

Prevalence and diversity of resistance genes identified in *Escherichia coli* isolated from raw bovine milk: a multicontinental systematic review

Prevalência e diversidade de genes de resistência identificados em *Escherichia coli* isolada de leite bovino cru: uma revisão sistemática multicontinental

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Highlights

Resistant *E. coli* was found in raw milk on four continents.

Indonesia had the highest prevalence of *E. coli* in raw bovine milk.

ESBL genes linked to β -lactam resistance hinder the treatment of *E. coli* infections.

Raw milk can be a vector for the spread of antimicrobial resistance.

Controlling antimicrobial resistance is essential in dairy production systems.

Abstract

Escherichia coli is a bacterium found in the gastrointestinal tract of animals and humans that can cause zoonotic infections. Strains producing extended-spectrum beta-lactamases (ESBLs) are emerging globally as an important cause of resistance to β -lactam antibiotics. This systematic review aimed to investigate the prevalence of *Escherichia coli* and the diversity of antimicrobial resistance genes in isolates obtained from raw bovine milk. Articles available in the scientific databases PubMed, LILACS, SciELO, Scopus, and Web of Science from the last five years were evaluated. A total of 16 studies from 13 countries across four continents Asia, Africa, North America, and South America were included in this review. The highest prevalence of *E. coli* was reported in Indonesia, with two distinct results: 91.81% (101/110) in one study and 70.05% (141/200) in another. In Turkey, the prevalence was 78.33% (47/60), and in Iran, 72.09% (62/86). Some antibiotics showed higher resistance rates, such as amoxicillin (59

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cases; 85.51%) and ceftriaxone (109 cases; 47.41%). The analysis revealed differences in the prevalence of resistance genes. The *tetA* gene was the most frequent, detected in 49 cases (73.14%), while *mcr-1* had the lowest prevalence, with nine cases (3.47%). The presence of resistance genes such as *bla_{TEM}*, *bla_{CTX-M}*, *bla_{SHV}*, *tetA*, and *mcr-1* in *E. coli* strains from raw milk poses a significant public health risk, highlighting the need for targeted policies to regulate antibiotic use, monitor antimicrobial resistance, and implement good agricultural practices.

Key words: Antimicrobial resistance. *E. coli*. ESBL. Genes. Raw milk.

Resumo

Escherichia coli é uma bactéria do trato gastrointestinal de animais e humanos que pode causar infecções zoonóticas. Cepas produtoras de beta-lactamases de espectro estendido (ESBL) estão emergindo globalmente como uma importante causa de resistência a antibióticos β -lactâmicos. Objetivou-se investigar a prevalência de *Escherichia coli* e a diversidade de genes de resistência antimicrobiana em isolados obtidos de leite bovino cru, por meio de revisão sistemática. Foram avaliados artigos disponíveis nas bases científicas PubMed, LILACS, SciELO, Scopus e Web of Science dos últimos cinco anos. Um total de 16 estudos selecionados de 13 países, distribuídos por quatro continentes: Ásia, África, América do Norte e América do Sul, foram incluídos nesta revisão sistemática. A prevalência de *E. coli* foi maior na Indonésia, com dois resultados distintos: 91,81% (101/110) em um estudo e 70,05% (141/200) em outro. Na Turquia foi de 78,33% (47/60) e, no Irã, de 72,09% (62/86). Alguns antibióticos demonstraram maiores taxas de resistência, como a amoxicilina (59 casos; 85,51%) e a ceftriaxona (109 casos; 47,41%). A análise revelou prevalências distintas entre os genes de resistência. O gene *tetA* foi o mais prevalente entre os analisados, identificado em 49 casos (73,14%), enquanto o *mcr-1* apresentou a menor prevalência, com nove casos (3,47%). A presença de genes de resistência, como *bla_{TEM}*, *bla_{CTX-M}*, *bla_{SHV}*, *tetA* e *mcr-1*, em cepas de *E. coli* provenientes de leite cru representa um risco significativo para a saúde pública, destacando a necessidade de políticas específicas para regulamentos ou uso de antibióticos, monitorar a resistência antimicrobiana e implementar boas práticas agropecuárias.

Palavras-chave: *E. coli*. ESBL. Genes. Leite Cru. Resistência antimicrobiana.

Introduction

In recent years, the consumption of raw milk has gained popularity due to its potential benefits, such as high nutritional value and the presence of beneficial bacteria (Luo et al., 2024). However, the potential presence of pathogens and their toxins capable of causing foodborne illness makes raw milk consumption a significant public health concern. Over the last decade, the incidence of foodborne microbial diseases

has increased considerably in several countries (Hassani et al., 2022).

The most common pathogens found in raw milk and dairy products include *Staphylococcus aureus*, *E. coli* (including the O157:H7 strain), *Salmonella* spp., *Listeria monocytogenes*, and *Brucella* spp. Contamination during milking or processing, as well as intramammary infections, makes raw milk an important route for zoonotic transmission. Treating

infections caused by these microorganisms involves the use of antimicrobials, which are also used prophylactically in livestock. However, excessive and inappropriate use of antibiotics in both human medicine and animal production has contributed to the increasing prevalence of multidrug-resistant pathogens, complicating therapeutic management and raising mortality rates (Belayneh et al., 2025; Loo et al., 2019).

Antimicrobial resistance occurs when microorganisms undergo changes upon exposure to antimicrobials, becoming capable of resisting drugs that were previously effective against them. The increasing prevalence of antimicrobial resistance is a serious threat to global health. Extended-spectrum beta-lactamases (ESBLs) are among the main resistance mechanisms in *Enterobacteriaceae*, hydrolyzing penicillins, cephalosporins, and aztreonam (monobactams), which are classes of antimicrobials commonly used to treat bacterial infections (Silva et al., 2020; Tóth et al., 2020).

ESBL-producing *E. coli* strains are emerging globally as a major cause of resistance to β -lactam antibiotics. *E. coli* are Gram-negative bacteria widely present in the gastrointestinal tract of animals and humans, making them a reliable indicator of fecal contamination in food. Furthermore, this bacterium can act as a zoonotic agent, causing infections (Ombarak et al., 2021; Saei et al., 2022).

The most common genes associated with ESBL production are *bla*_{SHV} (SHV), *bla*_{TEM}

(TEM), and *bla*_{CTX-M} (CTX-M). β -lactamases of the TEM and SHV types are recognized as the main resistance factors in *E. coli* strains. However, genes from the CTX-M family have been reported in several studies, typically in the *bla*_{CTX-M-1}, *bla*_{CTX-M-2-like}, *bla*_{CTX-M-9-like}, *bla*_{CTX-M-8-like} and *bla*_{CTX-M-25-like} subgroups (Andrade & Darini, 2017).

Antimicrobial resistance is recognized as one of the greatest threats to global health, with direct implications for human, animal, and environmental health, consolidating its position as one of the main challenges under the One Health approach. The consumption of animal products such as raw cow's milk can serve as a vector for transmitting antimicrobial resistance genes between animal and human environments (Kaplan, 2024).

Investigating the prevalence and diversity of resistance genes in *E. coli* isolated from raw cow's milk provides essential information for understanding the dynamics of antimicrobial resistance transmission at the animal-human health interface. Such knowledge is crucial for supporting integrated strategies to control and reduce antimicrobial resistance within the One Health framework, contributing to public health protection and the sustainability of animal production systems (Parker et al., 2024).

Therefore, this systematic review aimed to compile and analyze evidence on the prevalence of *E. coli* isolated from raw cow's milk and the diversity of antimicrobial resistance genes.

Material and Methods

Study type and data source

A systematic literature review was conducted following the protocol recommended in the PRISMA guidelines by Moher et al. (2009), which included the

following steps: (i) literature search to identify potentially relevant articles, (ii) assessment of article relevance, (iii) quality assessment, and (iv) extraction of predefined data (Figure 1). The study was registered with the International Prospective Registry of Systematic Reviews (PROSPERO) platform.

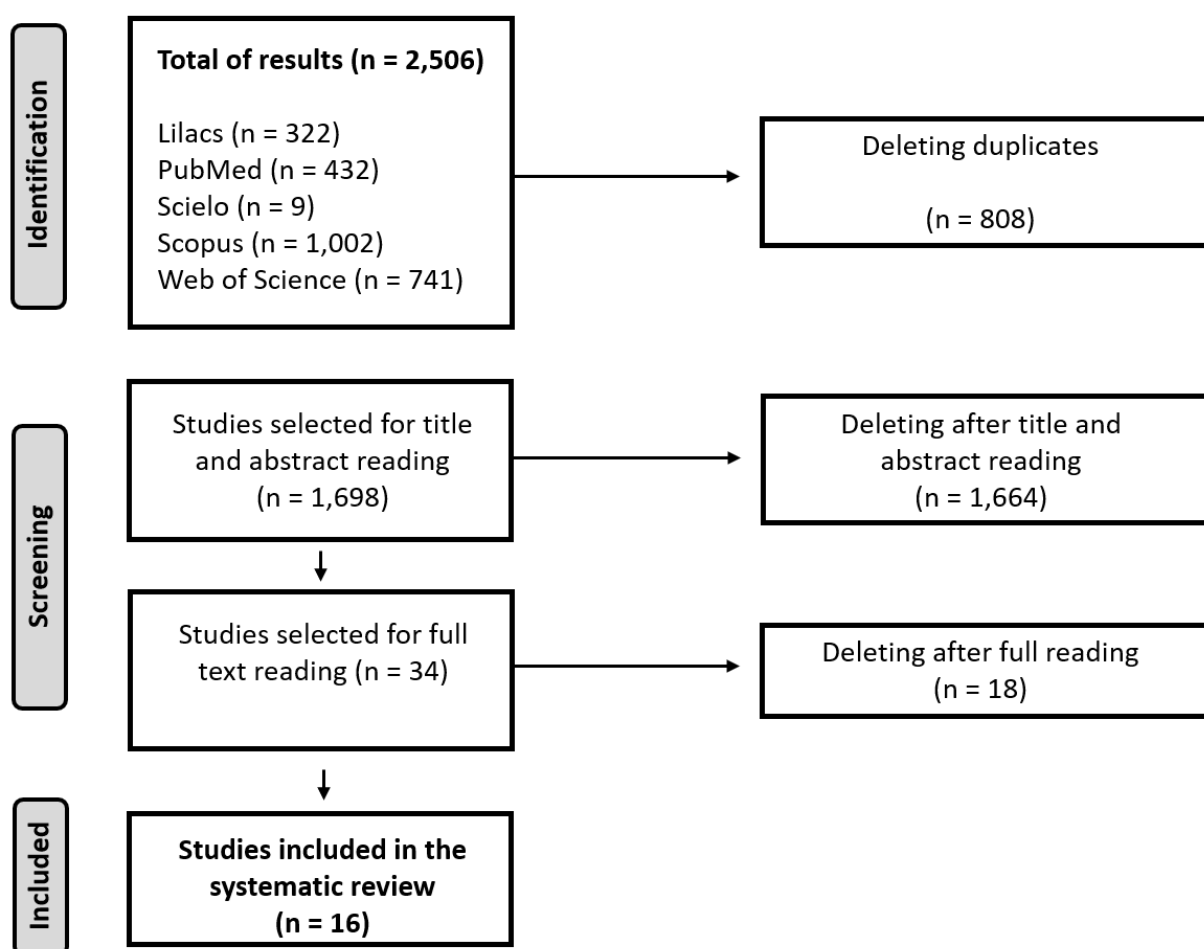


Figure 1. Flowchart of identification and selection of articles for systematic review on the prevalence and diversity of resistance genes identified in *E. coli* present in raw bovine milk.

Searches for published studies on antimicrobial resistance in *E. coli* from raw milk were conducted between November 5 and 10, 2024, using the following platforms: PubMed, LILACS, SciELO, Scopus, and Web of Science. The descriptors used in the inclusion process were "*Escherichia coli*," "milk," and "gene," and all retrieved publications were exported as BibTeX or RIS files to the Mendeley bibliographic manager (Mendeley software version 1.19.8, Mendeley Ltd., London, UK). Articles published in the last five years, in English, and without restrictions on country of origin were selected.

Criteria for article inclusion and exclusion

In the preliminary screening, all duplicates, theses, dissertations, final papers, review articles, and conference abstracts were excluded. Subsequently, the titles and abstracts of each article were read. In the final stage, the full texts were assessed, and articles that did not meet the established inclusion criteria were excluded (Figure 1).

For inclusion, studies published between 2019 and 2024 that investigated the presence of *Escherichia coli* in raw milk and employed molecular analysis to identify resistance genes were selected. In addition, studies needed to provide sufficient information on the prevalence and diversity of detected resistance genes to be considered in this review.

For exclusion, studies classified as gray literature, studies focused on foods other than raw milk, or those that did not use molecular analysis to identify resistance genes were removed. Publications dated

prior to 2019 and articles written in languages other than English were also excluded.

After applying the inclusion and exclusion criteria and screening the records obtained from the databases, 16 studies containing the required information (authors, country of origin, number of samples, *E. coli* prevalence, and resistance gene diversity) were included. These studies were from 13 countries across four continents Asia, Africa, North America, and South America reflecting the geographic scope of the review.

Data analysis

Data extracted from the included studies were analyzed using descriptive statistics. The combined prevalence was calculated for each antibiotic and resistance gene based on the total number of positive samples relative to the total number of samples tested across all studies. To represent the uncertainty associated with the estimates, 95% confidence intervals (95% CIs) were calculated, allowing interpretation of the expected variation in prevalence. All calculations were performed using Microsoft Excel (version 2016).

Assessment of heterogeneity and publication bias

Heterogeneity between studies was assessed using measures of central tendency, with standard deviation calculated to quantify variability and demonstrate the dispersion of data around the mean combined prevalence.

Determination of resistance genes

The word cloud method was used to determine the most frequent bacterial resistance genes and antimicrobial agents, applying the wordcloud function.

Results

India, Indonesia, and Turkey each accounted for the highest number of studies, with 12.5% (2/16) of the total (Table 1).

The prevalence of *E. coli* was highest in Indonesia, with two distinct results: 91.81% (101/110) in one study and 70.05% (141/200) in another. In Turkey, prevalence reached 78.33% (47/60), and in Iran, 72.09% (62/86). Analysis of the word cloud revealed that tetracycline and ampicillin were the most frequently mentioned antimicrobials. The most frequent resistance genes were *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{SHV} (Figure 2).

The combined prevalence varied widely among antimicrobials, indicating differences in observed resistance. Some

showed high resistance rates, such as amoxicillin (59/85.51%) and ceftriaxone (109/47.41%), while meropenem (7/1.44%) and colistin (4 cases; 2.02%) showed very low prevalences (Table 2).

Gene prevalence analysis yielded results that differed from the most frequent ones observed in the word cloud (Figure 2). The *tetA* gene, associated with tetracyclines, was the most prevalent among all those analyzed, at 73.14%, followed by *bla*_{TEM}, associated with β -lactams, at 24.90%. The *bla*_{CTX-M} gene had a prevalence of 14.10%, while *aadA1*, associated with aminoglycosides, had a prevalence of 11.94%. Among quinolones, the *qrrB19* gene was detected in 10.46% of cases, and polymyxin resistance, represented by *mcr-1*, was the least frequent, at 3.47% (Table 2).

The study revealed heterogeneity in the results, as indicated by the standard deviations and wide confidence intervals in some cases, suggesting variations in methods and sample populations among the evaluated studies (Table 2).

Table 1
Identification of authors, country, samples analyzed and positive for *E. coli*, antimicrobials tested, genotypic results and resistance genes detected

Reference	Country of origin	Number of samples	Positive samples for <i>E. coli</i> (%)	Antimicrobials tested	Positive samples in genotypic tests	Resistance genes identified
Gelalcha et al., 2023.	USA	33	08 (24.24%)	Ampicillin, ceftriaxone, ceftiofur, amoxicillin/clavulanic acid, meropenem, gentamicin, streptomycin, sulfisoxazole, trimethoprim + sulfamethoxazole, azithromycin, chloramphenicol, ciprofloxacin, nalidixic acid and tetracycline.	07	blaCTX-M and blaTEM-1-like
Rajib et al., 2023.	India	44	26 (59.09%)	Beta-lactams - oxacillin, cephalosporins, cefotaxime, cefepime and meropenem.	17	blaCTX-M
Patange & Thorat, 2022.	India	106	42 (39.62%)	Imipenem, ciprofloxacin, amoxicillin/clavulanic acid, ceftriaxone, tetracycline and amikacin.	10	blaTEM
Taniguchi et al., 2021.	Japan	1.549	293 (18.91%)	No report	21	blaCTX-M-15-like, blaTEM-1-like and blaSHV-1-like
Liu et al., 2020.	China	2.038	249 (12.21%)	Ampicillin, amikacin, chloramphenicol, ciprofloxacin, cefotaxime, ceftriaxone, ceftiofur, kanamycin, tetracycline and trimethoprim.	05	mcr-1-like and blaCTX-M-15-like
Abro et al., 2024.	Pakistan	100	44 (44%)	Ampicillin, levofloxacin, tetracycline, ceftiofur and ceftriaxone.	11	blaCTX-M-1-like
Anshari et al., 2021.	Indonesia	200	141 (70.05%)	Tetracycline, streptomycin, chloramphenicol, trimethoprim, aztreonam, amoxicillin-clavulanate, cefotaxime, and ceftazidime.	05	blaCTX-M and blaTEM
Widodo et al., 2023.	Indonesia	110	101 (91.81%)	Tetracycline, streptomycin, chloramphenicol, trimethoprim, aztreonam, cefotaxime, ceftazidime and amoxicillin-clavulanate.	04	blaTEM and blaCTX-M

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Sierra et al., 2023.	Brazil	257	14 (5.44%)	Cefotaxime, ceftazidime, ceftriaxone, and amoxicillin/clavulanic acid.	08	blaSHV and blaTEM
Kürekcı et al., 2021.	Turkey	105	59 (56.19%)	Ampicillin, amoxicillin/clavulanic acid, aztreonam, ceftazidime, ceftriaxone, cefoxitin, cefuroxime, cefotaxime, enrofloxacin, levofloxacin, norfloxacin, nalidixic acid, imipenem, chloramphenicol, gentamicin, tetracycline, colistin and trimethoprim-sulfamethoxazole.	42	blaCTX-M and blaTEM
Ceren & Gozde, 2024.	Turkey	60	47 (78.33%)	Aztreonam, gentamicin, imipenem, meropenem, levofloxacin, tobramycin, ceftazidime, cefotaxime, ampicillin, nalidixic acid, trimethoprim/sulfamethoxazole and piperacillin/tazobactam.	11	blaTEM, blaSHV and blaCTX-M
Saei et al., 2022.	Iran	86	62 (72.09%)	Ceftriaxone, gentamicin, cotrimoxazole, ceftazidime, cefotaxime, ampicillin, cefepime, cefoxitin and amikacin.	43	blaTEM, blaCTX-M-14-like, blaCTX-M-3-like, blaOXA and blaDHA
Duc et al., 2024.	Vietnam	400	139 (34.75%)	Ampicillin, tetracycline, streptomycin, gentamicin, colistin, azithromycin, trimethoprim/sulfamethoxazole, florfenicol, meropenem, cefotaxime, cefoxitin, cefepime, ceftazidime, ciprofloxacin, and nalidixic acid.	07	blaTEM and blaCTX-M-1-like
Amosun et al., 2021.	Nigeria	168	09 (5.35%)	Ampicillin, amoxicillin/Clavulanic Acid, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, tetracycline and trimethoprim/sulfamethoxazole.	05	blaCTX-M-15-like and blaTEM
Mwasinga et al., 2023.	Zambia	418	214 (51.19%)	Amoxicillin-clavulanic acid, ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, imipenem, nalidixic acid, tetracycline, and trimethoprim-sulfamethoxazole.	10	blaCTX-M and blaTEM
Aly et al., 2024.	Egypt	200	60 (30%)	Ofloxacin, amoxicillin, levofloxacin, erythromycin, azithromycin, gentamicin, ciprofloxacin, doxycycline, cefuroxime, rifampicin, cefoperazone, cefotaxime, ceftriaxone, and amikacin.	07	blaTEM and blaCTX-M

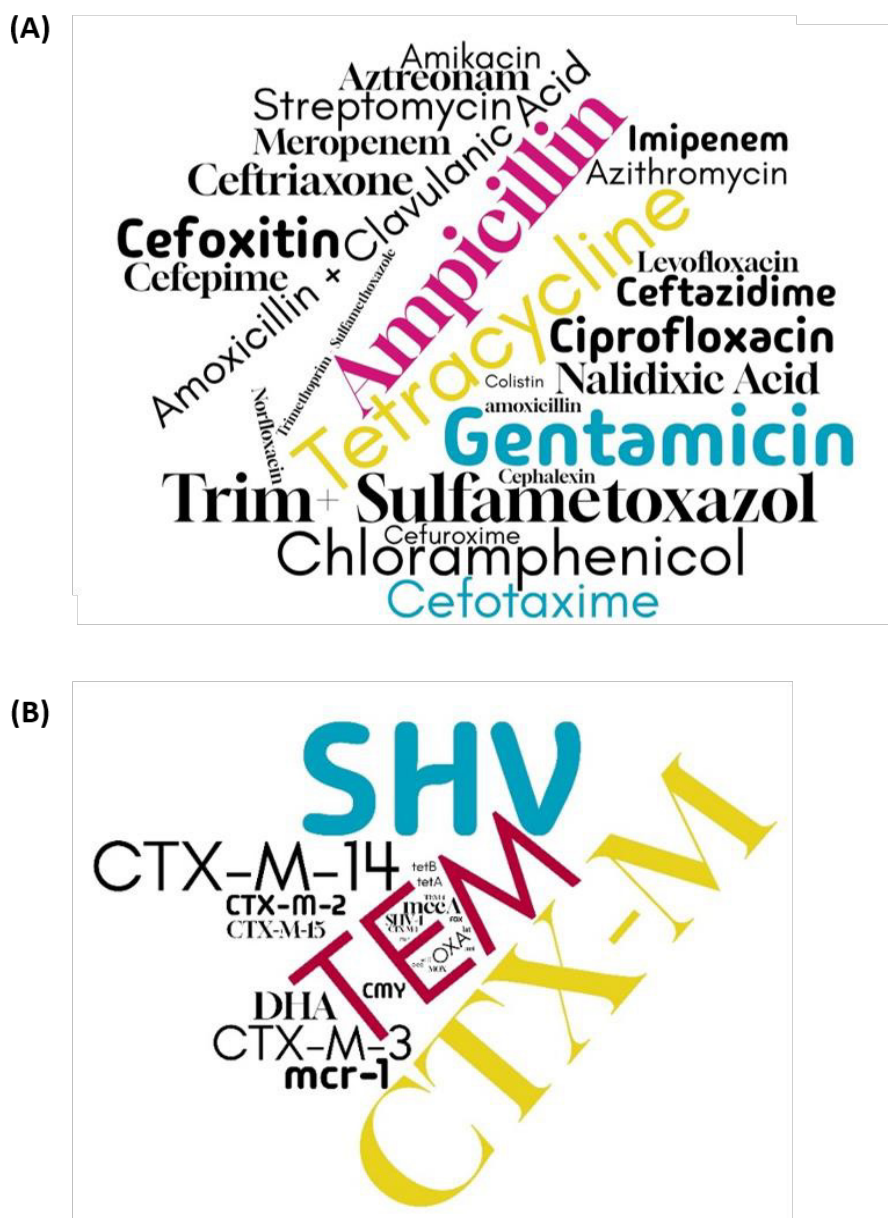


Figure 2. Word clouds extracted from the 16 articles based on antimicrobials (A) and bacterial resistance genes (B).

Table 2
Combined prevalence of antibiotic resistance in *E. coli* present in raw bovine milk

Antibiotics	Number of studies included	Number of samples tested	Number of positive samples	Combined prevalence (%)	Standard deviation	95% confidence interval	References
Nalidixic Acid	6	716	116	16.21	33.53	14.89 - 45.87%	Liu et al., 2020; Ansharieta et al., 2021; Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde 2024.
Amikacin	4	337	72	21.36	34.67	14.88 - 46.92%	Rajib et al., 2023; Kürekci et al., 2021; Saei et al., 2022; Mwasinga et al., 2023.
Amoxicillin + Clavulanic Acid	5	390	105	26.93	32.01	12.31 - 41.89%	Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde, 2024; Mwasinga et al., 2023.
Amoxicillin	2	69	59	85.51	17.20	64.85 - 80.75%	Rajib et al., 2023; Patange & Thorat, 2022.
Ampicillin	9	831	300	36.10	28.89	44.33 - 71.02%	Gelalcha et al., 2023; Patange & Thorat, 2022; Liu et al., 2020; Ansharieta et al., 2021; Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde, 2024; Saei et al., 2022.
Aztreonam	4	221	9	4.07	7.93	04.10 - 11.43%	Taniguchi et al., 2021; Liu et al., 2020; Sierra et al., 2023; Aly et al., 2024.
Azithromycin	4	557	79	14.18	40.04	12.47 - 49.46%	Rajib et al., 2023; Ansharieta et al., 2021; Widodo et al., 2023; Kürekci et al., 2021.
Cephalexin	2	293	25	8.52	21.73	13.69 - 33.76%	Abro et al., 2024; Rajib et al., 2023.
Cefepime	4	494	51	10.32	29.04	09.35 - 36.18%	Abro et al., 2024; Ansharieta et al., 2021; Kürekci et al., 2021; Saei et al., 2022.
Cefotaxime	8	826	118	14.30	26.75	10.23 - 34.95%	Rajib et al., 2023; Liu et al., 2020; Abro et al., 2024; Ansharieta et al., 2021; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde, 2024; Saei et al., 2022.

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Ceftriaxone	6	230	109	47.41	32.20	34.75 - 64.50%	Gelalcha et al., 2023; Rajib et al., 2023; Widodo et al., 2023; Saei et al., 2022; Duc et al., 2024; Mwasinga et al., 2023.
Cefoxitin	6	561	48	08.56	19.58	04.58 - 22.67%	Gelalcha et al., 2023; Ansharieta et al., 2021; Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Saei et al., 2022.
Ceftazidime,	6	517	27	5.22	24.80	09.76 - 32.68%	Patange & Thorat, 2022; Liu et al., 2020; Ansharieta et al., 2021; Sierra et al., 2023; Kürekci et al., 2021; Duc et al., 2024.
Cefuroxime	3	163	73	44.79	28.95	31.87 - 58.62%	Rajib et al., 2023; Abro et al., 2024; Sierra et al., 2023.
Ciprofloxacin	7	721	88	12.21	32.23	10.18 - 39.95%	Rajib et al., 2023; Patange & Thorat, 2022; Ansharieta et al., 2021; Widodo et al., 2023; Kürekci et al., 2021; Ceren & Gozde, 2024; Mwasinga et al., 2023.
Chloramphenicol	7	654	55	08.39	23.44	16.93 - 38.58%	Patange & Thorat, 2022; Taniguchi et al., 2021; Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde 2024; Aly et al., 2024.
Colistin	2	198	4	2.02	1.44	00.77 - 2.11%	Ansharieta et al., 2021; Sierra et al., 2023.
Streptomycin	5	271	65	23.99	30.24	34.36 - 62.30%	Patange & Thorat, 2022; Taniguchi et al., 2021; Ansharieta et al., 2021; Widodo et al., 2023; Aly et al., 2024.
Gentamicin	9	847	57	6.74	13.08	06.26 - 18.34%	Rajib et al., 2023; Patange & Thorat, 2022; Liu et al., 2020; Ansharieta et al., 2021; Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde, 2024; Saei et al., 2022.
Imipenem	4	362	42	11.59	11.87	06.47 - 17.43%	Liu et al., 2020; Sierra et al., 2023; Ceren & Gozde, 2024; Mwasinga et al., 2023.
Levofloxacin	4	210	83	39.52	26.19	26.19 - 50.39%	Gelalcha et al., 2023; Rajib et al., 2023; Liu et al., 2020; Sierra et al., 2023.

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Meropenem	5	487	7	01.44	5.96	00.23 - 5.73%	Liu et al., 2020; Abro et al., 2024; Ansharieta et al., 2021; Widodo et al., 2023; Kürekci et al., 2021.
Norfloxacin	2	308	61	19.80	46.45	26.99 - 69.91%	Sierra et al., 2023; Kürekci et al., 2021.
Tetracycline	10	879	263	29.91	33.66	38.73 - 69.83%	Gelalcha et al., 2023; Patange & Thorat, 2022; Taniguchi et al., 2021; Ansharieta et al., 2021; Widodo et al., 2023; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde 2024; Mwasinga et al., 2023; Aly et al., 2024.
Trimethoprim	2	115	9	7.83	24.01	14.90 - 37.08%	Taniguchi et al., 2021; Aly et al., 2024.
Trimethoprim + Sulfamethoxazole	7	779	190	24.39	26.18	25.30 - 49.48%	Patange & Thorat, 2022; Liu et al., 2020; Ansharieta et al., 2021; Sierra et al., 2023; Kürekci et al., 2021; Ceren & Gozde, 2024; Saei et al., 2022.

Discussion

The global prevalence of *E. coli* in raw bovine milk and the observed diversity of antimicrobial resistance genes reflect marked differences between continents and countries. Asian nations showed the highest prevalence rates, which may be linked to a combination of factors that directly affect milk production conditions and favor contamination with microorganisms such as *E. coli* (Kaplan, 2024; World Animal Protection [WAP], 2023).

Management practices and industrial techniques involving antimicrobial use vary significantly across regions. Differences in antimicrobial resistance between countries are related to geographic location, the antimicrobials authorized for use, and the agricultural management systems in place. These variations can also be influenced by production methods, personal hygiene, sanitary conditions, and other practices along the food chain (Song et al., 2020; Seo & Lee, 2018).

By 2020, Asia and Eastern Oceania accounted for 87.13% of the 97,784 t of antimicrobial agents consumed in animal production. Developing countries often lack effective regulations on the import, sale, and use of these drugs, applying different protocols for antimicrobial use on farms (World Organisation for Animal Health [WOAH], 2020; Anyanwu et al., 2021).

The highest combined prevalence of phenotypic resistance was observed for amoxicillin (85.51%) and ceftriaxone (47.41%), both belonging to the β -lactam class and widely used in human and veterinary medicine due to their efficacy

against bacterial infections. Such extensive use can lead to selective pressure, which may explain the high resistance rates for these antimicrobials (Silva et al., 2020; Loo et al., 2019).

Antimicrobials such as meropenem and colistin showed very low or no prevalence of resistance, likely due to their more restricted use. According to Anyanwu et al. (2021), carbapenems are reserved as last-line treatments for severe infections caused by resistant microorganisms.

Studies have reported a significant prevalence of resistance genes in *E. coli* isolated from raw milk, with bla_{CTX-M} , bla_{TEM} and bla_{SHV} being the most frequently identified. The combined prevalence of β -lactam resistance genes was notable, indicating a significant spread of resistance among isolates from the livestock sector (Chaudhary et al., 2023).

This dissemination can occur through plasmids or other mobile genetic elements, broadening the problem beyond the agricultural environment and into wider ecological niches, including humans (Kaplan, 2024; WAP, 2023).

In India, Rajib et al. (2023) reported the highest prevalence of the bla_{CTX-M} gene at 65.38% (17/26), while Patange and Thorat (2022) found bla_{TEM} in 23.8% (10/42) of isolates. Similar findings were obtained by Ansharieta et al. (2021) and Widodo et al. (2023) in Indonesia, both confirming the presence of bla_{CTX-M} and bla_{TEM} in resistant *E. coli* samples.

In Japan, Taniguchi et al. (2021) detected bla_{CTX-M} , bla_{TEM} and bla_{SHV} including variants such as $bla_{CTX-M-15-like}$, $bla_{TEM-1-like}$ and $bla_{SHV-1-like}$. Similarly, Ceren and Gozde (2024)

in Turkey identified genes from the *bla*_{CTX-M'}, *bla*_{TEM'} and *bla*_{SHV} families in *E. coli* isolated from raw milk.

The study by G. Liu et al. (2020) in China identified *bla*_{CTX-M-15} and *mcr-1*, associated with resistance to polymyxins, particularly colistin. This gene was first reported in Brazil in 2015 in *E. coli* from pigs, pork products, and humans. In China, colistin had been widely used in livestock as a growth promoter and for disease prevention, facilitating the gene's spread. Plasmid-mediated horizontal transfer further increases the risk of contamination in animal products such as raw milk (Y. Y. Liu et al., 2016; Z. Liu et al., 2021).

In Brazil, Sierra et al. (2023) reported an intermediate prevalence of *bla*_{CTX-M'} detected in 57.1% (8/14) of samples. In Pakistan, Abro et al. (2024) exclusively identified *bla*_{CTX-M-1-like} in resistant *E. coli* from buffalo milk with mastitis, results similar to those obtained in Northeast Brazil (Sierra et al., 2023).

Saei et al. (2022) in Iran observed wide genetic diversity, including *bla*_{TEM'}, *bla*_{CTX-M'}, *bla*_{OXA'} and *bla*_{DHA'}, with variants such as *bla*_{CTX-M-14-like} and *bla*_{CTX-M-3-like}, highlighting the need for both local and global control strategies.

Amosun et al. (2021), Mwasinga et al. (2023), and Aly et al. (2024) conducted studies in Nigeria, Zambia, and Egypt, respectively, identifying *bla*_{CTX-M} and *bla*_{TEM'} with the *bla*_{CTX-M-15-like} variant reported in Nigeria.

Kürekci et al. (2021), Gelalcha et al. (2023), and Duc et al. (2024) in Turkey, the USA, and Vietnam, respectively, also reported *bla*_{CTX-M} and *bla*_{TEM'} with differences in variants: *bla*_{CTX-M-1-like} in Vietnam and *bla*_{TEM-1-like} in the USA.

The use of antimicrobials in livestock for both treatment and prevention can accelerate the emergence of resistant bacterial strains, which may be transmitted to humans via contaminated food, animal contact, or environmental exposure (De Heus, 2023). These findings emphasize the need for a global approach to monitoring and control that accounts for regional specificities.

Therefore, the detection of resistance genes in *E. coli* from raw milk represents a significant public health risk, given the possibility of transferring these genes to human pathogens through dairy consumption or contact with infected animals. Resistance to β -lactams is of particular concern due to their importance in treating severe human infections, highlighting the need for policies on antibiotic regulation and use, antimicrobial resistance monitoring, and the adoption of good agricultural practices.

Conclusion

This study demonstrated that raw milk is an important reservoir of bacteria resistant to multiple antimicrobial drugs, especially those most relevant to human health, such as β -lactams. Studies on the genotypic prevalence of *E. coli* in raw bovine milk provide essential scientific data to guide sustained efforts and strategic measures aimed at preventing the spread of resistance genes. The findings highlight the urgency of integrating standardized, internationally coordinated surveillance programs.

International organizations have emphasized priority actions such as strengthening global surveillance systems

for antimicrobial resistance and use, enforcing regulations on antibiotic use in human and animal health, and investing in research and public education on responsible antimicrobial use. Only continuous, collaborative monitoring can curb the spread of resistance genes in animal food production and safeguard public health.

Acknowledgments

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for its financial support, which was essential for the development of this research, including data collection and analysis.

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