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Relevant increase of CTX-M-producing *Escherichia coli* carriage in school-aged children from rural areas of the Bolivian Chaco in a three-year period

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ABSTRACT

Objectives: The aim of this study was to perform two cross-sectional surveys on the fecal carriage of CTX-M–producing Enterobacterales in school-aged children from rural areas of the Bolivian Chaco (2016 vs 2019).

Methods: A total of 757 fecal samples were collected from school-aged children living in nine indigenous communities (n=337, 2016; n=420, 2019). After a first passage onto MacConkey agar (MCA), samples were plated onto MCA plus cefotaxime 2 µg/mL (MCA-CTX), and a loopful of the bacterial growth was used as a template for the detection of group 1, 2, 8/25, and 9 bla_{CTX-M} variants by multiplex reverse transcriptase polymerase chain reaction . Positive samples were tested again for detecting, identifying, and characterizing CTX-M-positive isolates.

Results: Growth onto MCA-CTX was obtained with 208 samples (27.5%; 62/337, 2016; 146/420, 2019), of which 201 (96.6%) were positive for bla_{CTX-M} genes. Overall, a relevant increase of fecal carriage of CTX-M–producing Enterobacterales was observed in the study period: 17,5% (59/337) in 2016 compared with 33,8% (142/420) in 2019, $p<0.01$. Nonetheless, the relative group distribution of CTX-M groups remained stable, with group 1 being the prevalent, followed by group 9 and group 8/25. Group 2 was not detected.

Conclusions: The present study demonstrated an alarming spread of CTX-M enzymes in rural areas of the Bolivian Chaco, where antibiotics consumption is limited. Further studies are encouraged to better understand the dissemination dynamics of such relevant resistance determinants.

Background

Extended-spectrum β-lactamases (ESBLs) have become endemic in Enterobacterales, in both hospital and community settings. CTX-M-type ESBLs have rapidly disseminated since the early 1990s and currently represent the most prevalent ESBLs among Enterobacterales worldwide (Peirano and Pitout, 2019). In particular, *Escherichia coli* has become the species most frequently associated with CTX-Ms, with some clones showing a pandemic dissemination (i.e., ST131 and ST1193 clonal groups) (Peirano and Pitout, 2019). The role of commensal *E. coli* as a reservoir of genes encoding ESBL has been recognized globally

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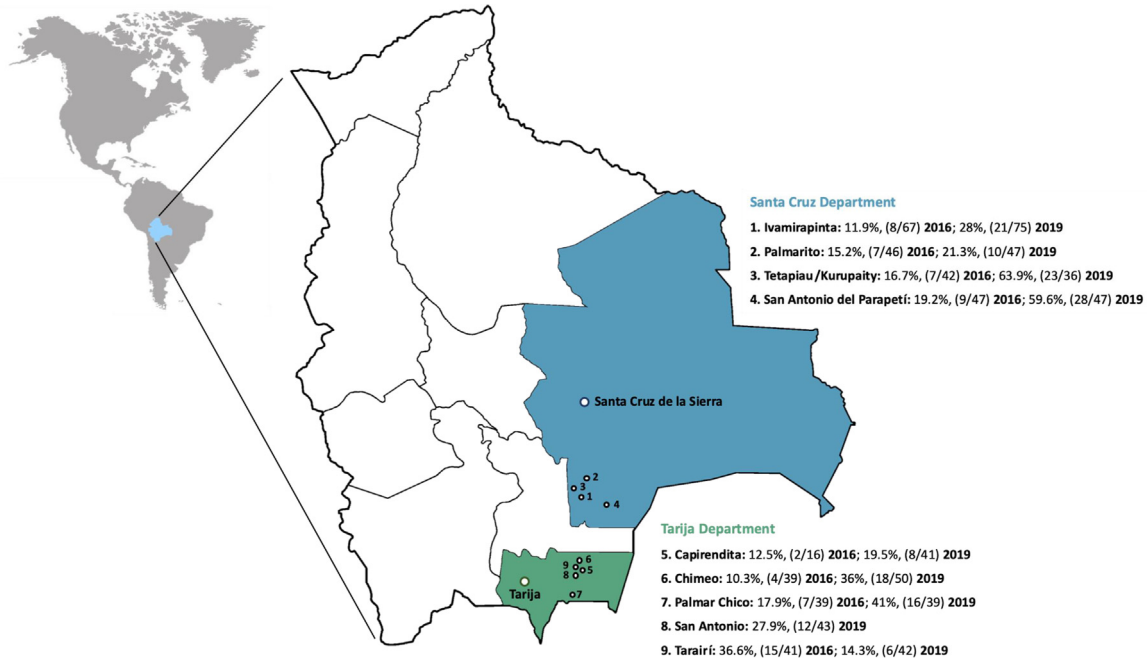

Fig. 1. Geographical area of the nine communities included in the study and percentages of children with CTX-M–positive *Escherichia coli*.

(World Health Organization WHO and GLASS, 2020), and several studies have reported high prevalence of CTX-M-type ESBLs in commensal isolates from healthy adults and children in the community setting (Woerther *et al.*, 2013).

In low/medium-income (LMI) settings, antimicrobial resistance rates have been demonstrated to be even higher than in higher-income countries, for complex factors mainly related to poverty (e.g., poor access to healthcare, poor sanitation, and unreliable water supplies), with a relevant impact on morbidity and mortality rates, especially in childhood (World Health Organization WHO and GLASS, 2020; Murray *et al.*, 2022).

In this study, we performed two cross-sectional surveys (i.e., 2016 vs 2019) to investigate the fecal carriage of CTX-M-producing Enterobacterales in school-aged children living in nine indigenous communities in rural areas of the Bolivian Chaco.

Methods

The study population was represented by school-aged children (i.e., aged 6–14 years) living in nine indigenous communities in rural areas of the Bolivian Chaco (Fig 1). Administration of antibiotics during the 15 days preceding the survey was investigated by a questionnaire administered to parents.

A total of 757 fecal samples (337 in 2016; 420 in 2019) were collected and transferred to the Laboratories of Camiri or Villa Montes Hospitals within six hours, for immediate plating onto MacConkey agar (MCA; Oxoid LTD, UK). After incubation at 35 °C for 18 hours, the bacterial growth (representative of the total enterobacterial microbiota) was collected using fecal swabs (Copan, Brescia, Italy), shipped to Italy, and preserved at 4°C until processed (within 30 days) (Giani *et al.*, 2018). For detection of CTX-M–producing enterobacteria, fecal swabs were plated onto MCA plates plus cefotaxime 2 µg/ml (MCA-CTX). After incubation at 35 °C for 18 hours, a loopful of the bacterial growth (taken either from confluent growth or from isolated colonies of different morphologies) was used as a template for the detection of group 1, 2, 8/25, and 9 $bla_{\text{CTX-M}}$ by mRT-PCR, as previously described (Giani *et al.*, 2017). To identify CTX-M–positive isolates, CTX-M–positive samples were again streaked onto MCA-CTX, and all colonies with a different appearance were re-isolated and subjected to i) a phenotypic test for ESBL production (using the double disk method with amoxicillin-clavulanate and cefotaxime), ii) characterization of $bla_{\text{CTX-M}}$ group by multiplex reverse transcriptase polymerase chain reaction (mRT-PCR), and iii) identification by the Bruker MS system (Bruker Daltonics, Germany; MBT reference library, version 2021) (Giani *et al.*, 2017).

Statistical analyses were performed using Pearson's Chi-square test with Yates' continuity correction with R version 4.0.5 for Windows. A *P*-value <0.05 was considered significant.

Results and discussion

In 2016, 337 children (mean age = 9.2 years, SD = 1.25; median age = 9 years; male:female ratio = 1:1.13) were included in the study (Table 1). Of the 337 samples, 61 grew on MCA-CTX (18.1%). Of these, 59 (96.7%) were found positive for $bla_{\text{CTX-M}}$ genes. The remaining two were found negative for ESBL production through phenotypic tests. Identification of CTX-M-producing isolates showed that all were *E. coli*, except for one *Enterobacter cloacae* complex. Some children (n=19, 32.2%) were found to be infected by more than one CTX-M-producing *E. coli,* for a total of 82 *E. coli* isolates, with one isolate carrying two $bla_{\text{CTX-M}}$ variants (i.e., $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ groups) (Table 1).

In 2019, 420 children were included in the study (mean age = 9.6 years, SD = 1.4; median age = 10 years; male:female ratio = 1.08:1). Of the 420 fecal samples collected, 146 (34.8%) grew on MCA-CTX, and 142 (97.3%) of these were found positive for $bla_{\text{CTX-M}}$ genes (Table 1). Only one of four CTX-M negative isolates showed a result of ESBL-producer through phenotypic testing. CTX-M–producing isolates were identified as *E. coli,* except for one *Raoultella ornithinolytica*. A totoal of 40 children (28.2%) were infected by multiple CTX-M–producing *E. coli*, for a total of 190 CTX-M–producing isolates, with five isolates carrying two $bla_{\text{CTX-M}}$ variants (i.e., n=4, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ groups, and n=1, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-8/25}}$ groups) (Table 1).

Usage of antibiotics was found to be very limited, with only two children (0.6%) in 2016 and 21 children (5%) in 2019 report-

Table 1
Features of the study population sorted by communities and by year with features of CTX-M-positive *Escherichia coli* isolates.

Community		Year	No. of studied children			Antibiotic consumption[a,b]	Tot children with CTX-M[a]	*p* value	CTX-M producing *E. coli*	Tot CTX-M detected	CTX-M-groups		
			M[a]	F[a]	Total						1[a,c]	9[a,c]	8/25[a,c]
Tarija Department	**Capirendita**	2016	9 (56.3)	7 (43.8)	16	-	2 (12.5)	0.8	2	2	-	2 (100)	-
		2019	23 (56.1)	18 (43.9)	41	-	8 (19.5)		11	12	7 (58.3)	5 (41.7)	-
	Chimeo	2016	18 (46.2)	21 (53.8)	39	-	4 (10.3)	0.01	4	4	3 (75)	1 (25)	-
		2019	25 (50)	25 (50)	50	5 (10)	18 (36)		21	21	9 (42.9)	12 (57.1)	-
	Palmar Chico	2016	18 (46.2)	21 (53.8)	39	-	7 (17.9)	0.05	8	10	6 (60)	4 (40)	-
		2019	21 (53.8)	18 (46.2)	39	1 (2.6)	16 (41)		19	19	13 (68.4)	6 (31.6)	-
	San Antonio	2016	-	-	-	-	-	-	-	-	-	-	-
		2019	24 (55.8)	19 (44.2)	43	-	12 (27.9)		14	15	12 (80)	2 (13.3)	1 (6.7)
	Tarairí	2016	18 (43.9)	23 (56.1)	41	-	15 (36.6)	0.03	21	22	18 (81.8)	4 (18.2)	-
		2019	19 (45.2)	23 (54.8)	42	-	6 (14.3)		7	7	3 (42.9)	3 (42.9)	1 (14.3)
Santa Cruz Department	**Ivamirapinta**	2016	35 (52.2)	32 (47.8)	67	-	8 (11.9)	0.03	12	12	9 (75)	3 (25)	-
		2019	41 (54.7)	34 (45.3)	75	4 (5.3)	21 (28)		35	35	26 (74.3)	6 (17.1)	3 (8.6)
	Palmarito	2016	19 (41.3)	27 (58.7)	46	1 (2.2)	7 (15.2)	0.6	9	9	-	3 (33.3)	6 (66.7)
		2019	21 (44.7)	26 (55.3)	47	5 (10.6)	10 (21.3)		14	15	9 (60)	5 (33.3)	1 (6.7)
	Tetapiau/ Kurupaity	2016	18 (42.9)	24 (57.1)	42	1 (2.4)	7 (16.7)	<0.0001	9	10	8 (80)	2 (20)	-
		2019	20 (55.6)	16 (44.4)	36	6 (16.7)	23 (63.9)		30	30	19 (63.3)	8 (26.7)	3 (10)
	San Antonio del Parapetí	2016	23 (48.9)	24 (51.1)	47	-	9 (19.2)	0.0001	13	14	9 (64.3)	5 (35.7)	-
		2019	24 (51.1)	23 (48.9)	47	-	28 (59.6)		39	41	22 (53.7)	15 (36.6)	4 (9.8)
	Total	2016	158 (46.9)	179 (53.1)	337	2 (0.6)	59 (17.5)	<0.0001	82	83	53 (63.9)	24 (28.9)	6 (7.2)
		2019	218 (51.9)	202 (48.1)	420	21 (5)	142 (33.8)		190	195	120 (61.5)	62 (31.8)	13 (6.7)

[a] number (%)
[b] during the 15 days preceding the survey
[c] over the total CTX-M

ing antibiotic consumption during the 15 days preceding the survey (Table 1).

Overall, despite the relevant spread of CTX-M enzymes over the study period (17.5%, 2016 vs 33.8%, 2019, $p<0.001$), the relative prevalence of each CTX-M group remained stable. The CTX-M-1 group represented the most prevalent one (n=53, 63.9% in 2016; n=120, 61.5% in 2019), followed by the CTX-M-9 group (n=24, 28.9% in 2016; n=62, 31.8% in 2019) and the CTX-M-8 group (n=6, 7.2% in 2016; n=13, 6.7% in 2019) (Table 1). Group 2 was not detected.

This study reported a notable increase of carriage of CTX-M–producing *E. coli* among healthy children living in rural communities of the Bolivian Chaco, where antibiotic usage remains scarce. Discordant data were only found in one community, where a decreasing trend was observed (Table 1).

The results of the present survey were consistent with the few similar studies that have been performed so far in rural communities from other LMI settings (Araque and Labrador, 2018; Purohit *et al.*, 2017).

Our study has some limitations. The number of children in each community was not representative of the total number of the population. Indeed, only children aged between six and 14 years were included. Moreover, it would be interesting to investigate the allelic variants of CTX-M ESBLs to better understand the dissemination dynamics of these enzymes.

Previous large-scale surveys conducted by our group in small urban areas of the Bolivian Chaco had demonstrated a dramatic increase of fecal carriage of CTX-M–producing *E. coli* in healthy children during the last two decades, from 0.1% in 2002 to 12% in 2011, with a change in the molecular epidemiology of CTX-M enzymes characterized by the CTX-M-1 group outcompeting the initially prevalent CTX-M-2 group (Bartoloni *et al.*, 2013). Data from the present study are overall consistent with such scenario and demonstrate the rapid spread and maintenance of CTX-M–producing Enterobacterales even in indigenous communities with poor access to conventional medicine and antibiotics. Further studies are encouraged to better understand the dissemination dynamics of these resistance determinants.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Ethical approval

Fecal samples were obtained from enrolled children, after informed consent was obtained from parents or legal guardians. Full ethical clearance was obtained from the qualified local authorities (Convenio de Salud, Ministerio de Salud—Vicariato de Camiri) who reviewed and approved the study design.

Authors'contributions

SRB and MM analyzed the data and drafted the manuscript; SRB, MM, TDM and AM produced phenotypic data, molecular detection and handled the samples; SRB, TDM, ALV, TBM, CR, VP, HG,

MS and MS collected the samples and participated in the coordination of the survey; AB, GMR and LP coordinated the survey, analyzed the data and produced the final version of the manuscript.

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