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# 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

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**The spread of extended-spectrum- $\beta$ -lactamase-producing *Enterobacteriaceae* (ESBL-PE) in low-income countries, where the burden of neonatal sepsis is high, may have a serious impact on neonatal mortality rates. Given the potential for mother-to-child transmission of multiresistant bacteria, this study investigated the ESBL-PE rectal colonization among pregnant women at delivery in the community in Madagascar and estimated a prevalence of 18.5% (95% confidence interval, 14.5% to 22.6%). One strain of *Klebsiella pneumoniae* isolated was also a New Delhi metallo- $\beta$ -lactamase-1 (NDM-1) producer.**

Severe bacterial infections are responsible for a large number of neonatal deaths in low-income countries (LICs) (1, 2), with *Enterobacteriaceae* most frequently isolated in neonatal sepsis (3, 4). The production of plasmid-borne extended-spectrum  $\beta$ -lactamases (ESBL) is the main mechanism of resistance against expanded-spectrum cephalosporins (ESC) among *Enterobacteriaceae*, with the CTX-M-15 variant being the most common enzyme (5). In LICs, where neonatal sepsis caused by ESBL-producing *Enterobacteriaceae* (ESBL-PE) may be associated with higher fatality rates (6), colonized mothers could represent a source of transmission to neonates. The objectives of this study were to estimate the prevalence of ESBL-PE rectal colonization among pregnant women in Madagascar in an urban area, Antananarivo, and a

semirural area, Moramanga, and to identify associated risk factors.

This study was conducted in the context of an international pediatric cohort, the BIRDY (bacterial infections and antibiotic resistance disease among young children in low-income countries) program, approved by the Ethics Committees of Ministry of Health, Madagascar, and Institut Pasteur, France. All pregnant women living in the study areas were identified by medical or community workers and enrolled after giving written informed consent. A stool sample was taken at delivery for ESBL-PE screening. Among the women enrolled, 356 gave birth between June 2013 and March 2014 and were included in the present study. A risk factor analysis was conducted on a subset of 231 women who delivered during a 6-month period (June to September 2013 and January to March 2014). Sociodemographic characteristics, previous health care exposure, and antibiotic consumption (collected with prescription documents, remaining tablets, or visual aid) were recorded. Fresh stools were plated onto Drigalski agar supplemented with 3 mg/liter of ceftriaxone at the clinical laboratory of Institut Pasteur of Madagascar. Plates were incubated for 24 to 48 h at 37°C. Every oxidase and Gram-negative colony type was

TABLE 1 Characteristics of the participants in Madagascar, 2013 to 2014

Characteristic	Participant data <sup>a</sup>
Study area	
Antananarivo	149 (41.8)
Moramanga	207 (58.2)
Age (yr)	26 (21, 32)
Parity	1 (0, 2)
Marital status	
Single, divorced, or widow	18 (5.1)
Married or consensual union	338 (94.9)
Education	
Absent or primary school	98 (27.5)
Junior school graduate	184 (51.7)
High school or university graduate	74 (20.8)
Electricity access	
Yes	263 (73.9)
No	93 (26.1)

<sup>a</sup> The total no. of participants was 356. Data shown are median (interquartile range) or number (%).

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TABLE 2 Factors associated with ESBL-PE colonization among pregnant women in Madagascar, 2013 to 2014<sup>a</sup>

Factor	No. (%) of women by ESBL status		Univariate analysis <sup>b</sup>		Multivariate analysis	
	Positive (n = 36)	Negative (n = 195)	OR (95% CI)	P	OR (95% CI)	P
Study area						
Antananarivo	20 (55.6)	80 (41.0)	Ref			
Moramanga	16 (44.4)	115 (59.0)	0.6 (0.3–1.1)	0.11		
Age						
≤25 yr	13 (36.1)	109 (55.9)	Ref			
>25 yr	23 (63.9)	86 (44.1)	2.2 (1.1–4.7)	0.03		
Marital status						
Single, divorced, or widow	1 (2.8)	6 (3.1)	Ref			0.9
Married or consensual union	35 (97.2)	189 (96.9)	1.1 (0.1–9.5)			
Education						
Absent or primary school	6 (16.7)	59 (30.3)	Ref			
Junior school graduate	17 (47.2)	98 (50.3)	1.7 (0.6–4.6)	0.29		
High school or university graduate	13 (36.1)	38 (19.5)	3.4 (1.2–9.6)	0.02		
Electricity access						
Yes	28 (77.8)	141 (72.3)	Ref			
No	8 (22.2)	54 (27.7)	0.7 (0.3–1.7)	0.49		
Birth attendant						
Unskilled worker <sup>c</sup>	5 (13.9)	62 (32.3)	Ref			
Skilled worker <sup>d</sup>	31 (86.1)	130 (67.7)	3.0 (1.1–8.0)	0.03		
Type of house						
Shared land use with other families	16 (44.4)	64 (32.8)	Ref		Ref	
Individual house	20 (55.6)	131 (67.2)	1.6 (0.8–3.4)	0.18	2.2 (1.0–4.8)	0.06
Drinking water supply						
Public tap	16 (44.4)	118 (60.5)	Ref		Ref	
Spring, well	13 (36.1)	65 (33.3)	1.5 (0.7–3.3)	0.34	2.2 (0.8–6.2)	0.15
Private inside access	7 (19.5)	12 (6.2)	4.3 (1.5–12.5)	0.01	3.8 (1.2–11.6)	0.02
Toilet facilities						
Shared with neighbors or public toilets	24 (66.7)	156 (80.0)	Ref			
Private	12 (33.3)	39 (20.0)	2.0 (0.9–4.3)	0.08		
Farmyard animals						
No	27 (75.0)	144 (73.8)	Ref			
Yes	9 (25.0)	51 (26.1)	0.9 (0.4–2.1)	0.89		
Poultry consumption						
<1/wk	24 (66.7)	130 (66.7)	Ref			
≥1/wk	12 (33.3)	65 (33.3)	1 (0.5–2.1)	1.00		
Hospitalization in the last year						
No	34 (94.4)	187 (95.9)	Ref			
Yes	2 (5.6)	8 (4.1)	1.4 (0.3–6.8)	0.69		
Close relative hospitalized						
No	23 (63.9)	156 (80.0)	Ref			
Yes	13 (36.1)	39 (20.0)	2.3 (1.0–4.9)	0.04		
Antibiotic treatment in the last year						
No	22 (61.1)	130 (66.7)	Ref			
Yes	14 (38.9)	65 (33.3)	1.3 (0.6–2.6)	0.52		
Antibiotic treatment in the last 3 mo						
No	29 (80.6)	165 (84.6)	Ref			
Yes	7 (19.4)	30 (15.4)	1.3 (0.5–3.3)	0.54		

<sup>a</sup> ESBL-PE, extended-spectrum-β-lactamase-producing *Enterobacteriaceae*.

<sup>b</sup> OR, odds ratio; 95% CI, 95% confidence interval; Ref, reference.

<sup>c</sup> Includes traditional birth attendants, nurses, or no attendant.

<sup>d</sup> Includes doctors and midwives.

identified with the API 20E system (bioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM).

Inhibition diameters were read with the ADAGIO apparatus (Bio-Rad, Marnes-la-Coquette, France). Production of ESBL in ESC-resistant *Enterobacteriaceae* was confirmed by the double-disk synergy test (CASFM). When suitable, carbapenem MICs were obtained using Etest (bioMérieux, Marcy l'Etoile, France). Genomic DNA

was extracted with the GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). Plasmid-borne *bla*<sub>TEM</sub> (7), *bla*<sub>SHV</sub> (8), and *bla*<sub>CTX-M</sub> β-lactamase genes (9) were screened by PCR. For ESBL-producing isolates also resistant to cefoxitin, the presence of *bla*<sub>AmpC</sub> (10) and *bla*<sub>CMY-2</sub> genes (11) were determined. ESBL-producing isolates showing a decreased susceptibility to carbapenems were screened for *bla*<sub>OXA-48</sub> (12), *bla*<sub>KPC</sub> (13), and *bla*<sub>NDM-1</sub> (14) genes. All of the positive NDM-1 and half of the positive CTX-M PCR products were sequenced (Cogenics); for CTX-M-negative PCR results, SHV and TEM PCR products were sequenced. Statistical analyses were performed using Stata12 (Stata Corp., College Station, TX). All variables were analyzed by univariate logistic regression to identify factors associated with ESBL-PE colonization and, when associated with a *P* value of <0.2, were included in a multivariate model (manual backward logistic regression). Odds ratios (OR) with 95% confidence intervals (95% CI) were estimated, and the statistical significance was set at a *P* value of <0.05.

Among the 356 women included (Table 1), 66 were colonized with ESBL-PE, representing a prevalence of 18.5% (95% CI, 14.5% to 22.6%), with no significant difference between Antananarivo (22.1%) and Moramanga (15.9%) (*P* = 0.14) but a significant difference according to the delivery period. The prevalence was 9.6% between June and September 2013, 25.4% between October and December 2013, and 25.6% between January and March 2014 (*P* = 0.001 between the three periods). The 66 ESBL-producing isolates included *E. coli* (*n* = 46), *Klebsiella* spp. (*n* = 11), *Enterobacter cloacae* (*n* = 5), *Citrobacter freundii* (*n* = 3), and *Morganella morganii* (*n* = 1). Forty-five isolates carried a *bla*<sub>CTX-M</sub> gene, 15 carried *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes, and 2 carried a *bla*<sub>SHV</sub> gene. All of the sequenced genes were SHV-12, CTX-M-15, and non-ESBL TEM-1. Cefoxitin resistance was associated with *bla*<sub>AmpC</sub> genes among 4 of these 62 ESBL-PE isolates: 1 *Enterobacter cloacae* (DHA), 1 *Citrobacter freundii* (CIT, FOX), 1 *E. coli* (MOX), and 1 *Morganella morganii* (DHA) isolate. The *E. coli*, *Citrobacter freundii*, and *Enterobacter cloacae* isolates also carried a *bla*<sub>CMY-2</sub> gene. A *bla*<sub>NDM-1</sub> gene was detected in a *Klebsiella pneumoniae* isolate exhibiting a reduced susceptibility to imipenem (MIC of 3 mg/liter). In 4 ESBL-producing *E. coli* isolates, no *bla*<sub>ESBL</sub> gene, and no cefoxitin resistance, was detected. High susceptibility to fosfomycin (97%) and amikacin (100%) was found, while 36% of isolates were resistant to ciprofloxacin.

Of the 231 women interviewed, 79 had taken antibiotics during the previous year, and 22 received more than 1 antibiotic course. Most antibiotics belonged to the β-lactam family (79.4%). Thirty-six women were colonized with ESBL-PE (27 *E. coli*, 4 *Klebsiella* spp., 4 *Enterobacter cloacae*, 1 *Morganella morganii*). Several factors were identified in univariate analysis (Table 2); only private inside access to drinking water and living in an individual house remained associated with ESBL-PE colonization in the multivariate logistic regression model (adjusted for delivery period, June to September 2013 or January to March 2014, and study area). Antibiotic consumption was not significantly associated with ESBL-PE colonization.

Our estimation of the ESBL-PE colonization rate among pregnant women in the community (18.5%) is similar to those reported in Tanzania (15% in 2013) (15) and India (15% in 2007 to 2009) (16). A previous community-based study conducted in Madagascar in 2009 estimated an ESBL-PE carriage rate of 10% (17). Our results suggest an increase in the prevalence of coloni-

zation, consistent with the worldwide increase of ESBL-PE carriage in the community (18). Data on ESBL-PE colonization rates in LICs are scarce, especially among pregnant women, despite a risk of transmission from mothers to neonates (19–21). The isolation of an NDM-1-producing *K. pneumoniae*, the first NDM-1-PE identified in Madagascar, is of great concern. Importantly, the woman colonized with this strain did not report any recent hospital contact or antibiotherapy, suggesting a community acquisition. Living in an individual house and having a private inside access to drinking water were associated with ESBL-PE colonization. These conditions reflect a higher socioeconomic status in a globally underprivileged population and may correlate with more frequent access to health care services or a higher antibiotic consumption (16). However, we did not find any association between individual antibiotic consumption and ESBL-PE colonization. Due to the cross-sectional design of the study, the interpretation of the risk factors must be cautious. Finally, we observed a difference in the prevalence of ESBL-PE colonization according to the delivery period. The period from October to March corresponds with the rainy season in Madagascar, which may be associated with an increase of bacterial diarrhea and ESBL-PE fecoral transmission.

This study highlights a significant prevalence of ESBL-PE colonization among pregnant women in the community in an LIC and describes an NDM-1-PE maternal carriage, indicating that pregnant women may represent a substantial source for transmission of multiresistant *Enterobacteriaceae* to neonates.

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