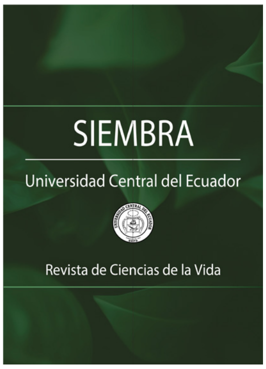
Presence and antimicrobial resistance of ESBL Escherichia coli from fecal samples of dairy cattle in northern Ecuador

Presencia y resistencia antimicrobiana de Escherichia coli BLEE en muestras fecales de bovinos productores de leche al norte de Ecuador



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Abstract

Escherichia coli causes colibacillosis in farm animals that act as reservoirs for pathogenic strains. Antimicrobial resistance of *E. coli* producing Extended Spectrum Beta-lactamases [ESBL] is a serious public health problem that can be attributed to factors related to food consumption and contact with domestic animals. This study aimed to determine the presence and patterns of antimicrobial resistance of ESBL *E. coli* isolated from fecal samples from dairy cattle in the Pichincha province. A total of 182 bovine feces samples were analyzed, 112 samples from cattle slaughtered at the official slaughterhouse in Quito-Pichincha province, and 70 samples from the collection of the Foodborne Diseases and Antimicrobial Resistance Research Unit [UNIETAR]. The isolation of ESBL *E. coli*, biochemical identification, and resistance tests using the main antibiotics were carried out at UNIETAR. It was possible to identify 93 positive samples for ESBL *E. coli* (51%), phenotypic analysis revealed that antibiotics such as amoxicillin plus clavulanic acid, cefepime, ceftazidime, ciprofloxacin, amikacin, tetracycline, presented resistance higher than 80%. Furthermore, low resistance to nitrofurantoin, cefoxitin, and ertapenem was observed, while no isolate was resistant to tigecycline. One hundred percent of the isolates presented multi-resistance phenotypes, with the most frequent pattern being composed of 7 families of antibiotics. In conclusion, these results suggest that *E. coli* from dairy cattle origin could be an important reservoir of ESBL genes.

Key words: *E. coli,* bovine, RAM, Ecuador, ESBL.

Resumen

La bacteria *Escherichia coli* causa la colibacilosis en animales de granja que actúan como reservorios de cepas patógenas. La resistencia antimicrobiana de *E. coli* productor de betalactamasas de espectro extendido [BLEE] es un grave problema de salud pública y se puede atribuir a factores relacionados con el consumo de alimentos y el contacto con animales domésticos. El objetivo de este estudio fue determinar la presencia y patrones de resistencia antimicrobiana de *E. coli* BLEE aislado en muestras fecales provenientes de bovinos productores de leche de la provincia de Pichincha. Se analizaron un total 182 muestras de heces de bovinos: 112 muestras de bovinos faenados en el Camal Metropolitano de la provincia de Pichincha y 70 muestras de la colección de la Unidad de Investigación de Enfermedades Transmitidas por Alimentos y Resistencias a los Antimicrobianos [UNIETAR], se realizó el aislamiento de *E. coli* BLEE, la identificación bioquímica y pruebas de resistencia a los principales antibióticos utilizados. Se logró identificar 93 muestras positivas a *E. coli* BLEE (51 %), el análisis fenotípico reveló que los antibióticos amoxicilina más ácido clavulánico, cefepime, ceftazidima, ciprofloxacina, amikacina y tetraciclina presentaron porcentajes de resistencia mayores al 80 %. Además, se observó una baja resistencia a la nitrofurantoína, cefoxitin y ertapenem, mientras que ningún aislado fue resistente a la tigeciclina. El 100 % de los aislados presentaron fenotipos de multirresistencia y el patrón más frecuente estuvo compuesto por 7 familias de antibióticos. En conclusión, estos resultados sugieren que *E. coli* originaria de bovinos lecheros podría ser un reservorio de genes BLEE.

Palabras clave: *E. coli,* bovino, RAM, Ecuador, BLEE.

1. Introduction

Escherichia coli is a facultative anaerobic bacterium and a commensal inhabitant of the intestine of warm-blooded animals (McVey et al., 2022; Winn et al., 2013). Some strains can cause intestinal and extraintestinal infections (Mueller & Tainter, 2023) and are capable of causing colibacillosis in farm animals, which additionally serve as reservoirs of the pathogenic strains (Bélanger et al., 2011). It has been reported that there are pathotypes that also carry genes for beta-lactamases resistance. (Tabaran et al., 2022; Yang et al., 2017).

Antimicrobial resistance [AMR] is a process through which microorganisms evolve to withstand the effects of antibiotics. (World Health Organization [WHO], 2021c). There are various factors that contribute to AMR being considered a serious public health issue. (WHO, 2021c). One of these factors is the indiscriminate use of antibiotics in veterinary settings for therapeutic, prophylactic, and/or growth-promoting purposes (Walsh, 2018). This behaviour promotes a greater selective pressure on microorganisms causing the spread of antibiotic-resistant bacteria (Palma et al., 2020). In hospital settings, the incidence of patients who do not respond to antibacterial drugs has been increasing (Genovese et al., 2020). *E. coli* BLEE (producer of extended-spectrum beta-lactamases) was reported as one of the bacteria causing hard-to-treat infections (WHO, 2021a). In Ecuador, resistant *E. coli* to cephalosporins was isolated in 40% of the public hospitals in 2017 (Instituto Nacional de Investigación en Salud Pública [INSPI, 2018). The economic impact of the AMR in the health system also needs to be considered, as it can increase the cost of medical care by up to 25 % (WHO, 2021b). Cross contamination of these pathogenic bacterial strains in animal slaughter or food processing could pose a serious risk to consumers' health (Yang et al., 2017).

In order to stop the progression of AMR, the use of antibiotics as growth promoters in food-producing animals was restricted in the European Union since 2006 (European Commission, 2005). According to the Pan American Health Organization (Organización Panamericana de La Salud [OPS], 2021), some country members have implemented control measures for the sale of veterinarian products containing colistin as one of the ingredients.

In general, the spread of *E. coli* BLEE can be attributed to factors related to food and the contact with domestic animals (Chong et al., 2018). Various studies have demonstrated the presence of *E. coli* BLEE in beef and dairy products (Cebeci, 2022; Dorado-García et al., 2018; Egervärn et al., 2014; Kaesbohrer et al., 2019). Therefore, the food chain of animal origin has a key role in the dissemination of this pathogen into the community (Doi et al., 2017). In Europe, *Enterobacteriaceae* producing beta-lactamases have been reported in cattle in 16 countries (Dantas Palmeira & Ferreira, 2020).

On the other hand, studies done in the South American region have detected the prevalence of *E. coli* BLEE in cattle from Brazil 18%, Peru 48%, and Chile 3% (Benavides et al., 2021).

Beta-lactamases can inactivate beta-lactam antibiotics such as penicillin, cephalosporins, monobactams, and carbapenems (Zhang & Cheng, 2022). Generally, *E. coli* BLEE is recognized by its enzymatic family, such as the TEM, SHV, CTX- M families (Castanheira et al., 2021).

The goal of this study was to determine the presence of *E. coli* BLEE, and to identify the patterns of isolated microbial resistance from fecal samples obtained from milk-producer cattle in the Pichincha province in Ecuador.

2. Materials and Methods

2.1. Study design

The research was observational, descriptive, and cross-sectional. The analysis covered a total of 182 fecal samples from cattle, 112 samples of beef meat slaughtered in the Metropolitan Camal of the Province of Pichincha and collected for 4 months, and 70 samples from the collection of the Foodborne Diseases and Antimicrobial Resistance Research Unit (*Unidad de Investigación de Enfermedades Transmitidas por Alimentos y Resistencias a los Antimicrobianos* [UNIETAR], in Spanish), which were taken from the project called "Estudio de resistencias a los antibióticos de *Escherichia coli* BLEE/AmpC aislado de muestras fecales de ganado bovino lechero en tres provincias del Ecuador" in collaboration with AGROCALIDAD.

The isolation of *Escherichia coli* BLEE was carried out in UNIETAR, located in the Faculty of Veterinary Medicine and Zootechnic at the Central University of Ecuador.

2.2. Isolation of Escherichia coli BLEE

For the isolation, 25 grams of fecal sample were used, 225 ml of BPW (Buffered Peptone Water) broth were added, and it was incubated at 37 °C for 24 hours. The samples were streaked using the striations due to thermal fatigue method on TBX (Tryptone Bile X-Glucuronide) agar with cefotaxime and incubated at 37°C for 24 hours.

2.3. Biochemical Identification

The bacteria were inoculated with a platinum loop into the TSI [Triple Sugar Iron] medium. They were incubated at 37 °C for 24 hours. Then, the characteristic phenotypic reaction of *E. coli*. was confirmed. The isolates that were identified as *E. coli.* were refrigerated for future analysis.

2.4. Antibiogram using the Kirby-Bauer method.

Positive colonies of *E. coli* were cultured in nutrient Agar [NA] and incubated at 37 °C for 24 hours. The suspension was obtained from the bacterial growth in NA. Three to four similar colonies were taken using a sterile loop and suspended into a tube containing sterile saline solution (4-5 mL). The suspension was standardized with a densimeter to reach a density of 0.5 McFarland. Then, a sterile cotton swab was introduced into the suspension to remove the excess of liquid. The inoculum was spread over the entire surface of the MH agar, inoculating the sample in three directions (horizontal, vertical, diagonal), and extending to the edges of the plate.

Antibiotic discs were applied using a sterile applicator or forceps. The antibiotic discs used contained ceftazidime 30 µg, cefepime 30 µg, ciprofloxacin 5 µg, amikacin 30 µg, amoxicillin plus clavulanic acid 30 µg, fosfomycin 200 µg, nitrofurantoin 300 µg, trimethoprim plus sulfamethoxazole 25 µg, gentamicin 10 µg, ertapenem 10 µg, tigecycline 15 µg, tetracycline 30 µg, chloramphenicol 30 µg, and cefoxitin 30 µg. The plates were incubated at 37 °C for 16-20 hours.

The interpretation of sensitivity was performed by measuring the inhibition halos with an automatic caliper, and the diameters were interpreted using the breakpoints provided in the M100 manual of the Clinical and Laboratory Standards Institute (CLSI, 2023).

3. Results

The analysis was done on 182 fecal samples from milk-producer cattle in TBX agar Plus cefotaxime (TBX+C) (3 mg/L) for the isolation of *E. coli.* Ninety-three samples were positively identified to contain *E. coli* BLEE (51 %, IC95% = 43-58). Also, the growth of colonies with a blue-green coloration was observed. All of the selected colonies were confirmed in TSI (Figure 1).

3.1. Analysis of phenotypic resistance to antibiotics of the strain E. coli BLEE isolated from cattle

From the antibiograms, it was observed that the antibiotics with higher resistance percentage were amoxicillin with clavulanic acid, cefepime, ceftazidime, ciprofloxacin, amikacin, and tetracycline with resistance percentages between 80.6 % and 97.8 %. On the other hand, the antibiotics with the lowest resistance were ertapenem, cefoxitin, and nitrofurantoin, with resistance percentages ranging between 2.2 % and 10.8 %. No isolate was resistant to tigecycline (Table 1).

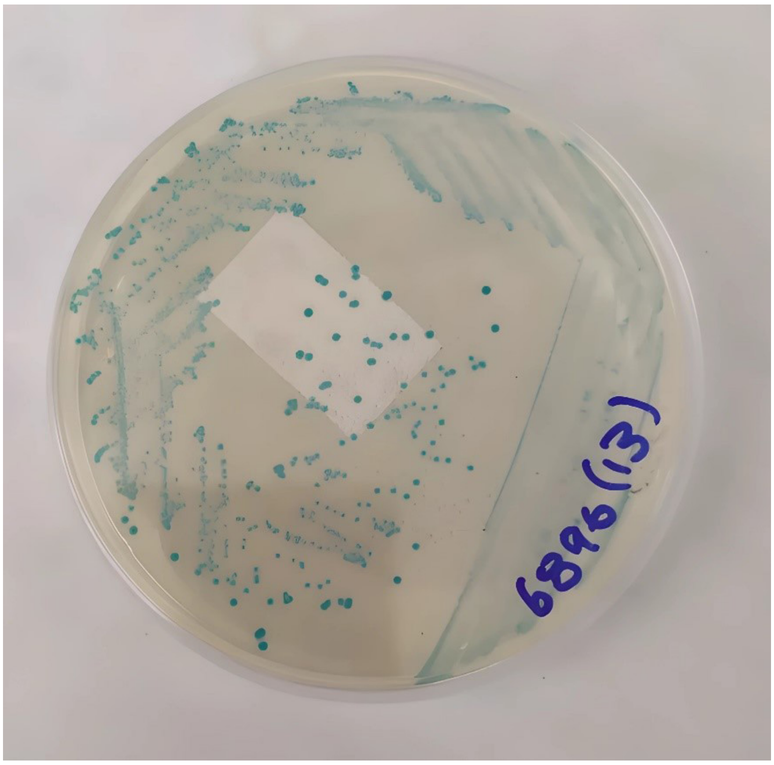

Figure 1. Strains of *E. coli* in greenish blue color in TBX+C agar.

Table 1. Results of the antibiogram analyses of 93 *E. coli* BLEE strains from cattle.

Family	Antibiotic	Resistances
Beta-lactams	cefepime	91 (97.8 %)
	ceftazidime	88 (94.6 %)
	cefoxitin	4 (4.3 %)
Aminoglycosides	amikacin	84 (90.3 %)
	gentamicin	44 (47.3 %)
β-LACTAM COMBINATION AGENTS	amoxicillin + clavulanic acid	91 (97.8 %)
Fluoroquinolones	ciprofloxacin	85 (91.4 %)
Tetracyclines	tetracycline	75 (80.6 %)
	tigecycline	0 (0.0 %)
folate inhibitor	sulfamethoxazole + trimethoprim	65 (69.9 %)
Phenicols	chloramphenicol	40 (43 %)
Fosfomycins	fosfomycin	40 (40 %)
Nitrofurans	nitrofurantoin	10 (10.8 %)
Carbapenems	ertapenem	2 (2.2 %)

3.2. Analysis of patterns and resistant genes in E. coli isolated from cattle

When analysing the phenotypic resistance of the 93 isolates, 35 resistance groups were obtained. The most prevalent ones were the isolates with resistance to 7 antibiotic families (10 groups with 29 isolates), shown in Table 2. Only one isolate showed resistance to 9 antibiotic families.

All the isolates analyzed showed multidrug-resistant phenotypes. The most frequent pattern was ABEFMTX (aminoglycosides, beta-lactams, phenicols, fluoroquinolones, combination of beta-lactam agents, tetracyclines, and folate pathway inhibitor) found in 15 isolates.

Table 2. Relationship between the number of antibiotic families present in the resistance patterns and number of *E. coli* strains from cattle.

No. of antibiotic families	Resistance pattern	No. of BLEE isolates (n = 93)
3	2	2
4	3	3
5	7	20
6	9	24
7	10	29
8	3	14
9	1	1
Total	35	93

4. Discussion

In this study, high percentages of antibiotic resistance were detected in strains of *E. coli* isolated from cattle. The Kirby-Bauer disk diffusion method was used to analyze the antibiotic susceptibility profile of the isolates. All the isolates studied (100 %) showed multidrug-resistant phenotypes [MDR] to 3 or more antibiotic families. Previous studies have reported high prevalence of MDR in developing countries, such as Pakistan, China, Mexico and Brazil (Jalil et al., 2023; Liu et al., 2022; Martínez-Vázquez et al., 2021; Tutija et al., 2022). The MDR obtained in this study could be related to the incorrect use of antibiotics in cattle farming (Martínez et al., 2023). A wide range of resistance was observed to third- and fourth-generation cephalosporins (ceftazidime and cefepime).

The resistance to tetracycline was 80.6 % which agrees with results previously published in the Netherlands, the United States and Mexico (Dorado-García et al., 2016; Hesp et al., 2019; Lee et al., 2020; Martínez-Vázquez et al., 2021). Tetracyclines are drugs commonly used in the production of livestock for consumption as prophylactics and/or growth promoters (Alonso et al., 2017). Tetracycline was mainly imported to Ecuador for veterinarian use in 2019 and the possibility that the administration of this antibiotic in the agricultural industry is associated with drug resistance cannot be ruled out (Amancha et al., 2023).

High values of resistance to ciprofloxacin (91.4 %) were also found. A systemic revision resulted in the documentation of higher resistance frequency to beta-lactams and quinolones in bacterial isolates from farm animals (Cota-Rubio et al., 2014). In a national context, the veterinarians prescribe third- and fourth-generation quinolones in cattle and poultry farms (Martínez et al., 2023). The resistance to quinolones might be associated to the production of BLEE from the family Enterobacteriaceae due to a gene co-transferring (Ortega-Paredes et al., 2020). This phenomenon could partially explain the resistance to quinolones in *E. coli* BLEE found in this study.

Regarding amikacin, the resistance was of 90.3 % in the *E. coli* BLEE isolates. Very low prevalences of resistance to amikacin were found in pigs (0.2 %) and calves (0.3 %) from some countries in the European Union (European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC], 2023; Organización Mundial de Sanidad Animal [OMSA], 2021). Resistance to amikacin in *E. coli* is related to the presence of aminoglycoside modifying enzymes [AME]. One type of AME is the 16S RNA methyltransferases (RMTases), which can be codified in the same plasmid as the enzymes type BLEE or AmpC

(Akova, 2016; EFSA & ECDC, 2023). Therefore, the presence of AME could result in the presence of different antibiotic resistance.

Another possible explanation for the increment of MDR in dairy cattle is the use of antibiotics for udder health (Oliver & Murinda, 2012). In Ecuador, antibiotics such as amoxicillin with clavulanic acid, cephalexin, ceftiofur, penicillin, streptomycin, tylosin, lincomycin, and neomycin are used for the treatment of mastitis (Jácome Mora, 2022). In addition, the prophylactic use of antibiotics is widely practiced to protect the udder from subclinical mastitis during the drying period (Öney et al., 2023). This has been reported as a factor that can increase the RAM (Dong et al., 2022; Oliver & Murinda, 2012). All of these factors could be related to the high values of MDR as well.

In the country, studies about the RAM in cattle are limited and more investigation is needed across various areas nationwide to contribute to the implementation of regulations towards a comprehensive surveillance and control of *E. coli* BLEE spread. Also, the implementation of alternatives and the optimization in the use of antibiotics for dairy cattle production should be prioritized to control the high levels of RAM reported in this study.

5. Conclusion

It was determined that the antibiotics with higher resistance levels in *E. coli* BLEE isolates from dairy cattle were amoxicillin with clavulanic acid, cefepime, ceftazidime, ciprofloxacin, amikacin, and tetracycline. It was also found that all the *E. coli* BLEE isolates presented multi-drug resistance patterns.

Contributor roles

- Pamela Carolina Calvopiña Montenegro: investigation, methodology, resources.
- Diana Sofía de Janon González: investigation, methodology, resources.
- José Luis Medina Santana: investigation, methodology, resources.
- Javier Vargas-Estrella: writing – original draft.
- Lenin Ron-Garrido: software, writing – original draft.
- Freddy Proaño-Pérez: conceptualization, investigation, validation, writing – review & editing.
- Christian Vinueza-Burgos: conceptualization, investigation, methodology, resources, writing – review & editing.

Ethical Implications

Ethics approval not applicable.

Conflicts of Interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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