

Extended-Spectrum Beta-Lactamases Producing *Escherichia coli* in South America: A Systematic Review with a One Health Perspective

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Purpose: Extended-spectrum beta-lactamase-producing (ESBL) Enterobacteriaceae, which includes *Escherichia coli*, has emerged as a global health threat. ESBL enzymes including CTX-M, TEM, and SHV are the most detected. Here, a systematic review was developed to assess the status of ESBLs in *E. coli* considering studies performed in the human, animal, food, and environmental realms in South America.

Methods: Following PRISMA guidelines, a systematic review was performed using the PubMed database as a primary source to identify studies containing data on ESBL-producing *E. coli* in South America. To obtain a comprehensive sample, studies in English, Spanish, and Portuguese were included from 1990 to April 2021. Inclusion such as the reporting of sample origin and diagnostic method and exclusion criteria such as review/letter articles were established to complete data extraction steps.

Results: Amongst 506 articles retrieved, 130 met the inclusion criteria. Brazil reported 65 (50%) of publications, followed by Argentina, and Ecuador with 11.5% each. According to the category of studies, human studies represented the 56%, animals the 20%, environmental the 11%, and food studies the 6%. Interestingly, studies assessing more than one category (ie, interdisciplinary) represented the 7%. Prevalence of ESBL producing *E. coli* in animal, food, and environmental studies was widely superior compared to human sources. In clinical studies, Brazil presented the greatest diversity in terms of ESBLs, featuring CTX-M, TEM, SHV, TOHO, OXA, and AmpC. CTX-M enzymes were the most frequent variants with 89.4% detections.

Conclusion: The present One Health review of 130 studies conducted over the past 21 years found ESBLs producing *E. coli* distributed across human, animal, food, and environmental samples across South America. There is a need to increment studies in underrepresented countries and to strengthen multi-sectoral antimicrobial resistance research and surveillance. This information can be used as basis for subsequent implementation of monitoring programs, targeting potential critical points of transmission sources.

Keywords: extended-spectrum beta-lactamase, *Escherichia coli*, South America, One Health

Introduction

The antimicrobial resistance phenomena existed long time before humans were implementing antibiotics.¹ Bacteria have several mechanisms to evade the action of antimicrobials. One of the most important in humans, animals, and the environment is the enzyme-mediated breaking of the beta-lactam ring of penicillin and its derivatives. Penicillins are the widest group of antibiotics.²

Enzyme-mediated resistance is a worldwide public health problem recognized by the World Health Organization (WHO) due to its rapid expansion and the generation of multidrug-resistant (MDR) bacteria that are increasingly difficult to eliminate.^{3,4} The current increase and dispersion of penicillin, carbapenem, and cephalosporin resistance is driven by a group of enzymes known as beta-lactamases. These enzymes, commonly found in well-known human environments,

have been discovered in apparently unsuitable localities such as soils or glaciers in Antarctica, which have probably never encountered beta-lactam antibiotics previously.⁵

Beta-lactamases enzymes were first described in 1940, England; isolated from an *E. coli*, which prompted antibiotic resistance research.^{6,7} In early 1980s, TEM-1, TEM-2 (isolated from a patient in Temoneira in Athens, Greece), and SHV-1 (sulfhydryl variable, active site) circulating beta-lactamases were found capable to hydrolyze the beta-lactamic ring of cephalosporins⁸ and therefore resistance was soon reported.⁹ Single-point mutations in these enzymes allowed beta-lactamases to break penicillin and its derivatives, as well as the first, the second, and third generation cephalosporins, and even monobactams.^{10–13}

In 1988 and 1989, the first isolate of SHV-ESBL was found in clinical samples from Argentina and Chile, respectively.¹⁴ Since then, different types of enzymes have been detected in South America with different predominating enzymes, namely, TEM and SHV, and CTX-M, the latter currently being the most widespread ESBL group in the region.⁷

Apart from human detections, beta-lactamases have been found in non-human specimens, animals, and the environment. The presence of ESBL genes in aquatic ecosystems has been studied in *E. coli* in different parts of the world, for example, in Mur River in Europe (ie, Austria)¹⁵ and in Yamato River in Asia (Japan)¹⁶ with *bla*_{CTX-M-1} and *bla*_{CTX-M-14} as the most prevalent ESBL genes, respectively.

Livestock and other animals used as food sources are a well-known reservoir of antibiotic-resistant microorganisms, despite the lack of literature exploring this topic. For example, few studies have explored veterinary sources of ESBLs, in stark contrast with the amount of data from humans.¹⁷ In 1988, ESBLs were detected for the first time in a dog in Japan with a strain of CTX-M-3-producing *E. coli*. ESBL types SHV-1, TEM-1, and OXA have been frequently described in *E. coli* and *Salmonella* spp. of animals and food of animal origin in Spain, Germany, the US, and the United Kingdom (UK).¹⁷

In South America, as in the rest of the world, human clinical studies of ESBLs in *E. coli* are abundant.¹⁸ Conversely, the current status of beta-lactam resistance in non-clinical scenarios such as their presence in healthy carriers, food matrices, animals, and the environment is scarce.¹⁹ Nevertheless, evidence suggests that limited access to public health services and lack of hygiene can contribute to the spread of ESBLs within communities.^{13,20} Moreover, the use of antibiotics as growth promoters in livestock animals favors the dissemination of different types of beta-lactamases CTX-M in food matrices.^{2,21} Finally, the poor management of hospital wastewater may result in discharge of multidrug-resistant coliforms (such as *E. coli* ESBL+) into natural waterbodies.²² All this evidence demonstrates the importance of conducting studies using a One Health approach, namely, understanding the dynamics surrounding human and animal health, and the environment, to develop strategies to monitor and control beta-lactam resistance.²³

Due to the aforementioned arguments, in this study we have aimed to develop a systematic review of the current status of ESBLs in one of the most relevant and ubiquitous bacteria, *E. coli*, in South America, considering studies performed in the human, animal, and environmental realms to present a comprehensive summary offering updated information for practitioners across different fields.

Methods

Protocol and Search Strategy

This systematic review was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.²⁴ The scientific literature was obtained from the NCBI-PubMed database on April 5th, 2021, including studies in English, Spanish, and Portuguese published since 1990 until 2021. Search terms included “*Escherichia coli*” AND “ESBL” OR “beta-lactamase” OR “β-lactamase”, plus the names of countries/territories that belong the South American region: ie, “Ecuador” OR “Peru” OR “Brazil” OR “Argentina” OR “Chile” OR “Colombia” OR “Venezuela” OR “Uruguay” OR “Paraguay” OR “Bolivia” OR “Suriname” OR “Guyana” OR “French Guiana”. All terms included the PubMed Title/Abstract criterion so that only studies that contained the searched keywords in their title and/or in their abstract were considered.

Additional articles found manually in Scopus, SciELO, and latindex databases were also included in this review. These articles were not found in the initial search because their title and/or abstract not included the search terms

mentioned above; however, they had other keywords such as “CTX-M”, “resistomes”, “multidrug-resistant” or “multi-resistant”. These studies presented relevant epidemiological information related to CTX-M beta-lactamases.

Study Selection

The selection of the studies was carried out by two separate reviewers (VV and AM) using the Rayyan QCRI bibliographic manager to review only titles and abstracts of the selected articles. The first phase consisted in the removal of duplicated studies and the inclusion of those related to *E. coli* and South America while excluding reviews/letters and studies not focused on ESBLs.

After the first round of selection, a detailed review of the selected articles was implemented. During this eligibility phase, only those studies conducted in humans, animals, and/or the environment with complete information (ie, sample origin and ESBL positive cases detected either by phenotypic or molecular tests) were included for the final analysis. At this stage, exclusion criteria allowed the rejection of [1] case reports, [2] studies in Enterobacteriaceae and other bacterial families that did not report data on ESBLs in *E. coli*, [3] articles unavailable in full-text, and [4] studies conducted in regions different from South America.

Data Extraction

Studies selected were tabulated and introduced in a Microsoft Excel 2016 spreadsheet with their general information (ie, author, year, country, and URL). Data to be evaluated included (i) detection methods, (ii) type and origin of samples, (iii) prevalence of *E. coli*, (iv) prevalence of *E. coli* with ESBL phenotype, (v) prevalence of *E. coli* with ESBL genotype, (vi) ESBL types, and (vii) identification of clones by multi-locus sequence typing (MLST) of clinical importance. The three-prevalence parameters (points iii, iv, and v) were independently reported for each South American country; these values were obtained by dividing the number of samples found in each category (*E. coli*, *E. coli* with ESBL phenotype, and *E. coli* with ESBL genotype) with the total sample size (n). Relevant data are presented in statistical graphs.

Data Analysis

Descriptive statistics were obtained for all the studied parameters (eg, sample origin and source, *E. coli* prevalence, etc.) and are shown with 95% confidence intervals when appropriate. ESBL types and CTX-M variants were further categorized using descriptive statistics and their proportions were depicted per country in maps of the region using barplots and pie charts according to each of the established categories, that is, human clinical cases, human healthy carriers, animal, food, and environmental studies using R programming language version 3.6.3 and QGIS 2.18 “Las Palmas”.

Results

Studies Included

The total number of articles found in PubMed from 1990 to April 2021 was 500; additionally, six articles were also included from SciELO and Latindex databases for a total of 506 articles. During the first screening phase, 321 studies were excluded because they were either reviews/letters or articles that deviate from the theme of this review. During the eligibility phase, 55 studies were discarded due to their lack of detail. A total of 130 articles were included in this review (Figure 1).

ESBLs Detection Methods for *E. coli*

From the 130 articles included, 25 used phenotypic tests, the three commonest being the disc diffusion, the minimum inhibitory concentration (MIC), and the VITEK system; the latter also used for bacterial identification. On the other hand, 14 articles used molecular tests including PCR (ie, either endpoint, multiplex, or quantitative) and sequencing to determine the presence of beta-lactamase coding genes. Ninety-one publications (70%) used both phenotypic and molecular tests for gene and bacteria identification.

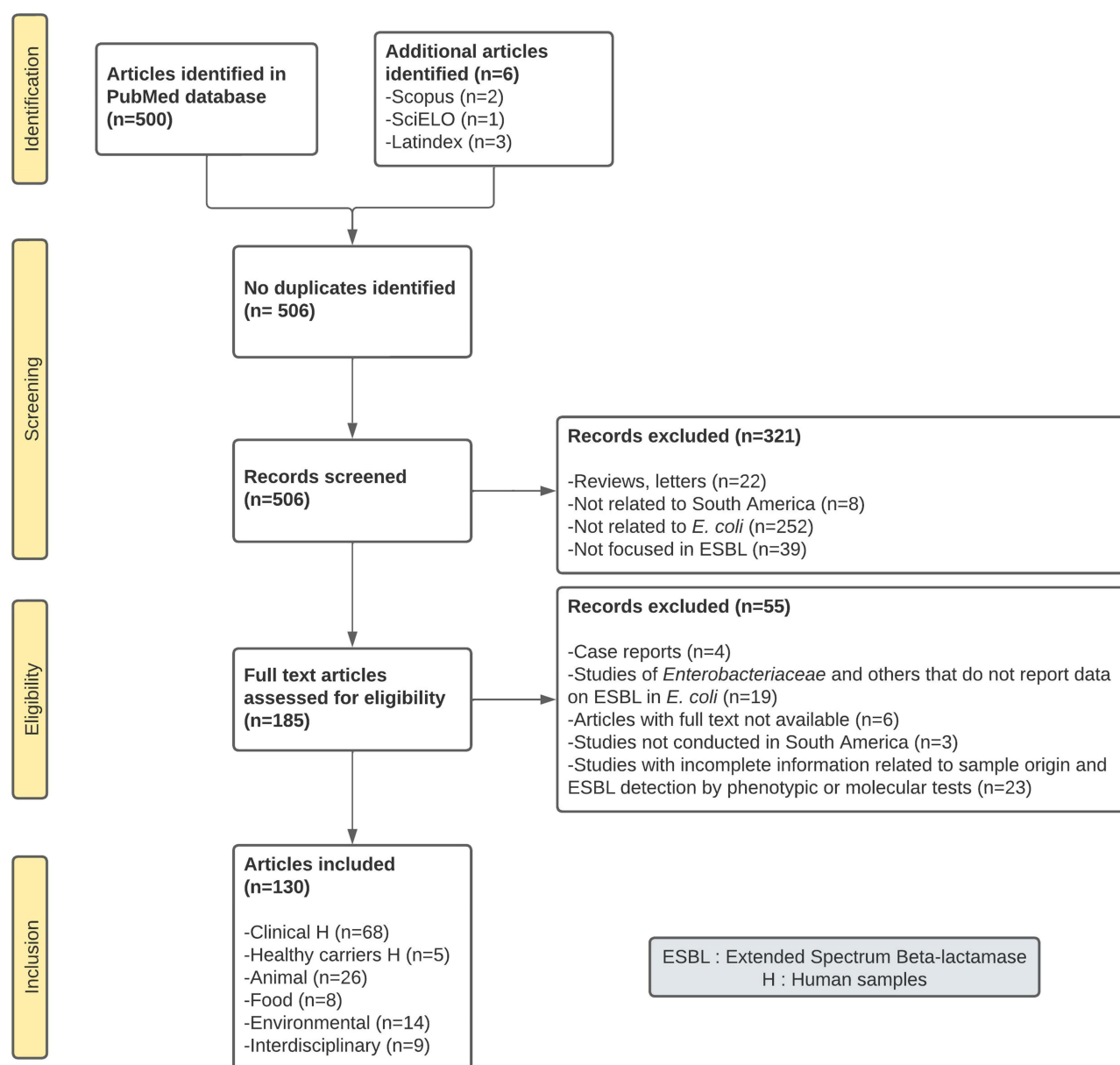


Figure 1 PRISMA flow diagram for study categorization and selection of the 130 studies included in this systematic review. Data came from PubMed and additional databases between 1990–2021.

Notes: Adapted from Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev*. 2021;10:89.25 Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/legalcode>).

Abbreviation: ESBL, extended spectrum beta-lactamases.

Classification of Studies

The studies were classified into six categories according to the origin of the sample: two categories for human studies (clinical cases and healthy carriers), followed by animal, food, environmental, and interdisciplinary studies, here labeled as those analyzing more than one category at the same time. More than half of the studies corresponded to human samples (56%; $n=73/130$). From them, 52% ($n=68/130$) corresponded to isolations from human clinical studies and 4% ($n=5/130$) corresponded to studies on human healthy carriers, the smallest category on our analysis. Samples isolated from animals and the environment corresponded to 20% ($n=26/130$) and 11% ($n=14/130$), respectively. Interdisciplinary studies corresponded to 7% ($n=9/130$). Samples isolated from food corresponded to the 6% of the publications studied ($n=8/130$).

In addition, animal samples were sub-classified into birds, companion animals, livestock, and wild animals (Figure 2). Birds were investigated in more than half of animal-oriented studies (51%; $n=18/35$), followed by domestic animals (23%; $n=8/35$), farm animals (20%; $n=7/35$), and wild animals (6%; $n=2/35$; Figure 2A). A subcategorization of birds showed that poultry was the most studied with 61% ($n=11/18$) of publications, followed by urban species such as doves and pigeons with 28% ($n=5/18$), and migratory bird data in 11% of studies ($n=2/18$; Figure 2B). A subcategorization of livestock showed that cattle and pigs represented 46% ($n=5/11$) and 36% ($n=4/11$) of the, respectively, farm animals studied (Figure 2C). It is important to mention that in some publications, more than one type of farm animal was studied.

ESBL Producing *E. coli* Studies per Country

Fifty percent of the studies ($n=65/130$) was carried out in Brazil, followed by Argentina, and Ecuador with 11.5% ($n=15/130$) each. As it can be seen, Brazil exceeds with 50 studies to all other countries (Figure 3). It is worth noting that a fraction of the studies identified ($n=7/130$) were developed in more than one country; thus, at least 5.4% of the studies occurred as international multicenter approaches.

As expected, a further categorization of sample type per country showed the predominance of samples from human clinical origin (Figure 3). For example, in Brazil, 46.2% ($n=30/65$) of the included studies corresponded to clinical isolations; moreover, in Venezuela, 100% of their research ($n=12/12$) were focused on nosocomial samples. Brazil, despite having the highest number of studies in the region, lacked studies on human healthy carriers. This pattern was similar across the rest of the countries analyzed, namely, predominance of human clinical samples followed by either one or two studies including any of the other studied categories (ie, healthy carriers, animal, or environmental sample types). Exceptions included French Guiana with a unique study focused on human healthy carriers, and Ecuador ($n=15$ studies) with at least one publication across each sample type category (Figure 3).

Prevalence and Distribution of ESBLs Producing *E. coli*

In South America, the prevalence of ESBLs at the level of the animal, food, and environment was larger than the prevalence from human sources (ie, either clinical cases or healthy carriers) (Table 1). The same pattern was evident in each country analyzed. Brazil presented the greatest number of samples of each category except for the healthy carriers, where data were absent. Details of these results per country are described in Supplementary Table 1.

ESBL Types and Enzymes Variants

Human Clinical Studies

The total number of ESBLs genes in clinical samples was 3509. The South American distribution of ESBLs based on the selected studies shows that Brazil was the country with the highest number of detections with 21.3% ($n=788/3701$). Also, Brazil presented the highest diversity of ESBL types reporting CTX-M, TEM, SHV, TOHO, OXA, and AmpC enzymes.

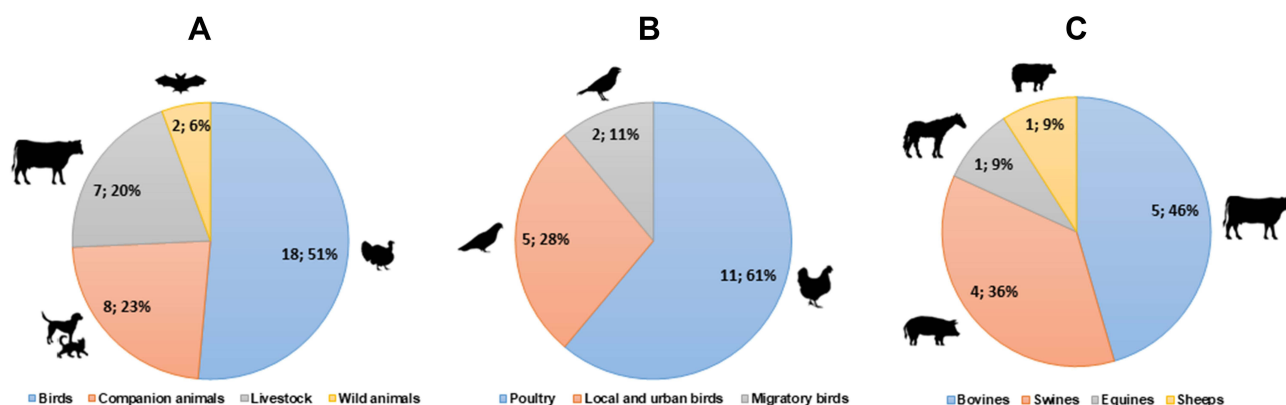


Figure 2 Classification and sub-classification of ESBLs producing *E. coli* studies developed in animal samples. (A) General classification of animals identified and their distribution in number of studies and percentages (n,%). (B) Animal studies in birds: sub-groups identified and their distribution in number of studies and percentages. (C) Animal studies in livestock: sub-groups identified and their distribution in number of studies and percentage.

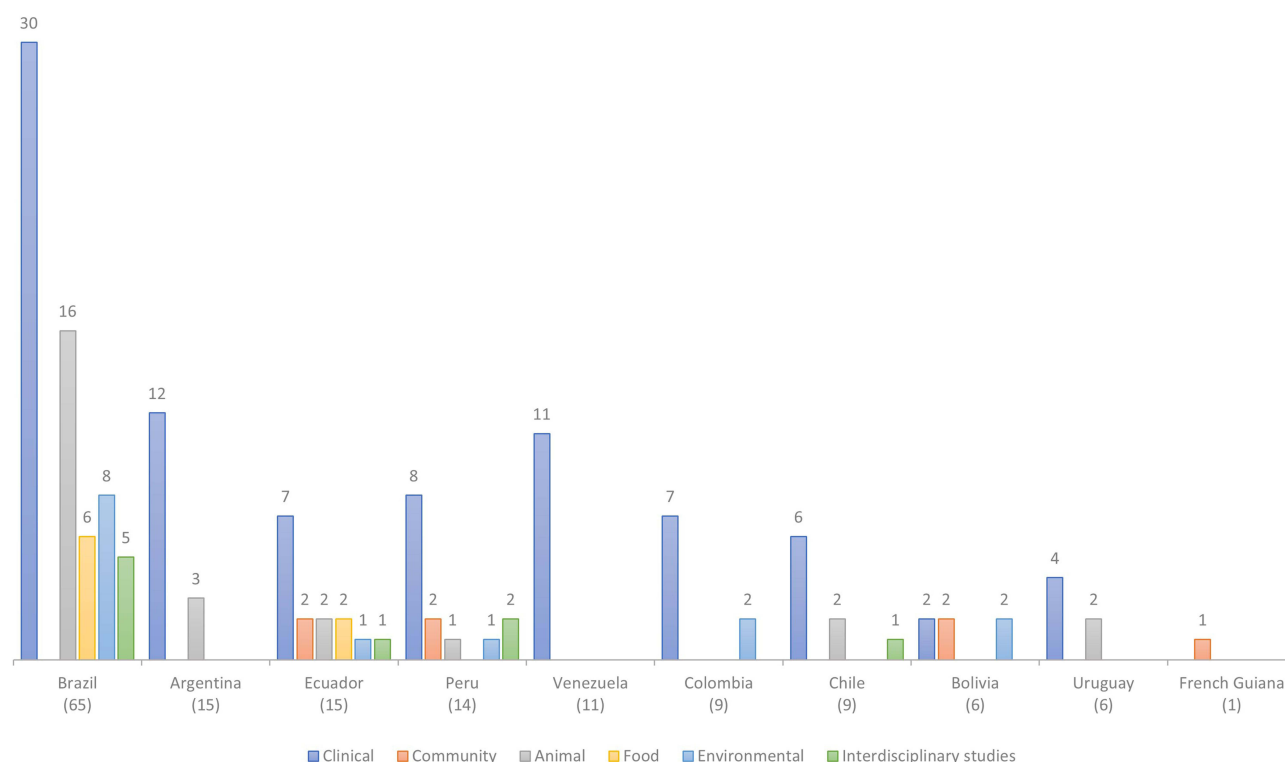


Figure 3 Categorization of 130 studies included in this systematic review from PubMed and additional databases between 1990–2021. Color bars represent the number of studies conducted in South American countries and number of studies per country categorized by sample type examined.

Surprisingly, PER-2 enzymes were only found in Uruguay. CTX-M enzymes were the most prevalent in South America with at least 89.4% (n=3309/3701) identifications. CTX-M enzymes variants recognized were usually reported as unspecified across countries/territories with few exceptional publications. For instance, multiple Bolivian studies reported the presence of CTX-M-1 (50.8%; n=67/132). Similarly, studies from Uruguay consistently reported the presence of CTX-M-15 (59.5%, n=47/79). These results are shown in more detail in [Figure 4](#) and [Supplementary Table 2](#).

Human Healthy Carriers Studies

Epidemiological data focused on isolations from healthy carriers in South America were limited. Only Ecuador, Peru, Bolivia, and French Guiana reported ESBLs from this category; thus, the total number of ESBLs in this context was 204. The enzymes CTX-M (96%; n=195/204) and TEM (4%; n=9/204) were the only ESBLs found. The CTX-M-2 enzyme variants were most prevalent in Peru (47.7%; n=31/65) and Bolivia (43.7%; n=21/48). In French Guiana, the enzyme CTX-M-1 was the most significant variant with a prevalence of 46.1% (n=6/13). Conversely, only CTX-M-55 enzyme

Table 1 Prevalence of *E. coli* Isolates According to Their Sample Source. Comparison of Prevalence of ESBL Determination by Phenotypic and Genotypic Methods Across Six Categories in 130 Studies from South America Between 1990 and 2021 (N=203057)

Categories of Sample	Sample Size (N)	<i>E. coli</i> Isolates			Phenotype of ESBL-Producing <i>E. coli</i>			Genotype of ESBL-Producing <i>E. coli</i>		
		(n)	%	[95% CI]	(n)	(%)	[95% CI]	(n)	(%)	[95% CI]
Clinical (H)	185,203	28,348	15.3	[0.151–0.155]	3719	13.1	[0.12–0.14]	3509	1.9	[0.018–0.02]
Healthy Carriers (H)	7861	1105	14.1	[0.133–0.149]	490	6.2	[0.057–0.067]	139	1.8	[0.02–0.2]
Animal	6616	3607	54.5	[0.533–0.557]	1418	21.4	[0.204–0.224]	1195	18.1	[0.1–0.2]
Food	1860	1401	75.3	[0.733–0.773]	193	10.4	[0.09–0.118]	580	31.2	[0.3–0.333]
Environment	1517	553	36.5	[0.341–0.389]	191	12.6	[0.109–0.143]	187	12.3	[0.106–0.14]

Abbreviations: N, total samples of each category; H, human samples; 95% CI, 95% confidence Intervals.

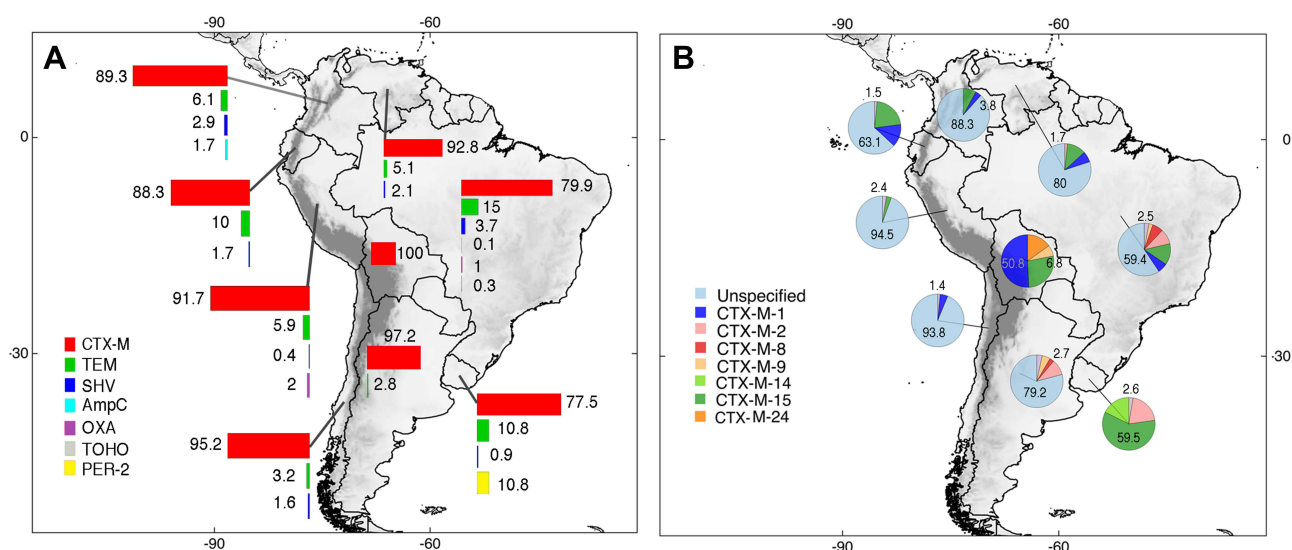


Figure 4 Distribution of ESBLs types (A) and CTX-M enzyme variants (B) in clinical human studies developed in South America. Unspecified = CTX-M enzyme present but variant unreported. Pie charts are showing the maximum and minimum percentages for each country. Complete information can be found in the main text and [Supplementary Table 2](#).

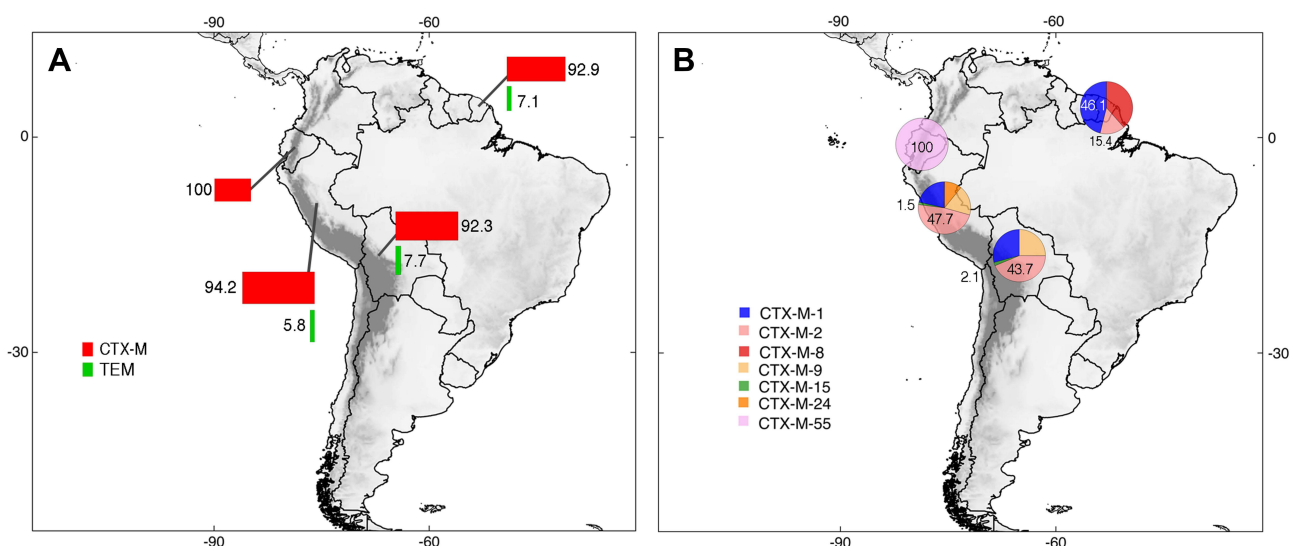


Figure 5 Distribution of ESBLs types (A) and CTX-M enzyme variants (B) identified in the context of healthy carriers human studies developed in South America. Pie charts are showing the maximum and minimum percentages for each country; complete information can be found in the main text and [Supplementary Table 2](#).

variants were reported in Ecuador ($n=69/69$). These results are depicted in more detail in [Figure 5](#) and [Supplementary Table 2](#).

Animal Studies

Six South American countries reported epidemiologic data in animals. The total number of ESBLs in animal samples was 1191. ESBL types identified included CTX-M, TEM, SHV, PER-2, and AmpC. CTX-M enzymes were the most prevalent (64.5%; $n=768/1191$). CTX-M enzyme variants featured included CTX-M-1 in Ecuador (28.4%; $n=50/176$) and Chile (63.3%; $n=124/196$); CTX-M-2 in Brazil (40.2%; $n=127/316$); and CTX-M-8 in Brazil (23.4%; $n=74/316$), Argentina (56.7%; $n=17/30$), and Uruguay (56.4%; $n=22/39$). Many CTX-M variants were recorded in Ecuador although the higher proportion (56.2%, $n=99/176$) of cases was represented by unknown variants. In Peru, only CTX-M-15 enzymes were reported ($n=11/11$). These results can be observed in more detail in [Figure 6](#) and [Supplementary Table 2](#).

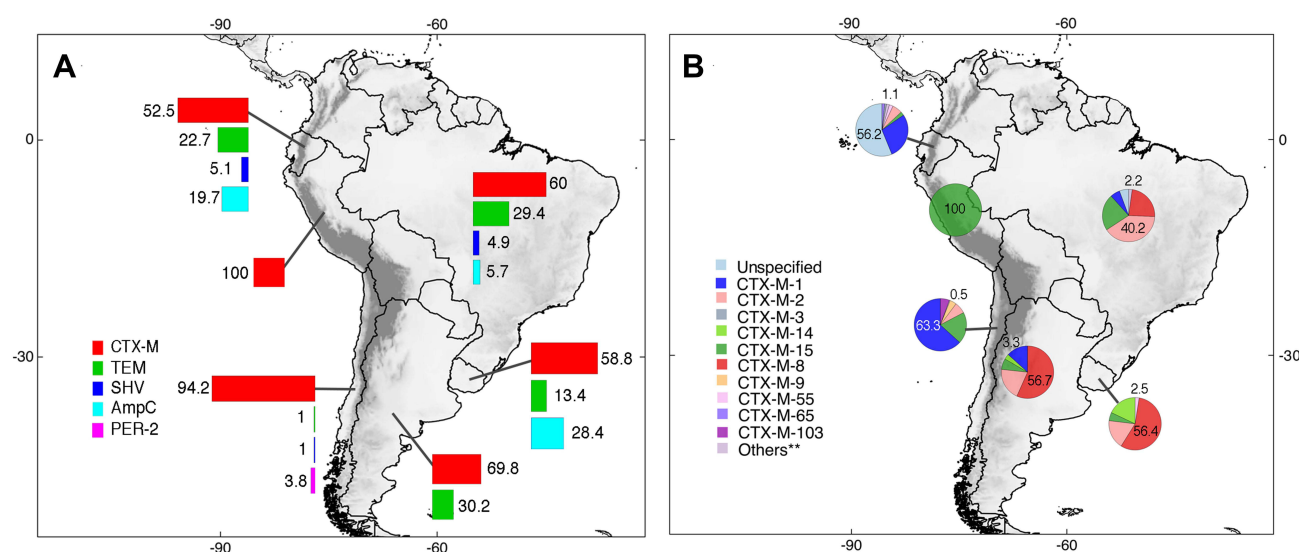


Figure 6 Distribution of ESBLs types (A) and CTX-M enzyme variants (B) identified in animals and developed in South America. Unspecified = CTX-M enzyme present but variant unreported. **Others = Variants of CTX-M enzymes with <1% of prevalence. Pie charts are showing the maximum and minimum percentages for each country; complete information can be found in the main text and [Supplementary Table 2](#).

Food Studies

Only two countries (Ecuador and Brazil) published studies about ESBL detection in food. The total number of ESBLs in food samples was 520, among them, CTX-M, TEM, SHV, and AmpC were identified. CTX-M enzymes were the most prevalent (73.6%; n=383/520). Considering CTX-M variants, CTX-M-1 in Ecuador with 66.1% (n=109/165) and CTX-M-2 in Brazil with 63.3% (n=138/218) were found. These results can be observed in detail through [Figure 7](#) and [Supplementary Table 2](#).

Environmental Studies

Environmental studies were found in five South American countries. The total number of ESBLs in environmental samples was 276. ESBL types identified included CTX-M, TEM, SHV, TOHO, OXA, and AmpC. Similar as it was

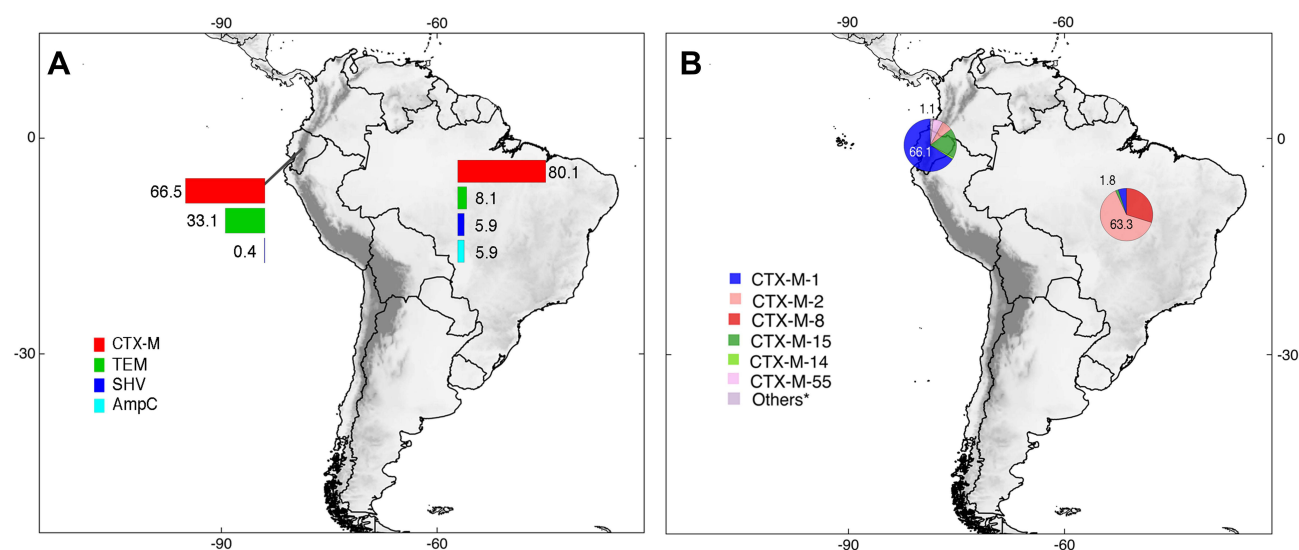


Figure 7 Distribution of ESBLs types (A) and CTX-M enzyme variants (B) identified in food and developed in South America. *Others = Variants of CTX-M enzymes with <1% of prevalence. Pie charts are showing the maximum and minimum percentages for each country; complete information can be found in the main text and in [Supplementary Table 2](#).

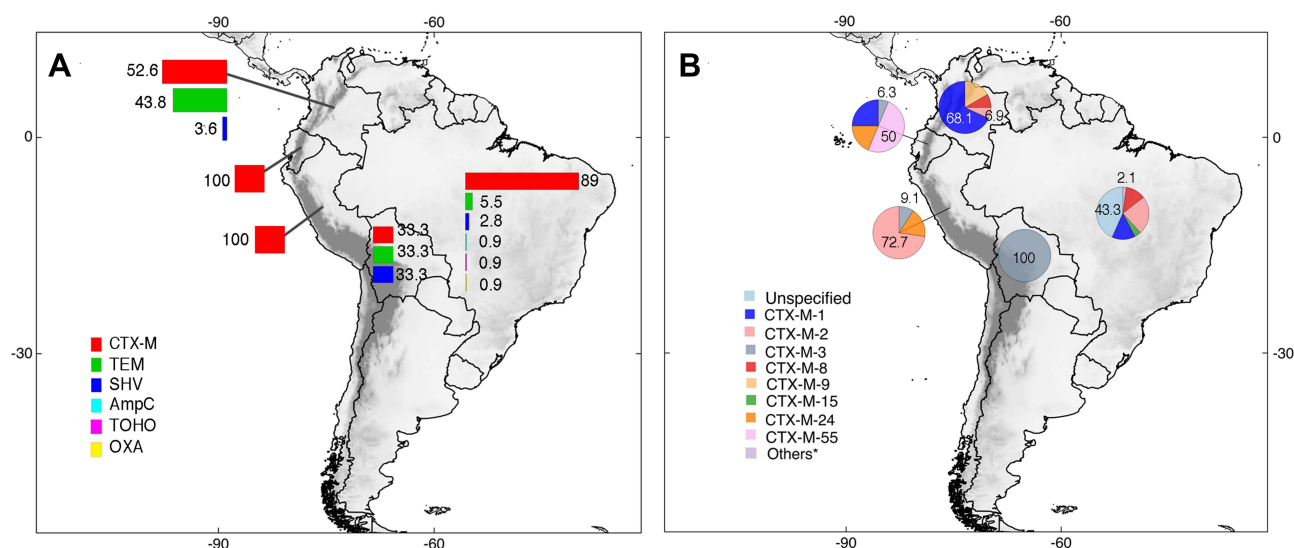


Figure 8 Distribution of ESBLs types (A) and CTX-M enzyme variants (B) identified in the environment and developed in South America. Unspecified = CTX-M enzyme present but variant unreported. *Others = Variants of CTX-M enzymes with <1% of prevalence. Pie charts are showing the maximum and minimum percentages for each country; complete information can be found in the main text and [Supplementary Table 2](#).

obtained in results from the other categories (ie, human clinical cases, human healthy carriers, animals, and food studies), CTX-M enzymes were the most prevalent (71.4%; n=197/276). Among CTX-M variants, CTX-M-1 in Colombia (68.1%; n=49/72), CTX-M-2 in Peru (72.7%; n=8/11), and CTX-M-55 in Ecuador (50%; n=8/16) were reported. In Brazil, CTX-M variants were unknown in most cases, representing 43.3% (n=42/97) of all detections. In Bolivia, CTX-M-3 was the variant detected in their unique report (n=1/1). These results can be traced in detail in [Figure 8](#) and [Supplementary Table 2](#).

Clones of Epidemiological Importance

A total of 59 different clones were described in 31 studies. The clone *E. coli* ST131 represented 48.4% of the total of detected clones in the studies (n=15/31), followed by ST10 with 29% (n=9/31). The clones ST405, ST648, and ST38 accounted for 13% (n=4/31) each; ST410 and ST744 for 10% (n=3/31) each; followed by clones ST90, ST117, and ST155 representing the 6.4% (n=2/31). All the remaining clones were represented by a single detection across the reporting studies (3%; n=1/31 each; [Supplementary Table 3](#)). It is worth to mention that the presence of these clones was conditioned to their detection per country; thus, Brazil was the country that reported more clones (55%; n=38/59) followed by Peru (17.4%; n=12/59), Ecuador, Uruguay (11.6%; n=8/59 each), Chile (5.8%; n=4/59), and single reports of clones from Colombia, Argentina, and French Guiana (1.4%; n=1/59 each).

Discussion

The present systematic review of the literature provides relevant information on the distributions of ESBLs in *E. coli* in South America. To our knowledge, this is the first attempt that addresses the presence of ESBLs across different categories, going beyond human-derived clinical samples, to also consider samples from human healthy carriers, animals, environmental, and food isolations, echoing calls of multidisciplinary, One Health approaches, to comprehend the presence of antibiotic resistance mechanisms.

Of the 130 studies included in this review, Brazil contributed with more than half the research of this topic (n=65/130; [Figure 3](#)). These results correspond with evidence showing different levels of scientific production in South America where Brazil is recognized as a regional leader.^{25–28} The other South American countries had at least one publication on ESBLs producing *E. coli*,²⁹ which is far from ideal and should prompt efforts to understand beta-lactam resistance and their importance in public health.^{2,3} Despite the low scientific production of Ecuador compared to the rest of South

American countries,^{27,28} Ecuador was the country with the second highest research contribution in the region in this review (n=15/130), together with Argentina (Figure 3).

Considering the limitations of many South American laboratories (eg, logistics, equipment, infrastructure, others),^{30,31} we expected that the inclusion of molecular tests that require higher costs and increased technical expertise³² would have been lower compared to phenotypic tests. Consequently, in this review, phenotypic tests (n=25/130) were implemented in 11 more publications than molecular tests (n=14/130); however, most of the publications used both phenotypic and molecular tests (70%; n=91/130) for identification, which allows a more precise detection and therefore improvement of ESBLs epidemiological surveillance in *E. coli*.³³ Some human clinical or environmental studies used molecular methods directly for ESBLs genes' detection. Ideally, both detection methods should be implemented since neither is exempt from limitations. First, methods such as PCR are less effective in the presence of unknown mutations of new unreported ESBL variants, especially when these mutations appear at primer hybridization sites.³³ Second, methods based on disk diffusion can report problems with interpretation when co-resistance events occur.³⁴ Thus, new techniques for identifying ESBLs are being developed and have proven to be more sensitive, specific, efficient, and even provide other advantages such as point-of-care detections.³⁵ One of the more recent options is the CRISPR-Cas9-based detection method with optical DNA mapping, which was used to identify *bla*_{CTX-M-15} and *bla*_{CTX-M-14} genes in *E. coli* from clinical urinary tract infections in Sweden.³⁶

Up to 52% (n=68/130) of studies corresponded to human clinical samples, which depicts the lack of research about antimicrobial resistance from non-human oriented sources.¹⁹ For example, for this review, Venezuela contributed with publications only within this category (Figure 3). Reviews such as that of Guzmán et al³⁷ expose the clinical situation in Venezuela but lack an analysis of antimicrobial resistance from animals, food products, or the environment.

Among the categories established in our review, ESBL detections from human healthy carriers, at the community level, were mostly underrepresented (n=5/130; Figure 3). Onduru et al evidenced a similar pattern in a review for African countries³⁸ where human clinical studies represented the 74%, while studies performed on healthy carriers contributed only with the 15%. It is known that healthy carriers are an important reservoir for the transmission of beta-lactamases and therefore act as spreaders to healthy individuals or environmental settings. Further studies including surveillance at these scales might unveil a hidden pattern for the epidemiology of bacterial resistance in human populations.^{39,40}

For this review, One Health studies were categorized as interdisciplinary, considering that they analyzed samples across different interfaces: human-animal,^{41–43} human-environment,^{44,45} human-food,⁴⁶ animal-environment,⁴⁷ human-animal-food,⁴⁸ and animal-food-environment.⁴⁹ The number of interdisciplinary studies included in this review was low (7%; n=9/130; Figure 3). Similarly, O'Neal et al review for Central America⁵⁰ and Escher et al review for Africa⁵¹ found small numbers of One Health-related studies. Thus, apart from South America, other world regions also struggle to incorporate One Health approaches to their experimental designs; a reality that might be tackled with international cooperation, executing multi-sectorial action plans as proposed by the WHO.⁵² Many of the clinical studies included in the present review were “international multicenter studies”, which are characterized by promoting joint research across several countries, albeit these alliances are usually focused on human clinical samples.⁵³ These types of studies might be a good example to follow to include a cooperative approach to address questions in the ecology and veterinary fields.

Considering animal publications, those for human consumption were more studied than other groups. Of these, 61% came from poultry in birds and 82% from bovines and swine in livestock. This can be explained due to awareness of the impact of antibiotic use as prophylactic treatment or most commonly as growth promoters for fattening. There is extensive evidence showing how this practice promotes the spread and therefore the risk of zoonotic antibiotic resistance mechanism contaminations through the food chain.^{54–56} Nevertheless, for this review, urban and migratory birds contributed with the 28% and 11% of ESBLs in *E. coli*, respectively. Recently, studies of migratory birds have incriminated their feces in the environment as possible contributors to the international spread of antimicrobial resistance.^{57–59} Studies focusing on this animal group should be encouraged to assess the validity of this hypothesis.

Veterinary publications have noticed a zoonotic transmission risk from pet animals, favored by their proximity to their owners.^{60–62} A similar trend can be stressed for the potential spillover of ESBLs; however, studies from pet animals were also underrepresented in our systematic review with only a 23%. Studies focused on wild animals were even less represented in our review (6%; n=2/35; Figure 2A). Wildlife might be an important source for the spread of resistance mechanisms as they act as bridges between the urban and sylvatic environments, especially mammals.⁶³ Another

example is that, in Brazil, fishes have been found to contribute to the spread of ESBLs in natural waterbodies and its marine fauna.^{64,65}

In South America, higher values of ESBL producing *E. coli* prevalence were obtained from animal, food, and environmental sources compared to human samples (Table 1). In our review, one of these results showed a prevalence of *E. coli* with ESBL genotype of 18.1% in animals and 12.3% in the environment compared to 1.9% in clinical studies. These results were analogous among the majority of South American countries and similar to observations in Tanzania, the Netherlands, and other regions of the world where ESBLs from animal and environmental sources showed between a 10 to 20% higher prevalence than humans.^{66–68} Therefore, although apparently many of the *E. coli* resistance mechanisms are acquired in the clinical setting, prevalence in animals and environmental sources predominate and should be further studied.

CTX-M enzymes were the most prevalent, with more than 50% detections in each country and category analyzed in this review (ie, human clinical samples, human healthy carriers, animal, food, and environment; Figures 4–8). Similar results have been reported in the rest of the world. For example, in Africa CTX-M prevalence reaches an 81.5%.³⁸ In Iran, CTX-M enzyme prevalence reached 31.2% followed by TEM with 27.6%.⁶⁹ It is worth to mention that the prevalence analyzed here only accounted for *E. coli*, thus it might be an underestimation if including other bacterial species such as *K. pneumoniae*, which usually harbor TEM or SHV enzymes.^{70–72}

Apart from the best-known enzymes (ie, TEM, SHV, and CTX-M), TOHO, reported for the first time in Japan in 1993,⁷³ was also found in South America among human clinical and environmental studies (Figures 4A and 8A). Thus, in little less than three decades, TOHO enzymes have spread to a completely different region albeit its low prevalence (<0.1%; n=1/3701). Other types of beta-lactamases that are worth highlighting are the OXA (<0.1%; n=17/3701) and AmpC (<0.1%; n=9/3701) enzymes. Both show a different resistance spectrum to the most common resistant enzymes. OXA enzymes can hydrolyze more effectively antibiotics such as carbapenems,⁷⁴ while AmpC is not inhibited by clavulanic acid.⁷⁵ The ESBL enzymes PER-2, with similar spectrum to TEM and SHV enzymes,⁷⁶ were only found in Uruguay with a low prevalence (Figure 4A). However, Celenza et al⁷⁶ have reported PER-2 isolated from Enterobacteriaceae in Bolivian hospitals, which suggests the existence of PER-2-carrying *E. coli* in this country. Although detected, CTX-M enzyme variants were poorly reported (Figure 4B). Detection of variants is one of the most important clinical data because each one has differences in their antibiotic response.^{77–79} It is worth noting that in other regions of the world such as Nigeria, Tunisia, and the Netherlands, CTX-M-15 variant isolated from clinical settings is usually detected with a prevalence ranging from 67.9% to 83.3%.^{80–82}

Considering animal studies, the presence of CTX-M-8 enzyme variant was frequently described in countries such as Brazil, Argentina, and Uruguay (Figure 6B). Because CTX-M-8 was first isolated from Enterobacteriaceae in Brazil,⁸³ the most likely scenario involves its spread across animals from neighboring countries, despite the lack of reports of CTX-M-8 in Chile, Peru, and Ecuador according to our review. For these countries, the variants CTX-M-1 and CTX-M-15 were detected, as has been seen in goat samples from Tunisia, pigs from Portugal, horses from the UK, and processed beef from Germany.^{84–87}

From food studies, the main types of CTX-M enzymes detected included the CTX-M-1 in Ecuador and the CTX-M-2 in Brazil (Figure 7B); results were consistent with reports from Germany^{88,89} and Algeria⁹⁰ where the CTX-M-1 variant is the most prevalent. However, as only few studies address the presence of antibiotic resistant enzymes in food-related sources, more research is granted to confirm their role as a mechanism of spread with public health consequences.

The enzymes of type TOHO, OXA, and AmpC from environmental studies were found only in Brazil (Figure 8A). These types of ESBLs had an overall low prevalence. For CTX-M enzyme variants from environmental sources, there was a great variability of detection across South American countries, namely, the enzyme CTX-M-1 in Colombia, CTX-M-3 enzyme in Ecuador/Bolivia, and the CTX-M-2 enzyme in Peru/Brazil (Figure 8B). Lines of research that go beyond the simple detection of ESBLs in the environment should be encouraged as has been done in other regions of the world. For example, in Nigeria, Lebanon, Vietnam, and India, there are studies assessing the impact of antibiotic release on hospital wastewater correlated with patterns on resistance acquisition in *E. coli*.^{91–94} Research from the UK, Tanzania, and the Dominican Republic^{95–97} have revealed the importance of IncF plasmids found in natural waterbodies. Being a conjugative plasmid, IncF is related to the dissemination of *bla*_{CTX-M-15} by horizontal gene transfer.^{98,99} Finally, other

exemplary studies show how activated sludge from wastewater treatment plants can be a source of high prevalence of CTX-M as demonstrated in Japan,¹⁰⁰ Austria,¹⁰¹ and India.¹⁰²

The *E. coli* clones ST131 and ST10 were found across South America (Supplementary Table 3). These results are consistent with a study conducted in Canada in which 96/209 (46%) *E. coli* strains corresponded to the clonal complex ST131¹⁰³ and with one developed in the Netherlands where from 112 strains belonging to *E. coli*, 21% belonged to ST131 and 17% to ST10,⁸² demonstrating the worldwide high prevalence of the ST131 clone. These clones are known to be a major reservoir of plasmids carrying resistant genes to multiple antibiotics, including *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}.¹⁰⁴ The influence of international travel on the dissemination of these clones has been studied in countries such as Germany, where the ST131 clone ranges between 19% and 30%, or the Netherlands, where the ST131 clone prevalence reaches a 21.4%.^{82,105} For the purposes of this argument, it is worth to mention that Woerther et al¹⁰⁶ reported the international clone ST10 in healthy carriers from a remote community at French Guiana, which begs the question on how this clone was acquired within an isolated population.

Limitations

Our results are based on a systematic review of published academic literature. Potentially, there is relevant information among the grey literature (eg, thesis) that might complement the results presented here; however, we rely on the quality of peer-review publications to assess the status of *E. coli* ESBLs in South America with certainty. Similarly, our analysis excluded articles in the category of “reviews” that sometimes present pieces of original research, but we believe that their overall contribution in the presented information may be negligible. Furthermore, while we comprehensively reviewed each of the 130 manuscripts included in this study, data heterogeneity across different countries and publications is a challenge that was overcome with some heuristic categorizations, which are the basis of any systematic review.

Finally, it is worth noticing that although manuscripts included in the present review were considered of high quality by the individual assessment of their results, we are aware that potentially some of them might be published in journals considered predatory. We believe that science should be judged by their findings instead of the journal in which it was published, and for this review we can trust in the reliability of the studies analyzed.

Conclusions

ESBLs producing *E. coli* in South America are widely distributed and show a high diversity of enzyme variants. ESBLs in human samples are the most studied, mainly in those linked to hospital environments (inpatient and outpatient). However, ESBLs in food and animal samples are the most prevalent. Countries such as Brazil present more studies on ESBL surveillance involving various sample sources. CTX-M enzymes are the most common and diverse of the types of beta-lactamases found and show a high prevalence across all the studied categories. ST131 and ST10 are the most widespread clones in the studies included in this review.

In order to fully characterize the situation of *E. coli* ESBLs in South America, a greater contribution from under-represented countries of the region should be encouraged. These contributions ideally should emphasize the role of sources different from human clinical settings such as animals, environmental, and food matrices, together with detections from human healthy carriers. Concerns related to transmission of resistant mechanisms among human healthy carriers, zoonotic sources, as well as the spread of ESBLs in aquatic ecosystems, reveal the importance of developing studies beyond the human clinical-centered view of health. Furthermore, the importance of animal and environmental health should be explored since ESBL genes in both realms have been detected in South America and the rest of the world.

Efforts to increase the epidemiological surveillance of the region to detect predominant types of ESBLs and the presence of new variants, as well as the distribution of ST clones, should be a priority considering their different roles in antibiotic resistance and their traceability to detect infection sources. At this point, the inclusion of both phenotypic and molecular tests, as well as the development of new detection techniques, should facilitate surveillance efforts to further the understanding of ESBL distribution in South America.

Finally, the information presented in this review can be used as basis for subsequent implementation of monitoring programs, targeting potential critical points of transmission sources. Following the One Health concept, the development

of contingency plans in different areas like hospitals, broiler breeding sites, and wastewater treatment plants might contribute to the identification of resistant enzymes and their spreading, which ideally should be controlled if not completely halted; within this objective, a multi-sectoral and multi-disciplinary cooperation will be of utmost importance.

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Supplementary Materials

To a better description of findings of this work, the following supporting information can be downloaded. [Supplementary Table 1](#): Number of samples identified (frequencies) and % prevalence of *E. coli*, *E. coli* with ESBL phenotype, and *E. coli* with ESBL genotype in South America from human clinical samples, human healthy carriers, animal, food, and environmental studies. Data from 130 studies included in systematic review between 1990 and 2021. [Supplementary Table 2](#): Percentages of different beta-lactamase enzymes identified across South American countries and CTX-M variants for human clinical samples, human healthy carriers, animal, food, and environmental studies. Data from 130 studies included in systematic review between 1990 and 2021. [Supplementary Table 3](#): *E. coli* ST clones found in studies in South America per country and author. Data from 130 studies included in systematic review between 1990 and 2021. [Supplementary Table 4](#): Summary Data Base and list of studies included in the systematic review and general data from 130 studies included between 1990 and 2021. [Supplementary Table 5](#): List of additional references included in the systematic review.^{107–225}

Disclosure

The authors report no conflicts of interest in this work.

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