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## Prevalence and factors associated with faecal carriage of extended-spectrum $\beta$ -lactamase-producing Enterobacterales among peripartum women in the community in Cambodia

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**Background:** In Southeast-Asia, where many conditions associated with dissemination of ESBL-producing Enterobacterales (ESBL-E) in the community are met, data from the community are scarce but show high ESBL-E carriage prevalence. Maternal ESBL-E colonization is considered a risk factor for neonatal colonization, which is the first step towards developing neonatal sepsis. Despite this, ESBL-E carriage prevalence and its risk factors during pregnancy or postpartum remain undefined in Southeast-Asia.

**Objectives:** To estimate the prevalence of ESBL-E faecal colonization among peripartum women in the community of an urban and a rural area in Cambodia, to investigate ESBL-E genomic characteristics and to identify associated risk factors.

**Methods:** Epidemiological data and faecal samples from 423 peripartum women were collected in an urban and rural areas in Cambodia (2015–16). Bacterial cultures, antibiotic susceptibility tests and ESBL gene sequencing were performed. Risk factor analysis was conducted using logistic regression.

**Results:** The prevalence of ESBL-E faecal carriage was 79.2% (95% CI 75.0%–82.8%) among which *Escherichia coli* ( $n=315/335$ , 94.0%) were most frequent. All isolates were multidrug resistant. Among 318 ESBL-E, the genes most frequently detected were *bla*<sub>CTX-M-15</sub> (41.5%), *bla*<sub>CTX-M-55</sub> (24.8%), and *bla*<sub>CTX-M-27</sub> (15.1%). Low income, undernutrition, multiparity, regular consumption of pork, dried meat, and raw vegetables, were associated with ESBL-E faecal carriage.

**Conclusions:** The high prevalence of ESBL-E carriage observed among peripartum women in Southeast-Asia and the identified associated factors underline the urgent need for public health measures to address antimicrobial resistance, including a ‘One Health’ approach.

## Introduction

The emergence of antimicrobial resistance (AMR) is a global health threat, with potentially 10 million deaths annually in 2050.<sup>1</sup> One of the main mechanisms of AMR is the production of an extended-spectrum  $\beta$ -lactamase (ESBL) enzyme by Enterobacterales (ESBL-*E*) conferring resistance to commonly used  $\beta$ -lactam antibiotics, such as cephalosporins (third- and fourth-generation) and also co-resistance to antibiotics from other drug classes.

In low and middle-income countries (LMICs), many conditions associated with dissemination of ESBL-*E* in the community are met, including suboptimal hygiene conditions, poorly controlled antibiotic consumption, and insufficient infection prevention control in healthcare settings. Data from the community are scarce and show high ESBL-*E* carriage prevalence, particularly in Southeast-Asia (ranging from 46% to 66%).<sup>2</sup> To date, very little data from the Cambodian community is available.

Maternal ESBL-*E* colonization is thought to be a risk factor for neonatal colonization, although pathways of mother-to-child transmission of resistant pathogens are incompletely understood.<sup>3,4</sup> Newborns who are colonized early with Enterobacterales are predisposed to neonatal septicemias where multidrug resistance, in particular mediated by ESBLs, is associated with an excess risk of mortality.<sup>5</sup> Pregnant women are also at higher risk of developing urinary tract infections, mostly due to Enterobacterales, and consequently suffering from bacteraemia with possibly poor obstetric outcomes.<sup>6</sup> Despite this, few studies have focused at the community level on ESBL-*E* carriage prevalence and its risk factors during pregnancy or postpartum. To our knowledge, none has been conducted in Southeast-Asian LMICs.

In Cambodia, we aimed to estimate at the community level the prevalence of ESBL-*E* faecal carriage among women at time of delivery, in order to identify factors associated with carriage and to document antimicrobial resistance patterns of ESBL-*E* isolates and their ESBL-encoding genes.

## Materials and methods

### Ethics

The study was approved by the relevant National Ethics Committee for Health Research in Cambodia (108) and the institutional review board of Institut Pasteur in France (IRB00006966). The BIRDY data collection has been declared to the Commission Nationale de l'Informatique et des Libertés (CNIL – French national data protection authority), in accordance with French law. All women (or guardians if they were minors) gave their informed consent prior to inclusion.

### Study design

The Bacterial Infections and antibiotic-Resistant Diseases among Young children in low-income countries (BIRDY) programme is an international and multicentric cohort aiming to assess the burden of bacterial infections and antimicrobial resistance in newborns and children <2 years old in three LMICs: Cambodia, Madagascar and Senegal.

The study design of the BIRDY programme has been detailed elsewhere.<sup>7</sup> Briefly, pregnant women were recruited in primary health centres during their third trimester of pregnancy and actively monitored to ensure the enrolment of neonates at birth. In Cambodia, for this cross-sectional study nested in the BIRDY programme, women from

Kampong Speu, a rural province, and Steung Meanchey, an urban district in Phnom Penh, were enrolled between April 2015 and December 2016.

All women were asked to provide a faecal swab within 7 days after delivery. Samples were stored at +4°C and transferred within 6 h to the Institut Pasteur (IP) in Cambodia microbiology laboratory.

At the time of faecal swab collection, data on maternal characteristics, antibiotic consumption and hospitalizations during pregnancy, delivery and birth conditions were collected. In addition, extensive epidemiological data (including dietary habits, animal contacts, and characteristics of the household environment) were collected for a subset of women who participated in ancillary studies during the same period.

### Microbiological analysis

Faecal swabs were spread on Drigalski plates supplemented with cefotaxime 2 mg/L (Bio-Rad, Marnes-La-Coquette, France) before overnight incubation in air at 37°C. ESBL production was screened by double-disc synergy test using the combination of cefotaxime, ceftriaxone, ceftazidime, aztreonam and amoxicillin/clavulanic acid, and was considered positive for any distortion or clear-cut increase around any antibiotic disc towards the disc of amoxicillin/clavulanic acid. The species of one ESBL-producing isolate per woman was identified by API 20E system (bioMérieux, Marcy l'Etoile, France). Isolates were stored at –80°C in a phosphate buffered saline medium supplemented with sucrose, glycerol and peptone (Cryobank™, Copan, Murrieta, USA).

Owing to substudy constraints, the isolates were divided in two subsets: a first subset ( $n=235$ ), on which AST and PCR were performed at IP Cambodia; and a second subset ( $n=83$ ) on which AST and WGS were performed at IP Paris.

After duplication, antimicrobial susceptibility testing was carried out on Mueller–Hinton agar (Bio-Rad) using the Kirby-Bauer disc diffusion method with interpretation according to EUCAST (2018; <http://www.eucast.org>) with a panel of 19 antibiotics (Bio-Rad). For isolates tested at IP Paris, the susceptibility to colistin was determined using the broth microdilution method, adapted from ISO protocol 20776-1. MDR was defined as non-susceptibility to one or more agent in three or more antimicrobial categories.<sup>8</sup>

### Molecular characterization

For the 235 isolates analysed at IP Cambodia, conventional PCR was used to detect *bla*<sub>CTX-M</sub> group 1 genes. If negative, PCR for *bla*<sub>CTX-M</sub> group 9 genes was performed. If still negative, PCRs for *bla*<sub>CTX-M</sub> group 2, *bla*<sub>CTX-M</sub> group 8, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> genes were performed simultaneously. Amplified gene products were sequenced by Macrogen, Korea.

For the 83 isolates analysed at IP Paris, WGS was performed on Illumina NextSeq-500 instruments using a 2 × 150 bp paired-end protocol, and assembled into draft genomes using SPAdes. Tools from the Center for Genomic Epidemiology were used to analyse the genomes. (<http://www.genomepidemiology.org>).

For all isolates, ResFinder v3.1.0 was used to screen genomes for  $\beta$ -lactamase or other acquired antimicrobial resistance genes where possible. Sequence data have been deposited in the European Nucleotide Archive under project number PRJEB25898 (Table S1, available as [Supplementary data](#) at JAC Online).

### Statistical analysis

We used Chi-square or Fisher's exact tests, and Student or Wilcoxon rank sum tests to compare characteristics between women carrying or not carrying ESBL-*E*.

To identify risk factors associated with maternal ESBL colonization, we considered a woman's age, the socio-economic conditions of their family, household environment, animal contacts, dietary habits, previous medical history, pregnancy history including antibiotic consumption,

hospitalizations, delivery and birth conditions. Drinking water sources were categorized using WHO-UNICEF definitions. Undernutrition was defined as a mid-upper-arm circumference <23 cm. Dietary habits regarding consumption of certain foodstuffs were categorized as follows: at least once per week, twice per month, once per month or less, or never. For data analysis, the categories have been adapted to the distribution.

We conducted univariate logistic regression to examine associations between all these variables and ESBL maternal carriage. All variables associated with maternal carriage in univariate analyses with  $P$  values <0.20 were then included in a multivariable logistic model. For variables that were highly correlated (parity, maternal age), the variable with the smallest  $P$  value in univariate analysis was included in the initial multivariable model to avoid collinearity. The final model was obtained using manual stepwise backward selection. A  $P$  value <0.05 was considered as statistically significant. We performed analyses using Stata software, Version 15.1 (StataCorp, College Station, TX, USA).

## Results

### Characteristics of the women

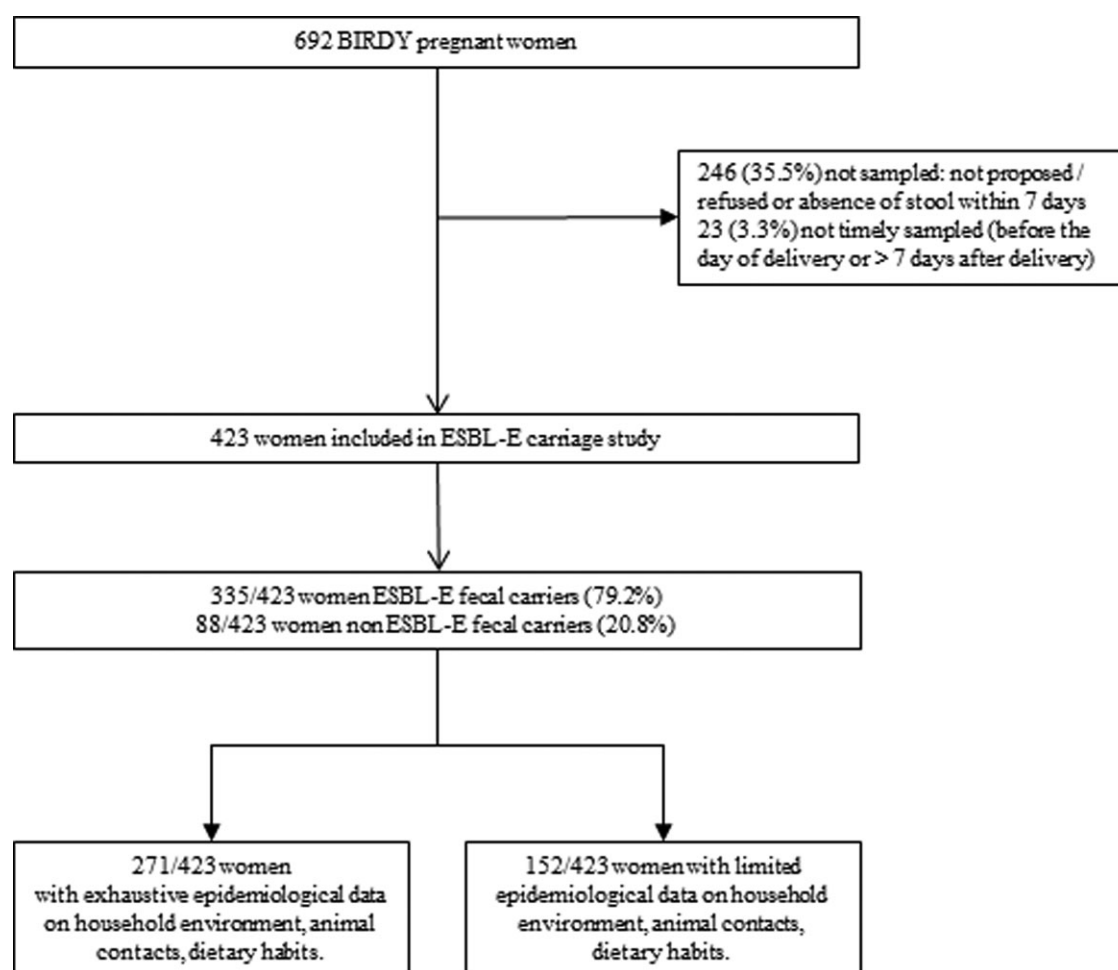
Overall, 692 women were eligible in the present study on ESBL carriage. Of which, 423 (61.1%) provided a stool sample on

time and were thus included in this analysis (Figure 1). Among the 269 women not included, 23 were not sampled in a timely manner and 246 were not sampled. The main characteristics of included ( $n=423$ ) and non-included women ( $n=269$ ) did not differ significantly regarding their age ( $P=0.18$ ), level of education ( $P=0.25$ ), undernutrition ( $P=0.21$ ), or parity ( $P=0.73$ ).

Among the 423 women who provided stool samples, the mean age was 27.8 years (range 14.5–44.7) with a majority from the rural area ( $n=232$ , 54.8%) and education frequently limited to primary school ( $n=239$ , 56.5%). One-third of women ( $n=143$ , 33.8%) were unemployed. Ninety pregnant women (21.4%) were suffering of undernutrition. A majority delivered in a public hospital ( $n=243$ , 57.5%), and 9.0% ( $n=38$ ) underwent a C-section (Table 1).

### Prevalence of ESBL-E faecal carriage, species and associated antibiotic resistance profiles

The overall prevalence of ESBL-E faecal carriage was 79.2% (95% CI 75.0%–82.8%) ( $n=335/423$ ), with no difference between rural and urban areas [80.2% (95% CI 74.5%–83.4%) versus 84.8% (95% CI 71.5%–83.4%)],  $P=0.59$ . Among the 335 isolates, species



**Figure 1.** Flow chart for study of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriales carriage in peripartum women in Cambodia 2015–16.

**Table 1.** Characteristics of peripartum women, 2015–16, Cambodia

Characteristics	All women	Urban women	Rural women	P value
Participants	423 <sup>a</sup>	191	232	
Environment				
Professions of the couple in family, N=419				<0.001
Mother unemployed and chief of family, manual worker or unemployed	134 (32.0)	86 (45.7)	48 (20.8)	
Both have manual jobs	231 (55.1)	90 (47.9)	141 (61.0)	
At least one is manager or works in an office	54 (12.9)	12 (6.4)	42 (18.2)	
Number of people per sleeping room, median (IQR)	3 (2–4)	3 (2.2–4)	3 (2–4)	<0.01
Toilet inside the house	211 (49.9)	142 (74.3)	69 (29.7)	<0.001
Women at time of delivery				
Age, years, mean (SD)	27.9 (5.3)	27.9 (5.4)	27.9 (5.2)	0.90
Education: at least secondary school	184 (43.5)	93 (48.7)	91 (39.2)	0.05
Multiparity	255 (60.3)	123 (64.4)	132 (56.9)	0.12
Undernutrition <sup>b</sup> , N=420	90 (21.4)	39 (20.6)	51 (22.1)	0.72
Hospitalization during pregnancy, N=422	9 (2.1)	5 (2.6)	4 (1.7)	0.74
Antibiotic intake during pregnancy	7 (1.7)	5 (2.6)	2 (0.9)	0.25
Delivery and birth				
ESBL-E faecal carriage	335 (79.2)	149 (78.0)	186 (80.2)	0.59
Place of delivery				<0.001
Health centre	72 (17.0)	72 (37.7)	0 (0.0)	
Hospital	243 (57.4)	69 (36.1)	174 (75.0)	
Home with traditional birth attendant	4 (0.9)	1 (0.5)	3 (1.3)	
Private clinic	104 (24.6)	49 (25.7)	55 (23.7)	
Skilled birth attendant	421 (99.5)	191 (100.0)	230 (99.1)	0.50
C-section	38 (9.0)	24 (12.6)	14 (6.0)	0.02
Antibiotic peripartum, N=406	87 (21.4)	31 (17.0)	56 (25.0)	0.05
Low birth weight (<2500 g)	17 (4.0)	9 (4.7)	8 (3.4)	0.51
Exhaustive epidemiological data				
Participants	271 <sup>c</sup>	114	157	
Type of water source, N=266				0.09
River, well, rainwater	18 (6.8)	4 (3.7)	14 (8.9)	
Improved water source	248 (93.2)	105 (96.3)	143 (91.1)	
Pork consumption weekly versus <1 per week	223 (82.3)	91 (79.8)	132 (84.1)	0.37
Dried-meat consumption at least twice per month	52 (19.2)	23 (20.2)	29 (18.5)	0.73
Raw-vegetable consumption	242 (89.3)	100 (87.7)	142 (90.4)	0.47
Contact with domestic animals (dog, cat, bird, rodent), N=270	33 (12.2)	20 (17.7)	13 (8.3)	0.02
Use of human or animal stool as fertilizer, N=270	33 (12.2)	2 (1.8)	31 (19.9)	<0.001

<sup>a</sup>Unless stated otherwise, the results presented are for 423 women.

<sup>b</sup>Undernutrition: medium upper arm circumference <23 cm.

<sup>c</sup>Unless stated otherwise, the results presented are for 271 women.

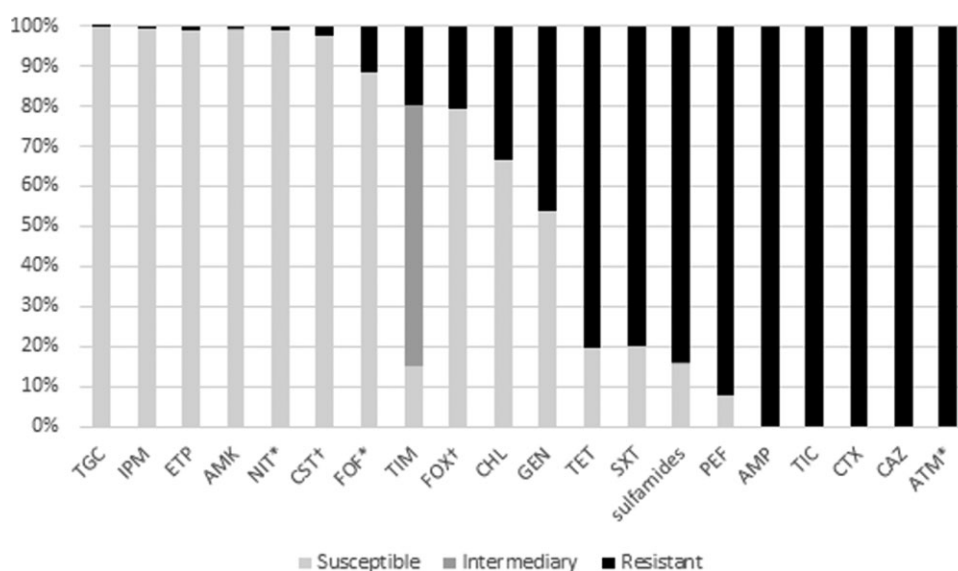
identified were *Escherichia coli*,  $n=315$  (94.0%), *Klebsiella pneumoniae*,  $n=19$  (5.7%) and *Citrobacter amalonaticus*,  $n=1$  (<0.1%).

Antimicrobial susceptibility testing was performed on 318/335 ESBL-E isolates that could be re-cultured after storage. All the 318 isolates were categorized as MDR with 234 (74%) resistant to  $\geq 7$  of 15 categories and 148 isolates (46.5%) resistant to  $\geq 8$  categories of antibiotics (Figure 2). Antibiotic susceptibility tests identified co-resistances in addition to ESBL production with 92.1% of isolates being resistant to pefloxacin ( $n=293/318$ ), 46.2% ( $n=147/318$ ) to gentamicin, 33.4% ( $n=103/318$ ) to chloramphenicol and 1.3% ( $n=4/318$ ) to ertapenem (Figure 2). Four isolates were resistant to ertapenem, with all four also resistant to pefloxacin, tetracycline, sulfonamide and trimethoprim/

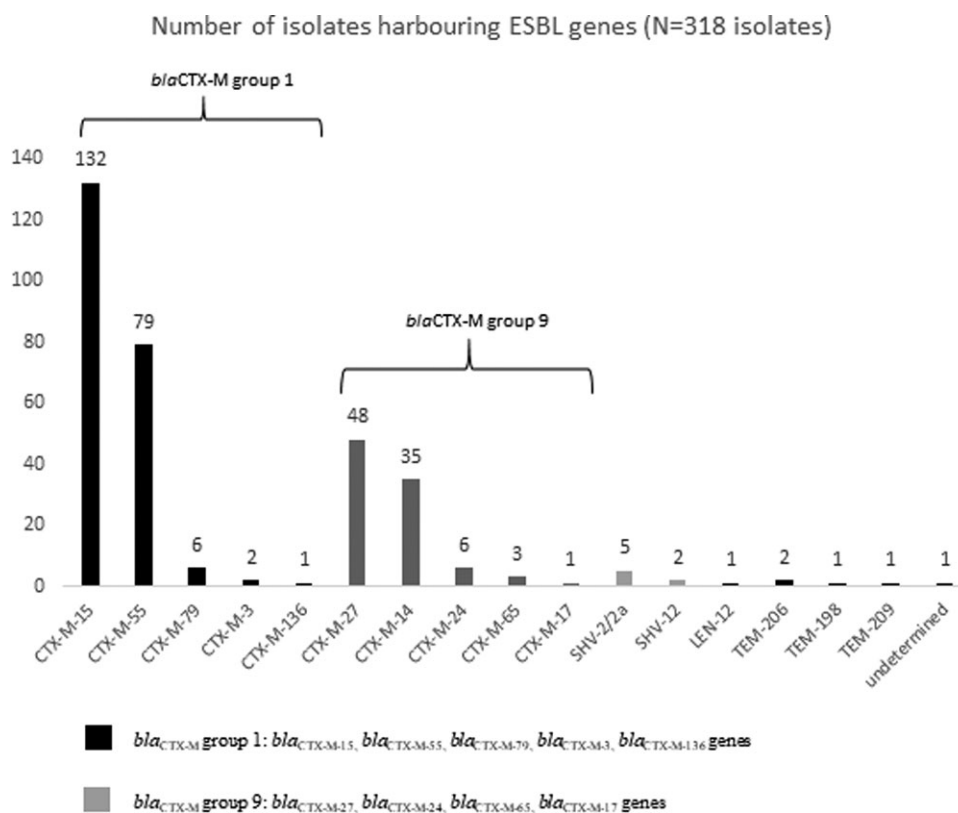
sulfamethoxazole, three were resistant to gentamicin, and one to chloramphenicol, whereas three isolates tested for colistin were susceptible (one isolate was not tested).

### Molecular characterization of resistance genes

Among the 318 ESBL isolates, 313 (98.4%) were positive for CTX-M-type genes, with  $bla_{CTX-M}$  group 1 genes being most frequent ( $n=220/318$ ; 69.2%), followed by group 9 ( $n=93/318$ ; 29.2%).  $bla_{CTX-M-15}$  was the most frequent gene detected ( $n=132/318$ ; 41.5%), followed by  $bla_{CTX-M-55}$  ( $n=79/318$ ; 24.8%),  $bla_{CTX-M-27}$  ( $n=48/318$ ; 15.1%) and  $bla_{CTX-M-14}$  ( $n=35/318$ ; 11.0%). Seven isolates expressed a  $bla_{SHV}$  gene, of which five *K.*



**Figure 2.** Phenotypic profile of 318 ESBL-producing isolates carried by peripartum women in Cambodia 2015–16. TGC, tigecycline; IPM, imipenem; ETP, ertapenem; AMK, amikacin; NIT, nitrofurantoin; CST, colistin; FOF, fosfomycin; TIM, ticarcillin/clavulanate; FOX, ceftoxitin; CHL, chloramphenicol; GEN, gentamicin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; PEF, pefloxacin; AMP, ampicillin; TIC, ticarcillin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam. \*ATM, NIT and FOF were tested only on 235 isolates at the Institut Pasteur de Cambodge. †FOX and CST were tested only on 83 isolates at the Institut Pasteur, Paris, France.



**Figure 3.** Molecular characterization of ESBL genes among 318 isolates carried by peripartum women in Cambodia 2015–16. The total number of genes are >318 as among the 83 isolates characterized by WGS, 8 isolates harboured two different ESBL genes, which are represented here (total number of genes is 326).

**Table 2.** Factors associated with ESBL-E faecal carriage in women at delivery in Cambodia

	Women (N=271) <sup>a</sup>		Univariate analysis		Multivariate analysis	
	Non-ESBL-E carrier (n=63)	ESBL-E carrier (n=208)	OR [95% CI]	P value	aOR [95% CI]	P value
Environment						
Site				0.66		
Rural	28 (44.4)	86 (41.3)	Ref			
Urban	35 (55.6)	122 (58.7)	1.14 [0.64–2.00]			
Professions of the family, N=267				0.06		0.02
Both have manual jobs	43 (68.3)	105 (51.5)	Ref		Ref	
Mother unemployed, and head of family manual worker or unemployed	13 (20.6)	69 (33.8)	2.17 [1.09–4.34]		2.57 [1.23–5.36]	
At least one is manager or works in an office	7 (11.1)	30 (14.7)	1.76 [0.72–4.30]		2.16 [0.83–5.58]	
Type of house, N=270				0.22		
Individual house or apartment	41 (66.1)	145 (69.7)	Ref			
House within a block shared by several households	11 (17.7)	45 (21.6)	1.16 [0.55–2.44]			
Rooms within a house or apartment shared with other families	10 (16.1)	18 (8.7)	0.51 [0.22–1.19]			
Mother use of soap after passing stool				0.1		
Yes, most often when washing hands	42 (66.7)	115 (55.3)	Ref			
Yes sometimes when washing hands	20 (31.7)	76 (36.5)	1.39 [0.76–2.54]			
No use of soap (even if washing hands)	1 (1.6)	17 (8.2)	6.21 [0.80–48.11]			
Women at time of delivery						
Age				0.48		
≤24 years old	17 (27.0)	50 (24.0)	Ref			
>24 to ≤31 years old	24 (38.1)	97 (46.6)	1.37 [0.68–2.79]			
>31 years old	22 (34.9)	61 (29.3)	0.94 [0.45–1.97]			
Education at least secondary school	31 (49.2)	82 (39.4)	0.67 [0.38–1.18]	0.17		
Multiparity	33 (52.4)	135 (64.9)	1.68 [0.95–2.97]	0.07	2.35 [1.25–4.42]	0.01
Undernutrition	9 (14.3)	53 (25.5)	2.07 [0.96–4.47]	0.06	2.66 [1.15–6.18]	0.02
Chronic diarrhoea	1 (1.6)	13 (6.3)	4.13 [0.53–32.23]	0.2		
Poultry consumption at least twice per month	35 (55.6)	143 (68.8)	1.76 [0.99–3.13]	0.05		
Pork consumption weekly versus <1 per week	47 (74.6)	176 (84.6)	1.87 [0.95–3.70]	0.07	2.17 [1.04–4.49]	0.04
Raw-meat consumption	8 (12.7)	43 (20.7)	1.80 [0.80–4.07]	0.15		
Dried-meat consumption at least twice per month	8 (12.7)	44 (21.2)	1.84 [0.82–4.16]	0.14	2.50 [1.05–5.99]	0.04
No dried-fish consumption	5 (7.9)	5 (2.4)	0.29 [0.08–1.02]	0.06		
Raw-vegetable consumption	52 (82.5)	190 (91.3)	2.23 [0.99–5.02]	0.05	2.55 [1.08–6.06]	0.03
Use of human stool as fertilizer, N=270	6 (9.7)	11 (5.3)	0.53 [0.19–1.50]	0.23		
Delivery and birth						
Penicillin peripartum, N=261	3 (4.8)	22 (11.1)	2.44 [0.71–8.46]	0.15		

<sup>a</sup>Unless stated otherwise, the results presented are for 271 women.

*pneumoniae* isolates expressed *bla*<sub>SHV-2/2a</sub> and two *E. coli* isolates expressed *bla*<sub>SHV-12</sub> (Figure 3). One *E. coli* expressed *bla*<sub>LEN-12</sub>, a rare gene, with 99% identity.

Of the 83 isolates analysed by WGS, eight *E. coli* (9.6%) harboured two different ESBL genes: three harbouring a double *bla*<sub>CTX-M</sub>, four harbouring a *bla*<sub>CTX-M</sub> plus a *bla*<sub>TEM</sub>, and one harbouring *bla*<sub>CTX-M-27</sub> plus *bla*<sub>SHV-12</sub>.

We identified three *E. coli* harbouring carbapenemase genes: one *bla*<sub>NDM-1</sub>, two *bla*<sub>NDM-5</sub> and one *K. pneumoniae* harbouring a *bla*<sub>NDM-6</sub> gene, and all were associated with the *bla*<sub>CTX-M-15</sub>

gene. Two of the 83 whole genome sequenced isolates harboured a resistance gene to colistin: *mcr-1* and *mcr-3*.

### Factors associated with maternal ESBL-E colonization

We present here the results of the risk factors analysis related to the 271 women with exhaustive epidemiological data. These data did not differ significantly compared with the remaining women in the study concerning the site ( $P=0.09$ ), mean age ( $P=0.79$ ), level of education ( $P=0.32$ ), or parity ( $P=0.66$ ).

In the multivariable analysis, factors independently associated with maternal ESBL-*E* colonization were families with the lowest incomes [woman unemployed and chief of family being unemployed or manual worker; adjusted odds ratio (aOR) 2.57, 95% CI 1.23–5.36]. Women who suffered from undernutrition (aOR 2.66, 95% CI 1.15–6.18) and who had at least one previous child (aOR 2.35, 95% CI 1.25–4.42) were more at risk of being ESBL-*E* colonized compared with women free of malnutrition and those who were primiparous, respectively. Dietary habits such as a consumption of pork at least once a week (aOR 2.17, 95% CI 1.04–4.49), dried meat at least twice per month (aOR 2.50, 95% CI 1.05–5.99), and raw vegetables at least once per month (aOR 2.55, 95% CI 1.08–6.06) were also found to be associated with ESBL-*E* colonization (Table 2).

We also performed analysis by including all 423 women; results were comparable except for dietary habits, which were not available.

## Discussion

### Prevalence of maternal ESBL-*E* carriage

In this cross-sectional study of peripartum women in Cambodia, we report one of the highest prevalences yet reported worldwide of ESBL-*E* faecal carriage in the community. Asia has been particularly affected by a high prevalence of ESBL-*E* carriage, although data from Cambodia is scarce. Karanika *et al.*<sup>2</sup> estimated in a meta-analysis an ESBL-*E* carriage prevalence of 46% (95% CI 29%–63%) in Asia (excluding China) with the highest rates ranging from 69% to 72% among adults in rural communities in Thailand and Laos.

In parallel with ESBL-*E* carriage, the incidence of ESBL-*E* infections has seriously increased over the last two decades worldwide, in particular in Asia. The peripartum period is particularly at risk for severe urinary infection and asymptomatic bacteriuria for women. Enterobacteriales are the most frequent pathogens responsible for these infections, which pose potential maternal and fetal/neonatal adverse outcomes, such as neonatal bacterial infections through mother-to-child ESBL-*E* transmission during delivery.<sup>3,9</sup> Given the risk of the morbidity/mortality caused by ESBL-*E* infections, the peripartum period is therefore a vulnerable period for both mother and child. Despite this, only a few studies have focused on peripartum maternal ESBL prevalence, particularly in low- and middle-income countries. In Madagascar, prevalence among mothers included in BIRDY study was considerably lower, 18.5% (95% CI 14.5%–22.6%), but consistent with other data from Africa: 17% (95% CI 10%–23%).<sup>10,11</sup> From India, studies have reported ESBL-*E* prevalence of 15.4% in peripartum women.<sup>12</sup> Studies from high-income countries (HICs) have shown considerably lower prevalence, for example 2.9% in Norway and 5.4% in Argentina, which may be attributable to different policies in antimicrobial use and infection control in these settings.<sup>13–15</sup>

### Factors associated with maternal ESBL-*E* carriage

We found that women from the poorest families were at increased risk of ESBL-*E* colonization. This finding is in line with previous studies in low-resource settings, which also identified poverty as being linked to ESBL-*E* colonization.<sup>16–18</sup> Low socio-

demographic conditions may encourage the use of lower quality or expired medications, shorter courses of therapy, medication sharing and also access to low hygiene level healthcare services with higher risk of nosocomial transmission.<sup>19</sup> All these conditions may contribute to acquisition or selection of ESBL-producing pathogens.

Undernutrition was also found to be independently associated with ESBL-*E* carriage. One plausible explanation is that undernutrition might be correlated to inadequate dietary intakes during pregnancy in a household with food insecurity as a consequence of poverty.<sup>20</sup> Causes of undernutrition are probably multifactorial and further research is needed to confirm the link between malnutrition and ESBL-*E* colonization. However, this finding is important as these women with microbial balance changes and a relative immunity alteration in both pregnancy and acute malnutrition will have an increased risk of Gram-negative infections requiring antibiotic treatment.<sup>20,21</sup>

We found that having at least one previous child was independently associated with the risk of ESBL-*E* colonization. Cambodian families frequently mutualize daycare of children to an older family member or neighbours. A Dutch study found that having a child attending daycare was associated with a higher risk of ESBL-*E* carriage among parents and family members.<sup>22</sup> We hypothesize that this may be a result of the close contacts between children, leading to increased risk of gastrointestinal and respiratory infections, antibiotic treatments for which may also facilitate further ESBL-*E* acquisition. This result might reflect close contact between a mother and her child, which may facilitate ESBL-*E* transmission.

The risk of the spread of AMR pathogens to humans after consumption of, or direct contact with, livestock or vegetables are higher in settings where regulations for the use of antimicrobial agents for growth promotion or treatment in animal husbandry or agriculture are not yet implemented.<sup>23</sup> In line with this, we found that regular consumption of pork and/or dried-meat (usually uncooked and sun-dried after a lime and spices mix marinade) were associated with ESBL-*E* carriage. This result is concordant with several studies that showed that the consumption of undercooked meat or pork meat is associated with faecal ESBL-*E* carriage.<sup>24</sup> In particular, we showed in a previous study that half of the ESBL-producing *E. coli* carried by 88 women included in the present study were phylogenetically similar to ESBL-producing *E. coli* from highly contaminated food samples (62%) of pork, chicken, and fish from two neighbouring markets.<sup>25</sup> This association was reinforced by the fact that 33% of isolates were resistant to chloramphenicol, an antibiotic not in use in human health in Cambodia up to 2015 but widely used in animal health, suggesting a possible link between human health and food chain or animal contact.

There are frequent reports of ESBL-*E* contamination of the food chain in Asian LMICs as well as in HICs, and there are descriptions of overlapping genes encoding ESBL and/or plasmids between sectors (human, farm animals, foods).<sup>26,27</sup> Most studies supports the idea of ESBL-*E* transfer from food animals and vegetables to humans, through consumption and/or manipulation. Although the mechanisms are still widely discussed, there is a need for 'One Health'-oriented studies in highly endemic LMICs with genomic data analysis.



## Antibiotic resistance profiles and molecular characterization

In case of infection, the presence of an ESBL would imply treatment failure to most antibiotics from the  $\beta$ -lactam group, which are recommended in Cambodia as first-line treatment in most infections (respiratory, digestive, urinary). Worryingly, all the ESBL-*E* isolates in our study were MDR and phenotypically resistant to multiple classes of antibiotics. Consequently, second-line antibiotics (e.g. fluoroquinolones and aminoglycosides) may also fail. The rates of co-resistances appeared higher than in other neighbouring countries such as Thailand or Laos.<sup>24,28</sup> Furthermore, we found high levels of resistance to colistin and chloramphenicol, which are last-option antibiotics along with carbapenems for some pathogens.

Molecular characterization of ESBL genes in our study shows that over two-thirds of isolates (68.9%) expressed ESBL genes belonging to the *bla*<sub>CTX-M</sub> group 1, with a majority of *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-55</sub>, confirming a trend towards a shift from *bla*<sub>CTX-M</sub> group 9 to *bla*<sub>CTX-M</sub> group 1 observed in Thailand by Niumsup et al.,<sup>24</sup> which is worrying since the first descriptions of *bla*<sub>CTX-M</sub>, in particular *bla*<sub>CTX-M-55</sub>, only date from 2007.

Only seven ESBL-producing isolates (2.2%) did not express a *bla*<sub>CTX-M</sub> gene: *bla*<sub>LEN-12</sub> (one case) and *bla*<sub>SHV</sub> (6 cases). *bla*<sub>SHV</sub> genes have previously been detected in animals, vegetables, aquatic environments, and in humans mostly in clinical settings.<sup>29</sup> Interestingly, none of the women with *bla*<sub>SHV</sub> genes in our study had a recent contact with healthcare settings other than antenatal visits, suggesting environmental transmission rather than healthcare facility transmission.

We reported four isolates harbouring both *bla*<sub>CTX-M</sub>-type and carbapenemases genes, all of *bla*<sub>NDM</sub> types. One of these women, who carried a *bla*<sub>NDM-6</sub> *K. pneumoniae*, had visited a relative in hospital during pregnancy, the three others seem to have been acquired in the community. This is consistent with previous reports where *bla*<sub>NDM</sub> genes of both *K. pneumoniae* and *E. coli* have been reported as sources of both nosocomial and community-acquired infections.<sup>30</sup> This is a first description in Cambodia, and the high potential of dissemination of NDM carbapenemases in communities must be taken into serious consideration.

## Limitations

Our work has some limitations. First, our study concerned only peripartum women, thus the results may not be generalizable to the entire Cambodian population. Nevertheless, pregnancy is not described as a risk factor for carriage by itself and our study population might be a good surrogate of the young adult population in Cambodia. Second, in contrast to previous studies, we did not find that antibiotic consumption or hospitalization were risk factors for ESBL carriage. Due to the retrospective collection of data on various exposures, there could have been a non-differential recall bias during pregnancy. However, in high endemicity settings such as Asia, exposure to food or other sources are likely to be more important than hospital contacts.

In addition, we cannot exclude that antibiotic consumption during pregnancy was higher. Along with recall bias, pregnant women in these settings may not be aware that they received an antibiotic in case of disease. However, to limit this bias, we

systematically asked for the prescription, if available, to check for antibiotics.

Finally, it is possible that the relatively small number of negative ESBL-*E* strains limited our multivariate analysis of risk factors.

## Conclusions

We found an alarmingly high prevalence of ESBL-*E* carriage in peripartum women and document for the first time the carriage in the community of *bla*<sub>NDM</sub>-type carbapenemases genes of high dissemination potential. These findings are worrisome as they are likely to mirror the carriage rate in the young adult population. Highlighting the burden of ESBL-*E* in the community, our work also emphasizes the needs to strengthen microbiological laboratory capacity, and to develop surveillance systems and antibiotic stewardship programmes in Cambodia.

Our results highlight that disentangling risk factors associated with the community ESBL-*E* reservoir in South-East Asia requires a 'One Health' approach, involving different sectors of human health (including food security), environment, animal health. The Cambodian authorities in the human, animal health and environment sectors have launched a multisectoral national action plan on AMR in December 2019 and now need to move forward with its effective implementation.

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## Transparency declarations

None to declare.

## Supplementary data

Table S1 is available as [Supplementary data](#) at JAC Online.

## References

- 1 O'Neill J. The Review on Antimicrobial Resistance. 2018. [https://amr-review.org/sites/default/files/160525\\_Final%20paper\\_with%20cover.pdf](https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf).
- 2 Karanika S, Karantanos T, Arvanitis M *et al*. Fecal colonization with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae and risk factors among healthy individuals: a systematic review and meta-analysis. *Clin Infect Dis* 2016; **63**: 310–8.
- 3 Chan GJ, Lee AC, Baqui AH *et al*. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. *PLoS Med* 2013; **10**: e1001502.
- 4 Denkel LA, Schwab F, Kola A *et al*. The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae (ESBL-E). *J Antimicrob Chemother* 2014; **69**: 2230–7.
- 5 Singh N, Patel KM, Léger M-M *et al*. Risk of resistant infections with Enterobacteriaceae in hospitalized neonates. *Pediatr Infect Dis J* 2002; **21**: 1029–33.
- 6 Kalinderi K, Delkos D, Kalinderis M *et al*. Urinary tract infection during pregnancy: current concepts on a common multifaceted problem. *J Obstet Gynaecol* 2018; **38**: 448–53.
- 7 Huynh B-T, Kermorvant-Duchemin E, Chheang R *et al*. Severe bacterial neonatal infections in Madagascar, Senegal, and Cambodia: a multi-centric community-based cohort study. *PLoS Med* 2021; **18**: e1003681.
- 8 Magiorakos A-P, Srinivasan A, Carey RB *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* 2012; **18**: 268–81.
- 9 Grette K, Cassity S, Holliday N *et al*. Acute pyelonephritis during pregnancy: a systematic review of the aetiology, timing, and reported adverse perinatal risks during pregnancy. *J Obstet Gynaecol* 2020; **40**: 739–48.
- 10 Chereau F, Herindrainy P, Garin B *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* 2015; **59**: 3652–5.
- 11 Bulabula ANH, Dramowski A, Mehtar S. Maternal colonization or infection with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in Africa: A systematic review and meta-analysis. *Int J Infect Dis* 2017; **64**: 58–66.
- 12 Pathak A, Chandran SP, Mahadik K *et al*. Frequency and factors associated with carriage of multi-drug resistant commensal *Escherichia coli* among women attending antenatal clinics in Central India. *BMC Infect Dis* 2013; **13**: 199.
- 13 Villar HE, Aubert V, Baserni MN *et al*. Maternal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in Argentina. *J Chemother* 2013; **25**: 324–7.
- 14 Rettedal S, Löhr IH, Bernhoff E *et al*. Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal-neonatal transmission. *J Perinatol* 2015; **35**: 907–12.
- 15 Jalilian N, Kooshkiforooshani M, Ahmadi S *et al*. Colonisation with extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in pregnant/post-partum women: Systematic review and meta-analysis. *J Glob Antimicrob Resist* 2019; **19**: 338–47.
- 16 Alsan M, Kammili N, Lakshmi J *et al*. Poverty and community-acquired antimicrobial resistance with extended-spectrum  $\beta$ -lactamase-producing organisms, Hyderabad, India. *Emerg Infect Dis* 2018; **24**: 1490–6.
- 17 Woerther P-L, Burdet C, Chachaty E *et al*. Trends in human fecal carriage of extended-spectrum  $\beta$ -lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 2013; **26**: 744–58.
- 18 Herindrainy P, Rabenandrasana MAN, Andrianirina ZZ *et al*. Acquisition of extended spectrum  $\beta$ -lactamase-producing enterobacteriaceae in neonates: A community based cohort in Madagascar. *PLoS One* 2018; **13**: e0193325.
- 19 Om C, Daily F, Vlieghe E *et al*. Pervasive antibiotic misuse in the Cambodian community: antibiotic-seeking behaviour with unrestricted access. *Antimicrob Resist Infect Control* 2017; **6**: 30.
- 20 Black RE, Allen LH, Bhutta ZA *et al*. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008; **371**: 243–60.
- 21 Schaible UE, Kaufmann SHE. Malnutrition and infection: complex mechanisms and global impacts. *PLoS Med* 2007; **4**: e115.
- 22 van den Bunt G, Liakopoulos A, Mevius DJ *et al*. ESBL/AmpC-producing Enterobacteriaceae in households with children of preschool age: prevalence, risk factors and co-carriage. *J Antimicrob Chemother* 2017; **72**: 589–95.
- 23 Okeke IN, Laxminarayan R, Bhutta ZA *et al*. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect Dis* 2005; **5**: 481–93.
- 24 Niumsup PR, Tansawai U, Na-Udom A *et al*. Prevalence and risk factors for intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai community. *Eur J Clin Microbiol Infect Dis* 2017; **37**: 69–75.
- 25 Nadimpalli M, Vuthy Y, de Lauzanne A *et al*. Meat and fish as sources of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli*, Cambodia. *Emerging Infect Dis* 2019; **25**: 126–31.
- 26 Boonyasiri A, Tangkoskul T, Seenama C *et al*. Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathog Glob Health* 2014; **108**: 235–45.
- 27 Zurfluh K, Nüesch-Inderbinen M, Morach M *et al*. Extended-spectrum- $\beta$ -lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican republic, India, Thailand, and Vietnam. *Appl Environ Microbiol* 2015; **81**: 3115–20.
- 28 Stoesser N, Xayaheuang S, Vongsouvath M *et al*. Colonization with Enterobacteriaceae producing ESBLs in children attending pre-school childcare facilities in the Lao People's Democratic Republic. *J Antimicrob Chemother* 2015; **70**: 1893–7.
- 29 Liakopoulos A, Mevius D, Ceccarelli D. A review of SHV extended-spectrum  $\beta$ -lactamases: neglected yet ubiquitous. *Front Microbiol* 2016; **7**: 1374.
- 30 Cornaglia G, Giamarellou H, Rossolini GM. Metallo- $\beta$ -lactamases: a last frontier for  $\beta$ -lactams? *Lancet Infect Dis* 2011; **11**: 381–93.