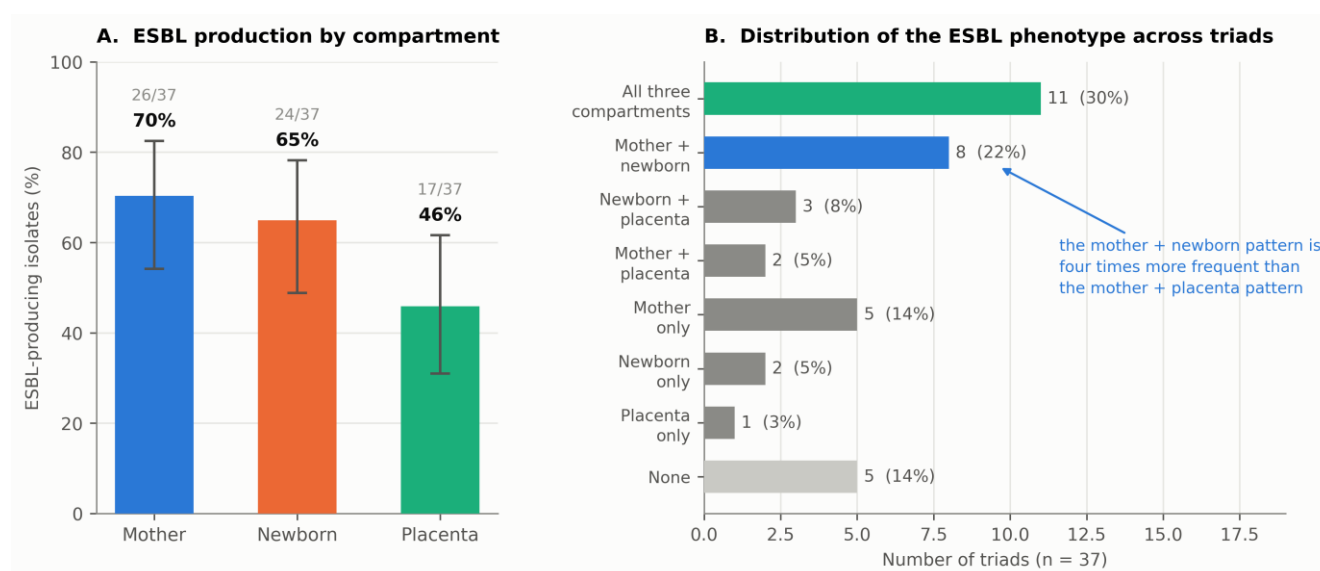


Figure 5. ESBL production by compartment (A) and distribution of the ESBL phenotype across the 37 concordant triads (B).

Of thirty-seven triads, thirty-two (86%) had at least one ESBL-producing compartment and eleven (30%) had three. The association of the ESBL phenotype between compartments went in the expected direction without reaching the significance threshold, power being limited by sample size: mother-newborn, concordance 25/37 (68%), OR = 3.26 ($p = 0.143$); placenta-newborn, 24/37 (65%), OR = 4.67 ($p = 0.082$); mother-placenta, 20/37 (54%), OR = 1.75 ($p = 0.495$). The pattern linking the mother and the newborn without the placenta was the most frequent in raw counts: eight triads, against two for the reverse pattern.

As an internal consistency check, the 67 ESBL-producing strains fall within the subset of 117 isolates (48.5%) resistant to at least one third-generation cephalosporin.

3.7. Molecular Identification and Phylogenetic Analysis

Amplification of the 16S rRNA gene produced single amplicons of about 1,500 bp for the sequenced isolates, the negative control remaining blank. A comparison of the sequences in BLAST confirmed the presumptive API 20E identifications and assigned the isolates to seven species: *Escherichia coli*, *Escherichia* sp., *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Enterobacter hormaechei*. The maximum-likelihood tree groups the isolates by species with high bootstrap supports, and the topology obtained by neighbour-joining is congruent with it (Figure 6).

16S phylogeny of Enterobacteriaceae isolates from mother-placenta-newborn triads

Maximum likelihood (IQ-TREE 2, HKY+F+R2, 1000 ultrafast bootstraps; node support $\geq 70\%$) · 52 sequences, 1410 positions · midpoint rooting
 The "Family" column is coloured by the degree of concordance of the triad; yellow boxes mark sequences whose 16S haplotype is also carried by an

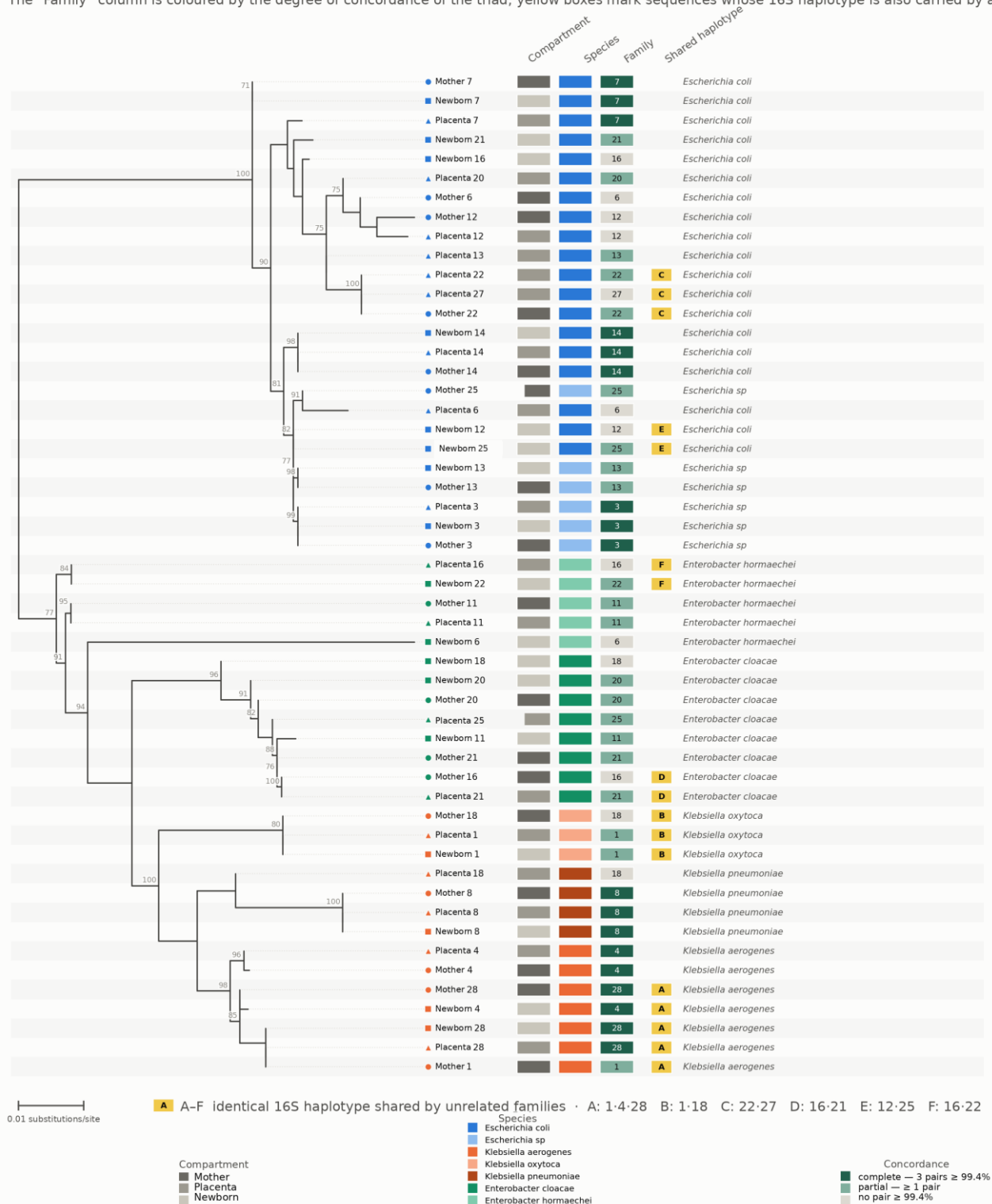


Figure 6. 16S rRNA gene phylogeny of the Enterobacteriaceae isolates from the mother, the placenta and the newborn (18 families, 52 sequences). The tree was reconstructed by maximum likelihood from a 1,410-position alignment, under the HKY+F+R2 model, with IQ-TREE 2. Node values are ultrafast bootstrap supports over 1,000 replicates; only those equal to or above 70% are shown. The tree is midpoint-rooted. Leaf symbols indicate the sampling compartment (circle, mother; triangle, placenta; square, newborn) and are coloured by genus. The colour of the "Family" column reflects the degree of concordance of the triad: dark green, the three compartments share the same species with at least 99.4% identity across the three pairs; light green, at least one pair reaches this threshold; grey, no pair reaches it. Yellow boxes mark the isolates whose 16S haplotype is strictly identical to that of an epidemiologically unrelated family. Sequenced triads are designated by a family number specific to this analysis, independent of the numbering used for the antibiotics.

Six families showed complete concordance at the 16S level, the same species being found in the three compartments with at least 99.4% identity across the three pairs: families 3, 4, 7, 8, 14 and 28. The others reached this threshold on one pair only, or on none. Grouping into haplotypes revealed six haplotypes shared by families with no link between them: A in families 1, 4 and 28; B in 1 and 18; C in 22 and 27; D in 16 and 21; E in 12 and 25; F in 16 and 22. Those of families 3, 7, 13 and 14 appeared in no other.

4. Discussion

4.1. Concordance as an Argument for Transmission

Of 79 complete triads, 37 (47%) show a strictly identical resistance profile in the three compartments, whereas fewer than one concordance would be expected if profiles were distributed independently. The internal control strengthens this reading: identity between isolates of different triads is 1.9%. All deliveries in this study took place vaginally. The most probable route is therefore intrapartum, through contact with maternal genital and intestinal flora during passage through the birth canal, a modality that falls fully within the definition of vertical transmission. Our results are consistent with the meta-analysis of Bulabula et al. [8], with the cross-sectional study of Matok et al. [26], which documented the passage of resistant bacteria from mother to newborn, and with the genomic analysis of Okomo et al. [15] in the Gambia, which established the vertical transmission of early-onset neonatal sepsis strains.

The phylogenetic analysis provides both support and a nuance. Six families carry the same species in the three compartments, which points to a shared strain. But the *Klebsiella aerogenes* haplotype common to the three compartments of family 28 is found in two families unrelated to it: the 16S rRNA gene does not discriminate strains at this level of resolution, and sequence identity within a triad remains compatible with vertical transmission without demonstrating it. The exceptions are the four families whose haplotype appears nowhere else, the strongest candidates for confirmation by MLST or by whole-genome sequencing.

4.2. Strengths and Limitations

The first strength of this work is to have sampled the three compartments in the same parturient, which measures the circulation of a strain within a triad and not between families. The second is to have quantified the chance component: the identity frequency between epidemiologically unrelated isolates, measured at 1.9%, provides a background level against which to compare the observed concordance, and the permutation test places the latter far beyond what an independent distribution of profiles would produce.

In order to rule out the hypothesis of contamination by vaginal flora during delivery, three measures were taken. The specimen was collected in the delivery room, just after expulsion of the placenta, to limit the risk of outside contamination. Next, the outer surface of the fetal side was disinfected with 90% alcohol before the inside was sampled with a swab. Finally, a sterile placenta handled under the same conditions was used as a negative control. Above all, 37% of placentas remained sterile in culture: if swabbing merely collected genital flora in passing, positivity close to that of the mothers, which is 100%, would be expected.

Four limitations must nonetheless be stated. The first concerns the nature of the marker: the antibiotype is not a clonal marker. The permutation test establishes that the observed concordance is not due to chance, but not that the isolates of a triad belong to the same strain, since common exposure to a shared environmental source would produce a comparable signal. Only molecular typing would confirm the clonal identity of the strains. This analysis is currently under way on the strains that were stored. The second bias concerns the mode of delivery: as all patients delivered vaginally, it is impossible to distinguish a contamination occurring during labour from an antenatal contamination. The third bias is linked to the placental specimen itself, which remains superficial. Although prior disinfection and the use of a negative control exclude contamination at the time of sampling, swabbing the inner surface does not allow a true colonisation of placental tissue to be distinguished from a simple bacterial deposit acquired during passage through the birth canal. Finally, the fourth bias concerns the scope of the search for ESBLs, limited to the concordant triads alone. As a result, the rate of 60.4% cannot be extrapolated to all isolates, nor interpreted as a carriage prevalence.

A further point must be underlined: the interval before neonatal sampling does not allow early postnatal acquisition to be entirely ruled out, whether from the hospital environment or from healthcare staff, whose involvement is already reported in the literature [5]. Three further limitations must be mentioned. First, isolates cannot be linked to a specific patient, since each triad is identified only by a serial number internal to the genus. Second, the 16S rRNA gene does not allow a strain to be identified with precision, as our own haplotype analysis confirms. Third, the antibiotic panel tested is restricted to seven beta-lactams. Adding aminoglycosides, fluoroquinolones and sulphonamides would strengthen the reach of the results.

4.3. Ecology, Resistance and the Placental Compartment

Concordance does not differ from one pair of compartments to another, at 52%, 53% and 57%, with widely overlapping confidence intervals and non-significant paired comparisons. No pair stands out, and the three compartments take part in the

same circulation without an order being established between them on the basis of antibiotype alone. The question of a resident placental microbiome lies beyond this work and remains debated: after Aagaard et al. [11], de Goffau et al. [12] showed that most bacterial signals detected in the placenta arise from reagent contamination or from intrapartum acquisition. Our sampling, superficial by nature, does not allow the matter to be settled, and the placental compartment should be read as a witness to the microbiological environment of this interface at birth.

Escherichia, *Enterobacter* and *Klebsiella* represent 90.3% of the identified isolates, a distribution comparable to that reported by Moyon et al. [16] in Brazzaville. Resistance rates are high: 48.5% of isolates resist at least one third-generation cephalosporin, in a community carriage population and not in hospitalised patients. This level fits the trend described by Woerther et al. [2] and matches regional observations [13, 17]. It bears directly on neonatal prophylaxis protocols based on ampicillin and gentamicin.

ESBL production concerned 60.4% of the isolates from concordant triads. This figure cannot be read as a carriage prevalence, since the strains were selected on their concordance; it does indicate that antibiotype concordance is very often accompanied by this mechanism, which is consistent with the community diffusion of CTX-M-type enzymes [2] and with regional observations in Nigeria [7] and Gabon [27]. Beaumont et al. [28] recently quantified, in two community cohorts in Madagascar and Cambodia, the contribution of maternal intestinal carriage to the neonatal acquisition of ESBL-producing Enterobacteriaceae; our data point the same way and add the placental compartment to it.

Carbapenem resistance deserves particular attention: 18.3% of isolates for imipenem, 14.5% for ertapenem, and up to 25% in *Klebsiella*. This level remains poorly documented in maternal-neonatal carriage, but it is not isolated in the subregion: Dos Santos et al. [27] report carbapenemase-producing Enterobacteriaceae in mother-newborn pairs in neighbouring Gabon, and Djuikoue et al. [29] describe mother-to-newborn transmission in two maternity units in Yaoundé. Our results point the same way and suggest that the spread of these strains outside the hospital setting is already under way in Central Africa. What is reported here is a phenotype, and the phenotype alone does not designate the mechanism: a carbapenemase and impermeability through porin loss combined with a cephalosporinase produce the same picture on agar. Molecular characterisation of the *bla*OXA-48, *bla*NDM and *bla*KPC genes forms the second part of this work and will settle the question.

5. Conclusions

In the mother-placenta-newborn triads of this Brazzaville maternity unit, Enterobacteriaceae are dominated by *Escherichia*, *Enterobacter* and *Klebsiella* and show high levels of beta-lactam resistance: a large share of isolates resist third-generation cephalosporins, and most strains from concordant

triads produce an extended-spectrum beta-lactamase. Nearly half of the complete triads carry a strictly identical resistance profile in the three compartments, whereas such concordance would be exceptional if profiles were distributed at random, and two epidemiologically unrelated isolates almost never share the same profile. This gap is the central finding of the study and supports peripartum mother-to-child transmission of resistant Enterobacteriaceae. Because the antibiotype is not a clonal marker, molecular typing is necessary before clonal identity can be asserted, and that work is under way. In the meantime, these results argue for systematic screening of maternal carriage of resistant Enterobacteriaceae at delivery and for a review of neonatal prophylaxis protocols in Brazzaville.

Abbreviations

AmpC	AmpC-Type Chromosomal Cephalosporinase
ATM	Aztreonam
BLAST	Basic Local Alignment Search Tool
CAZ	Ceftazidime
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CTX	Cefotaxime
ESBL	Extended-Spectrum Beta-lactamase
ETP	Ertapenem
FEP	Cefepime
IMP	Imipenem
MLST	Multilocus Sequence Typing
OR	Odds Ratio
PRL	Piperacillin
SH-	Shimodaira-Hasegawa Approximate
aLRT	Likelihood Ratio Test

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Conflicts of Interest

The authors declare no conflicts of interest.

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