Increase of antibiotic resistance in the gut microbiota of healthy children living in rural communities of the Bolivian Chaco during a 6-year study period

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Background:

Antibiotic resistance (AR) causes approximately 1.3 million deaths annually, primarily in low- and middle-income countries like South America.[1,2] Commensal bacteria, though not the primary targets of antibiotics, are constantly exposed to these drugs, making them important reservoirs for both resistant strains that can become pathogens and resistance genes that can transfer to pathogenic bacteria.[3] Sequence type (ST) 131 is among the most successful high-risk *Escherichia coli* clone, mostly associated with third-generation cephalosporins resistance. Recently, a new high-risk clone of *E. coli*, ST1193, has emerged and showed a propensity toward a pandemic dissemination.[4]

Here we present a large-scale survey on antibiotic resistance in gut microbiota of healthy children living in rural areas of the Bolivian Chaco during 6 years.

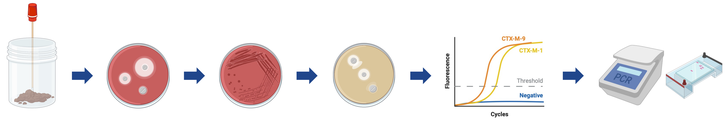
Rural communities included in the study:

Santa Cruz Department: 1, Ivamirapinta; 2, Palmarito; 3, Tetapiau/Kurupaity; 4, San Antonio del Parapeti.

Tarija Department: 5, Capirendita; 6, Chimeo; 7, Palmar Chico; 8, San Antonio; 9, Tarairi.

Methods:

Fecal samples were obtained from a total of 1989 school-age healthy children living in 9 rural communities of the Bolivian Chaco (i.e 4 in Santa Cruz Department and 5 in Tarija Department) between 2016 and 2022. Antibiotic use during the 15 days preceding the survey was investigated by a questionnaire administered to parents. A rapid screening method was used to detect antimicrobial resistance to ciprofloxacin, cefotaxime, and meropenem for samples from the two studied departments.[5] Further analysis was specifically carried out on isolated cefotaxime-resistant (CTX-R) *E. coli* from samples collected in the Tarija department.



Results:

Overall, prior antibiotic consumption was found to be very limited, ranging from 0.6% in 2016 to 6.8% in 2022. Sample analysis from rapid screening method (RSM) on commensal microbiota across the years revealed a significant increase in resistance rates to ciprofloxacin (from 39.5% in 2016 to 61.9% in 2022, *p* value <0.0001) and cefotaxime (from 17.5% in 2016 to 54% in 2022, *p* value <0.0001), while meropenem susceptibility was not found to be affected for the entire study period (Figure 1). Bacterial Identification of CTX-R samples indicated that they were primarily *E. coli*, with almost all (96 to 100%) confirmed as ESBL producers through phenotypic tests and testing positive for $bla_{\text{CTX-M}}$ genes. Across the study period we observed a significant increase in the prevalence of CTX-M determinats, ranging from 19.3% in 2016 to 60.1% in 2022 (*p* value < 0.0001), mirroring the dramatic increase of resistance to expanded spectrum cephalosporins in this aera. Despite the notable increase in CTX-M enzyme prevalence over the study period, the relative prevalence of each CTX-M group remained relatively stable (Table 1). Furthermore, molecular detection of *E. coli* pandemic clones ST131 and ST1193 revealed, starting from 2018, a notable prevalence of the two pandemic clones among CTX-M-producing commensal *E. coli*, even though clonal expansion might not be the primary driver of CTX-M ESBL spread among enterobacteria (Figure 2).

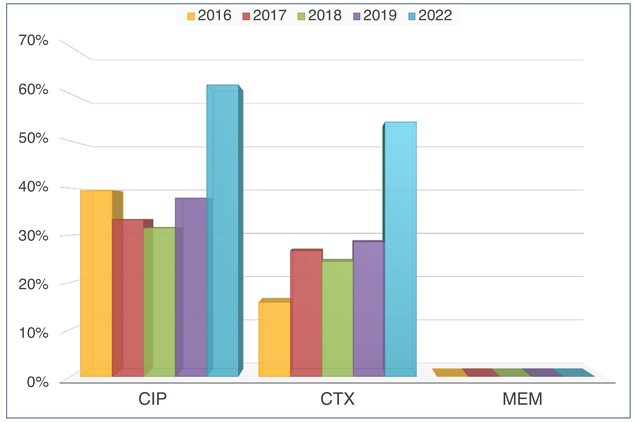


Figure 1. Resistance rates of ciprofloxacin (CIP), cefotaxime (CTX) and meropenem (MEM) in commensal microbiota trough a rapid screening method among the 1989 children of Bolivian Chaco

Table 1. Prevalence of children carrying the $bla_{\text{CTX-M}}$ genes and distribution of CTX-M-positive isolates in 2016-2022 in rural communities of Tarija Department (Plurinational State of Bolivia)

Years	No. of studied children	CTX-M-positive children	CTX-M-positive *E. coli*	Total CTX-M	CTX-M groups			
					CTX-M-1	CTX-M-2	CTX-M-9	CTX-M-8/25
2016	135	26 (**19.3**)	33	34	25 (73.5)	0	9 (26.5)	0
2017	247	85 (**34.4**)	97	98	78 (79.6)	2 (2)	15 (15.3)	3 (3.1)
2018	203	44 (**21.7**)	50	51	24 (47)	1 (2)	25 (49)	1 (2)
2019	215	60 (**27.9**)	70	71	42 (59.2)	0	27 (38)	2 (2.8)
2022	138	83 (**60.1**)	101	105	78 (74.3)	1 (0.9)	23 (21.9)	3 (2.9)

Results are presented as n (%) unless otherwise specified

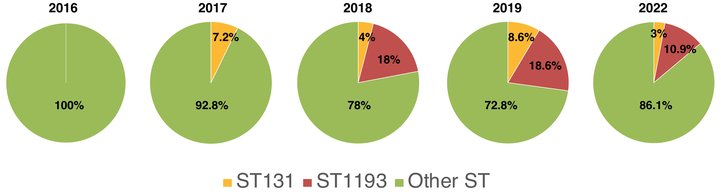


Figure 2. Clonal distribution among CTX-M-producing *E. coli*

Conclusions:

We observed a rapid increase in the prevalence of CTX-M determinants, significantly contributing to the rise in resistance to extended-spectrum cephalosporins in commensal *E. coli* among healthy children residing in rural communities of the Bolivian Chaco over a six-years period. This trend persisted despite limited antibiotic usage in the studied area. This study underlines the magnitude of the problem of AR in low-resource settings and the urgent need for surveillance and control activities.

References:

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