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Emergence of Extended-spectrum Beta-lactamase Producer and Colistin-resistant *E. coli* in Animal-origin Foods in Libya

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ABSTRACT

The increasing prevalence of *Escherichia coli* (*E. coli*) infection poses significant health challenges worldwide. Understanding the genetic, pathogenic, and antimicrobial resistance profiles of *E. coli* is crucial for effective diagnosis and treatment. The present study aimed to assess the prevalence and antimicrobial susceptibility patterns of *E. coli* isolated from different samples of food products from animals, with specific attention to identifying and characterizing extended-spectrum beta-lactamase (ESBL)-producing isolates. The present study analyzed 92 *E. coli* isolates obtained from 1120 food samples, including milk, dairy products, meat, and meat products, collected randomly from retail markets in Libya. The isolates were tested for antimicrobial susceptibility, and the antibiotic resistance profiles were evaluated using 32 antibiotics from 12 different classes. Multiple antibiotic resistance (MAR) and antibiotic resistance index were calculated, with MAR $\geq$ 0.2 indicating high antibiotic resistance. Isolates were categorized as multidrug resistant (MDR), extensively drug-resistant (XDR), or pan drug-resistant (PDR) based on standard definitions. The ESBL production was assessed using the double-disc synergy test, and colistin resistance was tested using the agar diffusion method. Antimicrobial susceptibility testing of *E. coli* isolates revealed 100% resistance to penicillin and cloxacillin, with high resistance rates observed against neomycin (93.4%), rifampicin (86.9%), and methicillin (75%). However, all isolates were susceptible to chloramphenicol, whereas carbapenems (imipenem, meropenem, ertapenem) indicated the lowest resistance (3.2%). Cefepime demonstrated the highest effectiveness among cephalosporins, with a resistance rate of 1.08%. The MAR ranged from 0.09 to 0.6, with the highest MAR value (0.6) observed in isolates resistant to 20 antibiotics. All isolates were MDR, but no XDR or PDR strains were detected. Among the 92 isolates, 43 were confirmed as ESBL producers, primarily originating from raw milk, lben (fermented milk), and other dairy products. In addition, 83 isolates demonstrated phenotypic resistance to colistin. The present study highlighted the significant presence of MDR *E. coli* in food products of animal origin, particularly raw milk, fermented milk, and chicken meat in Libya, emphasizing the urgent need for antimicrobial stewardship, stronger regulatory frameworks, and integrated One Health surveillance approaches to combat AMR in Libya.

Keywords: Antimicrobial resistance, Colistin, *Escherichia coli*, Extended-spectrum beta-lactamases, Food sample

INTRODUCTION

Food safety is a crucial component of public health and has been recognized as an effective measure for preventing foodborne diseases for over a century, owing to advancements in food production and the adoption of modern management philosophies, such as hazard analysis. However, several issues continue to persist, one of which is the high prevalence of foodborne illnesses caused by specific pathogens, which appear to have increased in recent decades.

Worldwide, foodborne diseases caused by contaminated food are rapidly spreading and can arise at any point in the food chain, from food production to delivery to consumption. Foodborne diseases may originate from a variety of environmental contaminants, such as unsafe or improper food processing, storage practices, and air, water, or soil pollution. According to the World Health Organization, 1 in 10 people falls ill each year due to contaminated food. Many of these illnesses are often linked to the consumption of contaminated undercooked meat and raw milk (Chaves et al., 2015). Furthermore, Faour-Klingbeil and Todd (2019) indicated that international commerce is impacted by food laws and regulations, which are implemented by nations as part of their control tactics against foodborne illnesses, which is a worldwide public health concern that affects people's health, livelihoods, and healthcare systems. *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., and *Escherichia coli* (*E. coli*) are some of the key bacterial

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pathogens responsible for causing foodborne illnesses, according to Habib and Mohamed (2022). Although Africa bears the highest burden of foodborne disease, there is a lack of understanding of the disease's prevalence in Africa due to the limited studies and surveillance programs (Desta, 2020), and Libya is not exceptional, where many cases are either dismissed as minor and self-limiting or go unconfirmed because of reporting, detection, and monitoring gaps.

Escherichia coli is considered a dangerous pathogen in dairy farm operations worldwide because it leads to significant economic losses (Allocati et al., 2013). There are several strains of *E. coli*, most of which are not hazardous, but a few cause serious foodborne illnesses in humans. Additionally, *E. coli* has emerged as an essential model organism for researching the spread of drug resistance within bacterial populations and serves as an indicator of the selective pressure caused by the widespread and unregulated use of antibiotics in animal agriculture (Petty et al., 2014). This has positioned *E. coli* as a key reference in global drug resistance monitoring programs (Sheikh et al., 2012). The insights derived from drug resistance monitoring can contribute to the development of strategies aimed at reducing the risk of these infections among the population (Guiral et al., 2011).

Currently, numerous antimicrobial drugs are utilized in food animal production to manage infections and serve as growth promoters, a practice that is increasingly contributing to the human food chain, raising substantial health concerns for both humans and animals (Hao et al., 2014). Common antibiotics such as tetracycline and amoxicillin are used to treat foodborne infections, but their overuse has led to rising resistance in bacteria such as *E. coli* (Miranda et al., 2014; Gwida and El-Gohary, 2015; Kapoor et al., 2017). Contributing factors to antibiotic resistance include misuse, incorrect prescription, extensive agricultural use, and inadequate regulation have been highlighted by Johnson et al. (2007) and Peterson and Kaur (2018). Developing nations, particularly those in Africa, do not have strong regulations for the use or management of antibiotics (Maron et al., 2013). According to the World Organization for Animal Health, many developing countries do not yet have legislation that addresses the proper use of antimicrobials in veterinary practices.

Bacteria have evolved numerous resistance mechanisms to oppose the effects of antibiotics. Certain antibiotics contain chemical bonds, including amides and esters, that are susceptible to hydrolytic cleavage. Certain enzymes, such as extended-spectrum beta-lactamases (ESBLs), weaken antibiotic effectiveness by breaking key chemical bonds. The ESBLs confer resistance to all penicillins, third-generation cephalosporins, and aztreonam, but not to cephamycins or carbapenems (Ćirić et al., 2018). In India, the rise of ESBL-producing strains has led to increased carbapenem use, potentially accelerating the spread of carbapenem-resistant bacteria (Nagshetty et al., 2021). In addition to the consequences of bacterial infection, the susceptibility of the causative agent to antibiotics is considered a risk factor. Drug-resistant *E. coli* strains were found in humans and food in Salvador, Brazil, showing resistance to multiple antibiotics (Melo et al., 2015). Similarly, 65 strains of *E. coli* from animals and animal products in Tunisia demonstrated resistance to eight antibiotics, including kanamycin, gentamicin, and amoxicillin (Badi et al., 2022). These strains may spread to humans via direct contact, contaminated food, or the environment.

Libya faces significant challenges in tackling bacterial food contamination due to limited awareness of food safety and security. Previous studies did not adequately cover the contamination of food from animal origin (meat and dairy) by pathogenic bacteria and their antibiotic resistance patterns.

Infections and diseases caused by antimicrobial drug-resistant pathogens have led to increased morbidity and mortality rates in health care facilities (Alonso et al., 2017). As this problem has grown dramatically, standardized definitions that classify and explain bacteria resistant to different types of antimicrobial drugs are necessary so that epidemiological data can be gathered and analyzed reliably across countries. Since this issue has grown substantially, the term multidrug-resistant (MDR) refers to bacteria that are unsusceptible to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012; Al-Hasani et al., 2023).

In Libya, data on antimicrobial resistance (AMR) of bacteria isolated from food and dairy products are lacking. Nevertheless, a key review article, conducted during the period from 1970 to 2011 and 2002 to 2021, revealed a scarcity of data on AMR, attributing it to the lack of surveillance studies (Ghenghesh et al., 2013; Atia et al., 2022). Another finding from a review of published data over 20 years is that urinary tract infection (UTI) is among the most frequently diagnosed clinical conditions. Notably, the predominant bacterial pathogen causing UTIs is *E. coli*, which displayed a significant resistance rate to commonly prescribed first-line treatments, such as nitrofurantoin and cotrimoxazole. This resistance raises a concern about potential complications, as patients with UTIs face an increased risk of developing renal damage (Hemolytic Uremic Syndrome) and future complications such as renal failure or hypertension, if their treatment is ineffective (Atia et al., 2022).

Developed nations typically have stringent regulations and thorough documentation regarding antibiotic use. However, in many African countries, veterinary antimicrobials are readily available without prescription (Mainda et al., 2015). Recent studies have increasingly focused on the epidemiology of AMR bacteria, especially those producing plasmid-mediated AmpC β-lactamases, carbapenemases, and ESBLs and these studies centered on *E. coli*, a key indicator of antibiotic resistance due to its wide host range and clinical relevance. Studying *E. coli* helped track resistance patterns across populations and the transfer of resistance between animals and humans (van den Bogaard and Stobberingh, 2000). Consumption of animal-derived food remains a significant pathway for spreading antibiotic-resistant pathogens. Therefore, the present study aimed to assess the antimicrobial susceptibility patterns of *E. coli* isolates obtained from different foods of animal origin, with particular focus on ESBL-producing isolates.

MATERIALS AND METHODS

Ethical approval

The current study was conducted according to the guidelines of the Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya.

Sampling, isolation, and identification

A total of 92 isolates of *E. coli* were utilized in the present study, which was a laboratory-based descriptive investigation conducted during the year 2024. Isolates of *E. coli* had been isolated previously from 1120 food samples and stored at -80^{o}C (Garbaj et al., 2016, 2017, 2022; Eshamah et al., 2020). Among the 1120 food samples, 500 were milk and dairy products, and 620 were meat and meat products, as illustrated in Table 1. The samples of food from animal origin were collected randomly from several retail markets from different Libyan cities, including Tripoli, Sabha, Tobruk, and Regdalin. The isolates were revived from storage at -80°C by removing one cryobead from each cryovial, placing it in 5 mL of peptone water, and then incubating it overnight at 35-37°C for further testing.

Table 1. Samples cultured on agar media for the detection of *Escherichia coli* in Libya during 2024

Sample type	No. of samples	No. of positive samples
Raw cows' milk	139	21
Fermented milk (Lben)	86	28
White soft cheese (Massora)	57	13
White soft cheese (Ricotta)	36	4
Goats milk	8	2
She camels' milk	15	2
Butter	4	3
Milk powder	36	2
Ice cream	24	4
Labanh	11	1
Skim milk powder	9	0
Cereal baby food	16	0
UHT milk	8	0
Yogurt	5	0
Growing up formula	18	0
Ready to feed baby formula	10	0
Full-cream milk powder	18	0
Chicken Meat	51	4
Chicken kabab	17	0
Chicken Burger	70	1
Chicken sausage	38	0
Ground chicken	42	0
Chicken liver	5	0
Ground beef	68	0
Beef	73	2
Camel's meat	107	1
Beef Burger	84	2
Beef Sausage	47	1
Beef Kabab	18	1
Total	1120	92

Table 2. Different antibiotic categories used in the present study

Categories	Antibiotics
Penicillin	Amoxicillin 30 µg
	Ampicillin 30 µg
	Amoxicillin Clavunate
	Penicillin 10 µg
	Methicillin 5 µg
	Piperacillin/Tazobactam
	Cloxacillin 5 µg
	Ticarcillin + Clavunate
Cephalosporins	Cefepime 30 µg
	Cefoperazone75µg
	Cefotaxime 30 µg
	Ceftriaxone 30µ
	Cefoxitin 30 µg
Carbapenems	Imipenem 10 µg
	Meropenem 10µ
	Ertapenem 10 µg
Aminoglycosides	Neomycin 10 µg
	Kanamycin 30 µg
	Gentamycin 10 µg
	Tobramycin 10 µg
	Strepromycin 10 µg
Tetracyclines	Tetracycline 30 µg
	Doxycycline 30 µg
	Oxytetracycline 30 µg
Fluoroquinolones	Levofloxacin 5µ
	Ciprofloxacin 5 µg
Glycopeptides	Polymixin 300units
Phenicols	Chloramphenicol 30 µg
Sulfonamides	Sulphamethoxazole/Trimethoprim
Monobactum	Aztreonam 30 µg
Nitrofurantoin	Nitrofurantoin 300 µg
Rifampicin	Rifampicin 5 µg

Antimicrobial susceptibility profile

Antibiotic susceptibility of the *E. coli* isolates was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA), following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2024). Thirty-two antibiotics from 12 classes (Antrim Technology Park, Antrim BT41, England) were tested, as described in Table 2. A sterile nutrient broth was first inoculated with 3-5 colonies from each isolate and then incubated at 37°C for 2-4 hours until it reached an optimum log phase. The suspension was standardized to a 0.5 McFarland turbidity level, then evenly spread onto an MHA plate. After allowing it to dry and absorb for five minutes, the plate was inoculated with a range of commercially available antibiotic discs.

Multiple antibiotic resistance index and antibiotic resistance index

The multiple antibiotic resistance **(**MAR) index and antibiotic resistance index (ARI) indices were estimated and interpreted according to Hinton and Linton (1983) as follows.

MAR index = a/b,

Antibiotic resistance index (ARI) = y / nx,

Where a is the number of antibiotics to which the isolates are resistant, b is the total number of antibiotics exposed, y is the number of resistant isolates, n is the number of isolates, and x is the number of antibiotics. A MAR value $\geq$ 0.2 indicated that antibiotics were ineffective.

Identification of multidrug resistance, extensively drug-resistant, and pan-drug-resistant

The isolates were considered as multidrug resistant (MDR) if they appeared resistant to at least one antimicrobial agent in three or more antimicrobial different classes, extensively drug-resistant (XDR) was defined as resistance to at least one agent in all except two or fewer antimicrobial categories, and pan drug-resistant **(**PDR) was defined as non-susceptibility to all agents in all antimicrobial categories according to Magiorakos et al. (2012).

Detection of extended-spectrum beta-lactamase producers

The incidence of antimicrobial resistance and ESBL-producing *E. coli* was phenotypically assessed by culture and antibiotic susceptibility testing of the isolates. The ESBL-producing *E. coli* was determined by double-disc synergy tests using amoxicillin-clavulanate, cefotaxime, ceftazidime, ceftriaxone, and cefoxitin (Amare et al., 2022).

Colistin susceptibility testing

Colistin resistance was phenotypically assessed using the agar diffusion method, with 4 mg/dL of colistin incorporated into the culture medium (Tartor et al., 2021).

RESULTS

Antimicrobial susceptibility profile of *Escherichia coli* isolates

A total of 32 commonly used antibiotics from 12 categories, including penicillin, cephalosporins, carbapenems, monobactams, aminoglycosides, tetracyclines, fluoroquinolones, glycopeptides, phenicols, sulfonamides, nitrofurantoin, and rifampicin, were used to evaluate the susceptibility of the isolates. The overall isolated *E. coli* susceptibility profiles are shown in Table 3. All *E. coli* isolates showed complete resistance (100%) to penicillin and cloxacillin. A high resistance rate was also observed for neomycin (93.4%), followed by rifampicin (86.9%) and methicillin (75%). In contrast, all isolates were fully susceptible to chloramphenicol. The lowest resistance was noted against carbapenems, imipenem, meropenem, and ertapenem, with a combined resistance rate of only 3.2%. Among the tested cephalosporins, cefepime was the most effective, exhibiting a resistance rate of just 1.08%. Additionally, low resistance was observed against the piperacillin/tazobactam combination (3.2%) and cefoxitin (4.3%). Resistance to nitrofurantoin, levofloxacin, ticarcillin/clavulanate, and kanamycin was similarly low, each at 5.4% (Figures 1, 2, and 3).

Multiple antibiotic resistance index and antibiotic resistance index

All tested isolates exhibited resistance to the antibiotics administered. Table 4 displays the MAR index values. The MAR index for *E. coli* isolates ranged from 0.09 to 0.6. The minimum MAR index value for *E. coli* was 0.09, corresponding to three antimicrobial agents. Conversely, the maximum MAR index value of 0.6 was associated with twenty antimicrobial agents. The calculated ARI values are provided in Table 3.

Multidrug resistance patterns of *Escherichia coli*

The prevalence of MDR bacteria was high based on the antibiotic classification. In the present study, all *E. coli* isolates were resistant to three or more antibiotic categories. For instance, *E. coli* isolate number E315 derived from raw milk was resistant to three categories (Figure 4). The current results indicated that E260 isolated from lben (fermented milk) and E52 isolated from chicken meat were resistant to nine antibiotic categories (Figures 5 and 6). However, XDR and PDR were not detected in *E. coli* isolates.

Phenotypic detection of extended-spectrum beta-lactamase production in *Escherichia coli*

Among the 92 isolates, 43 were phenotypically confirmed to be positive for ESBL. Among all types of food, 17 isolates were detected in raw milk, 10 were isolated from lben, and nine isolates were from dairy products. A total of 7 isolates were detected from meat and its products. All phenotypically ESBL-positive isolates are listed in Table 5.

Colistin-resistant isolates

A total of 83 *E. coli* were identified as phenotypically colistin-resistant isolates using the agar diffusion method.

Table 3. Antibiotic susceptibility profile and antibiotic resistance index of *Escherichia coli* isolates in Libya during 2024

Antibiotic	Susceptible (n)%	Intermediate (n)%	Resistant (n)%	ARI
Imipenem 10 µg	(63) 68.5	(26)28.2	(3) 3.2	0.0010
Meropenem 10 µg	(71) 77.2	(18)19.5	(3) 3.2	0.0010
Ertapenem 10 µg	(67) 72.8	(22)23.9	(3) 3.2	0.0010
Cefepime 30 µg	(64) 69.5	(27) 29.3	(1)1.08	0.0003
Cefoperazone 75 µg	(66) 71.7	(12) 13	(14) 15.2	0.0047
Cefotaxime 30 µg	(25) 27.1	(24) 26	(43) 46.7	0.0146
Ceftriaxone 30 µg	(49) 53.2	(29) 31	(14) 15.2	0.0047
Cefoxitin 30 µg	(76) 82.6	(12) 13	(4) 4.3	0.0013
Amoxicillin 30 µg	(41) 44.5	(32) 34.7	(19) 20.6	0.0064
Ampicillin 30 µg	(17) 18.4	(51) 55.4	(24) 26	0.0081
Amoxicillin Clavunate20/10 µg	(39) 42.3	(41) 44.5	(12) 13	0.0040
Ticarcillin + Clavunate75/10 µg	(58) 63	(29) 31	(5) 5.4	0.0016
Penicillin 10 µg	(0) 0	(0) 0	(92) 100	0.0312
Cloxacillin 5 µg	(0) 0	(0) 0	(92) 100	0.0312
Methicillin 5 µg	(2) 2.1	(21) 22.8	(69) 75	0.0234
Piperacillin/Tazobactam100/10µg	(51) 55.4	(38) 41.3	(3) 3.2	0.0010
Aztreonam 30 µg	(64) 69.5	(10)10.8	(18) 19.5	0.0061
Neomycin 10 µg	(1)1.08	(5) 5.4	(86) 93.4	0.0292
Kanamycin 30 µg	(38) 41.3	(49) 53.2	(5) 5.4	0.0016
Gentamycin 10 µg	(72) 78.2	(13) 14.1	(7) 7.6	0.0023
Tobramycin 10 µg	(46) 50	(36) 39.1	(10) 10.8	0.0033
Streptomycin 10 µg	(36) 39.1	(45) 48.9	(11) 11.9	0.0037
Levofloxacin 5 µg	(83) 90.2	(4) 4.3	(5) 5.4	0.0016
Ciprofloxacin 5 µg	(70) 76	(12) 13	(10) 10.8	0.0033
Oxytetracycline 30 µg	(1)1.08	(62) 67.3	(29) 31.5	0.0089
Tetracycline 30 µg	(74) 80	(1) 1.08	(17) 18.4	0.0057
Doxycycline 30 µg	(63) 68.4	(21) 22.8	(8) 8.6	0.0027
Sulphamethoxazole/Trimethoprim 25 µg	(80) 86.9	(1) 1.08	(11) 11.9	0.0037
Chloramphenicol 30 µg	(89) 96	(3) 3.2	0	0
Nitrofurantoin 300 µg	(73) 79.3	(14) 15.2	(5) 5.4	0.0016
Rifampicin 5 µg	(9) 9.7	(3) 3.2	(80) 86.9	0.0271
Polymixin 300units	(6) 6.5	(23) 25	(63) 68.4	0.0213
Colistin	(9) 9.7	0	(83) 90	0.0281

ARI: Antibiotic resistance index.

Table 4. Multiple antibiotic-resistant index of *E. coli* isolates in Libya during 2024

Antibiotics	Resistant isolates	MARI
20	1	0.6
16	2	0.5
15	1	0.4
14	5	0.4
12	3	0.3
11	4	0.3
10	5	0.3
9	7	0.2
8	17	0.2
7	15	0.2
6	16	0.1
5	4	0.1
4	2	0.1
3	4	0.09

MAR: Multiple antibiotic-resistant index

Table 5. Extended-spectrum beta-lactamase-positive isolates

ESBL-positive *E. coli* isolates	Sources of isolates
E49	Minced meat
E55	Beef kebab
E56	Beef burger
E57	Chicken burger
E59	Goats milk
E61	Maasorra
E63	Cow’s milk
E65	Lben
E66	Lben
E67	Lben
E75	Beef burger
E166	Cow’s milk
E168	Cow’s milk
E169	Cow’s milk
E170	Cow’s milk
E171	Cow’s milk
E173	Lben
E174	Maasorra
E180	Lben
E182	Lben
E188	Cow’s milk
E189	Cow’s milk
E194	Cow’s milk
E195	Cow’s milk
E199	Ricotta
E202	Cow’s milk
E203	Cow’s milk
E204	Butter
E209	Lben
E213	Lben
E221	Chicken meat
E222	Chicken meat
E223	Milk powder
E228	Cow’s milk
E238	Ricotta
E250	Camels’ milk
E252	Cow’s milk
E253	Cow’s milk
E260	Lben
E299	Maasorra
E317	Lben
E321	Ricotta
E323	Milk powder

ESBL: Extended-spectrum beta-lactamase.

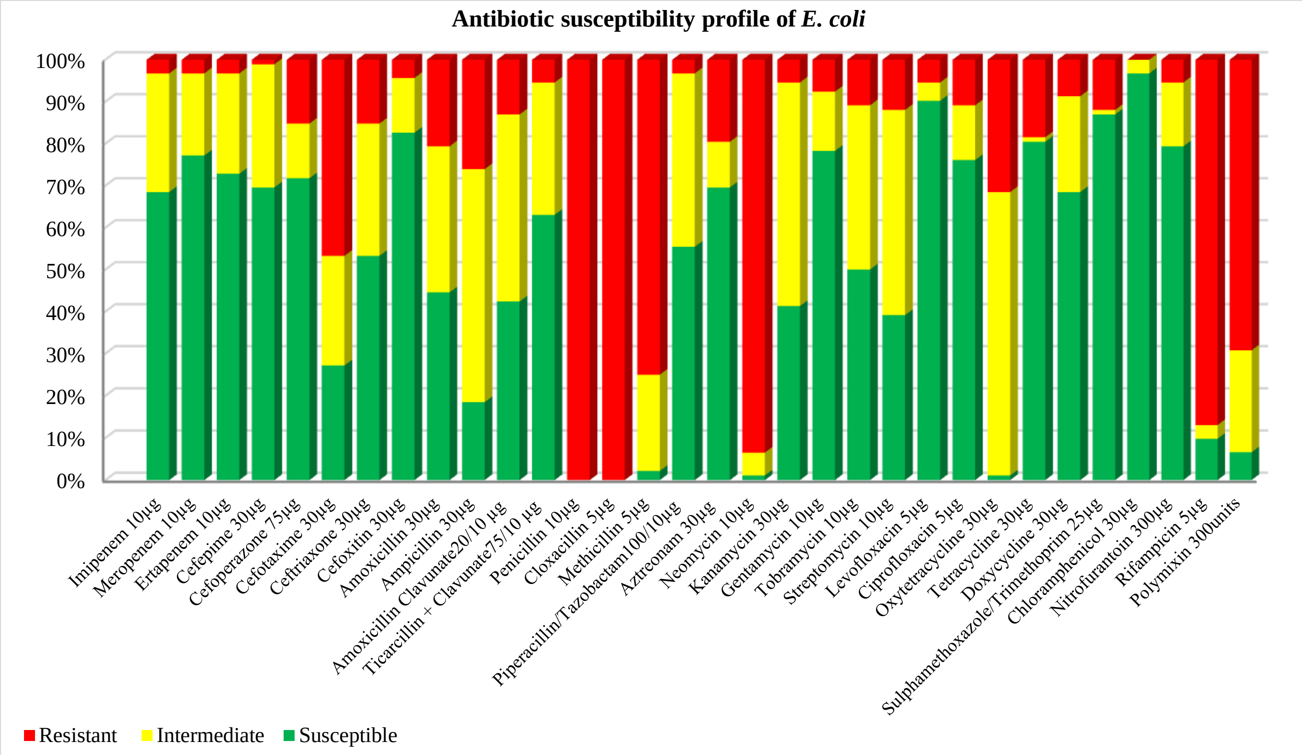

Figure 1. Antimicrobial resistance profiles of *Escherichia coli* isolates against 32 antimicrobial agents in Libya during 2024

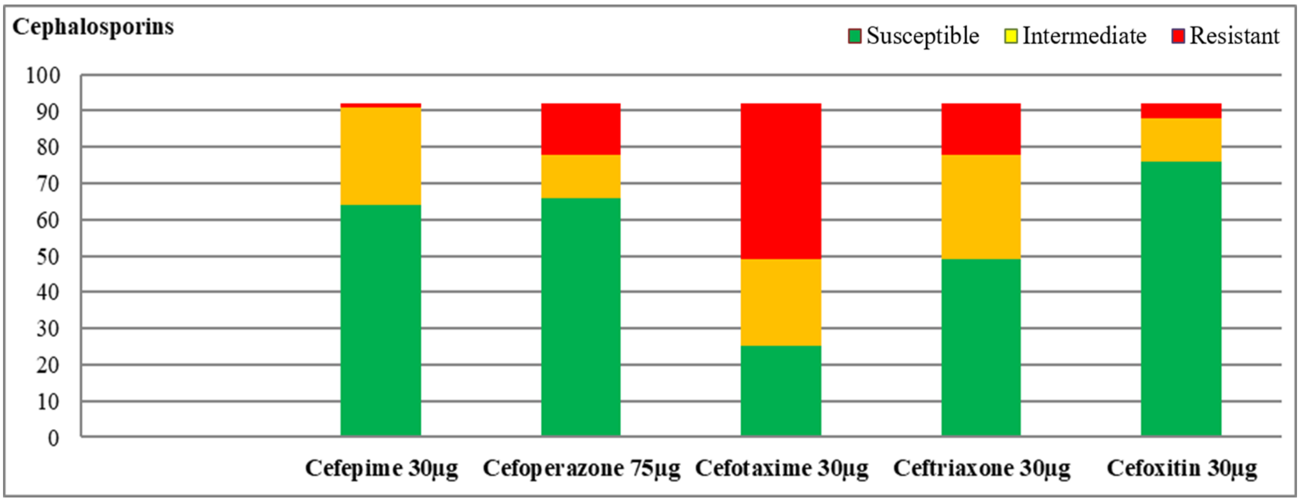

Figure 2. Antimicrobial resistance profiles of all *Escherichia coli* isolates against cephalosporins in Libya during 2024

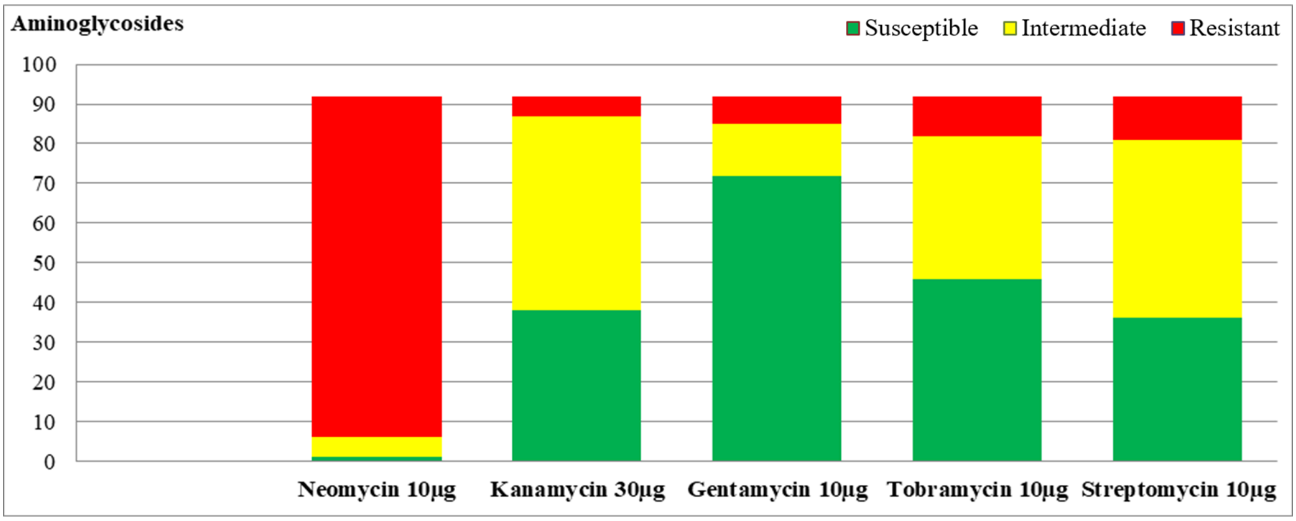

Figure 3. Antimicrobial resistance profiles of all *Escherichia coli* isolates against aminoglycosides in Libya during 2024

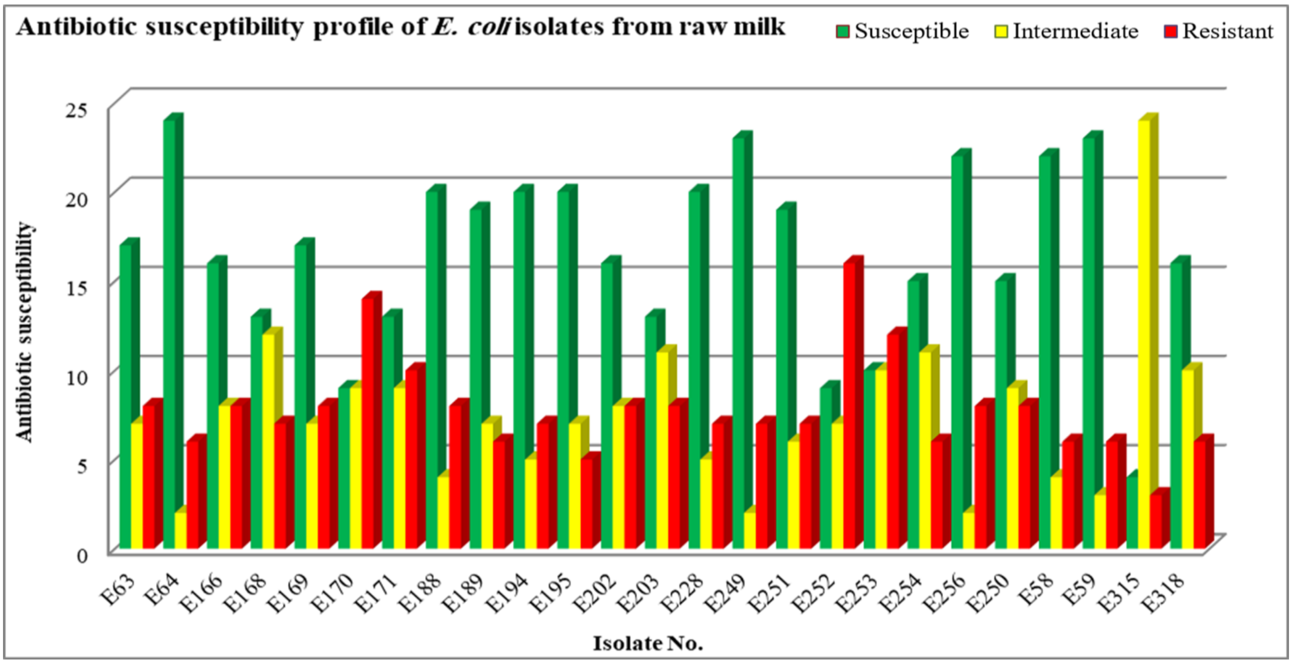

Figure 4. Antimicrobial resistance profiles of *Escherichia coli* isolated from raw milk in Libya during 2024

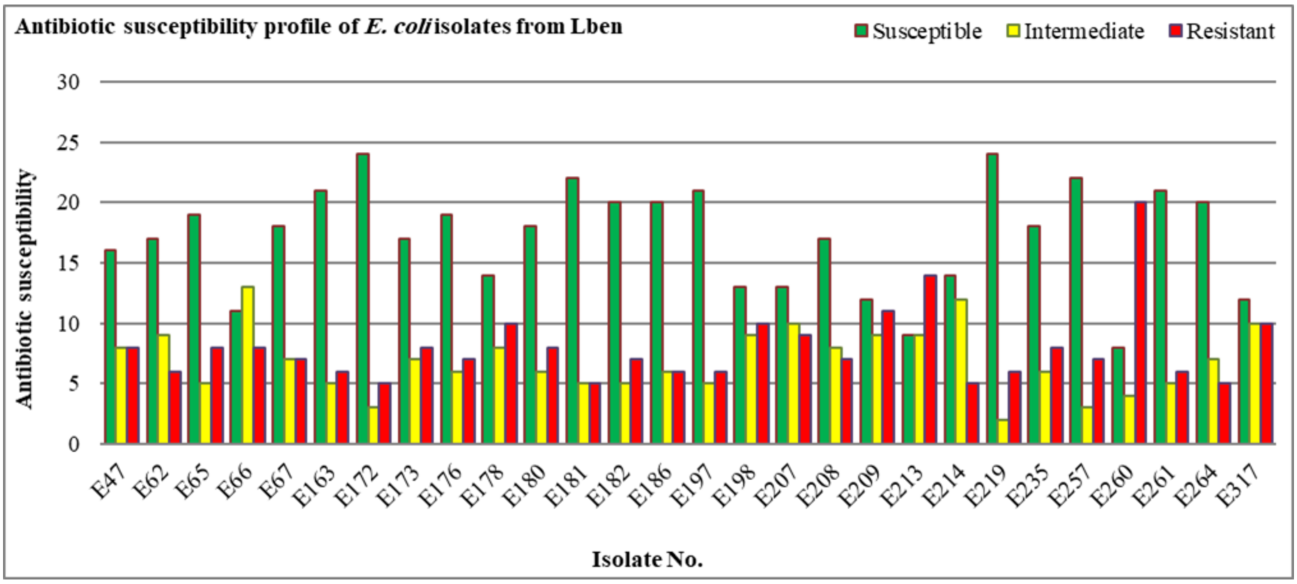

Figure 5. Antimicrobial resistance profiles of *Escherichia coli* isolated from Lben (fermented milk) in Libya during 2024

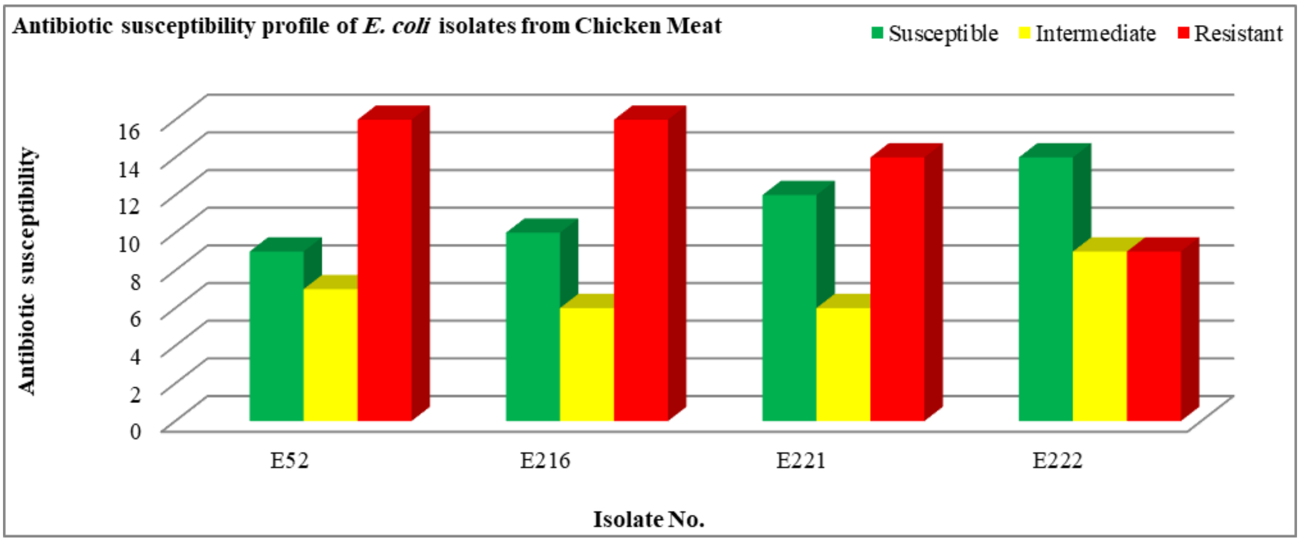

Figure 6. Antimicrobial resistance profiles of *Escherichia coli* isolated from chicken meat in Libya during 2024

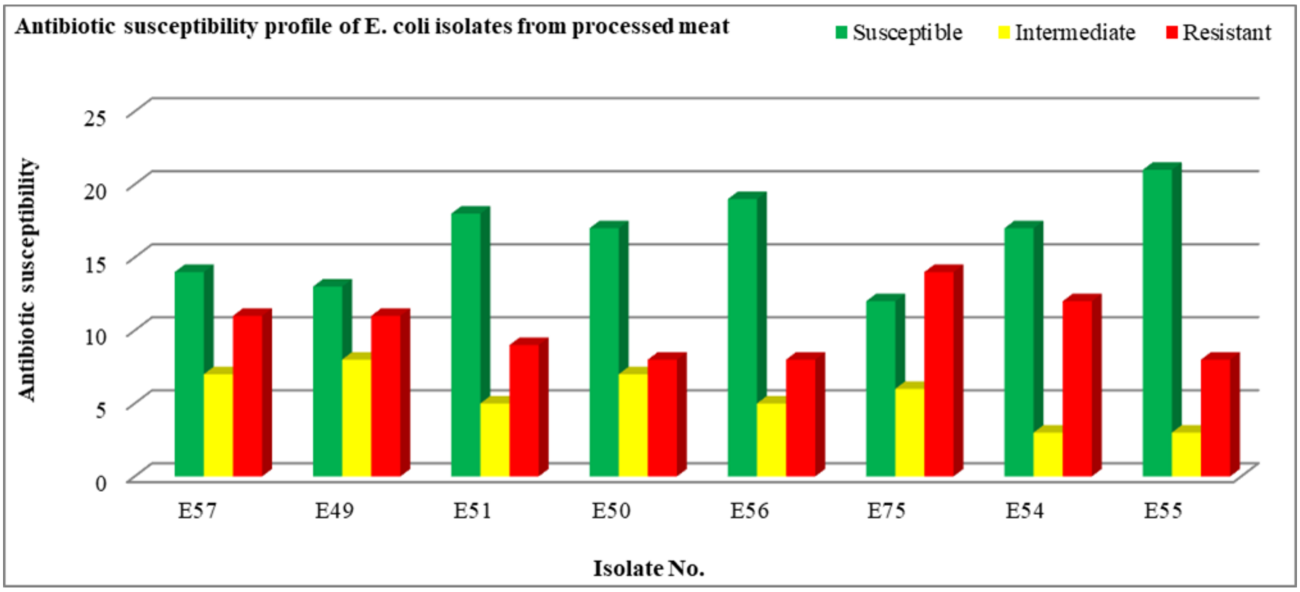

Figure 7. Antimicrobial resistance profiles of *Escherichia coli* isolated from processed meat in Libya during 2024

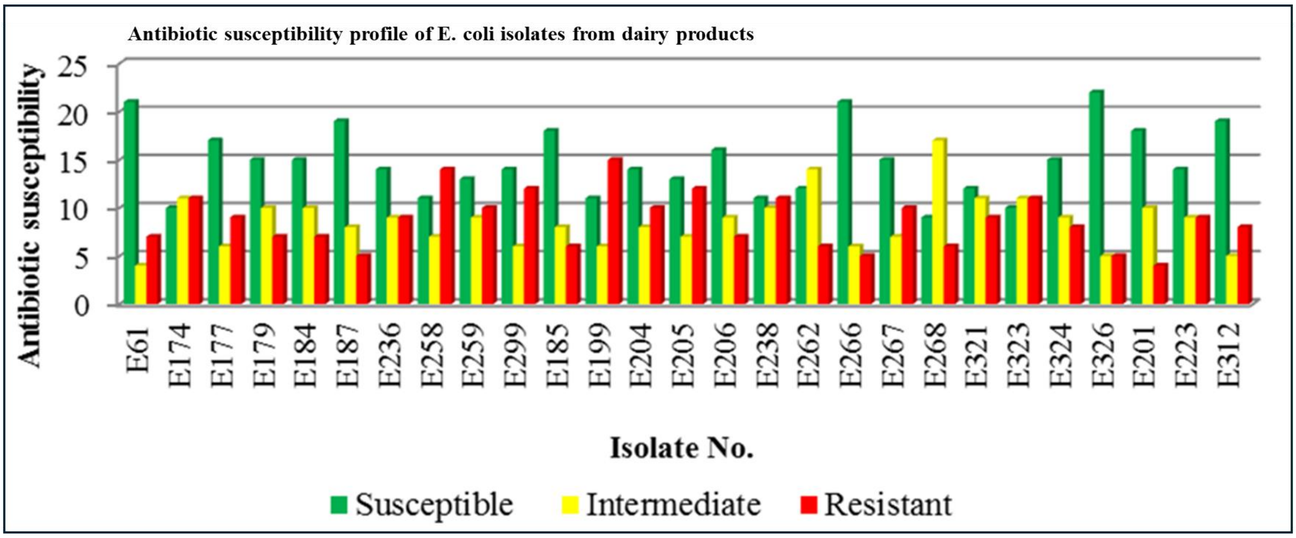

Figure 8. Antimicrobial resistance profiles of *Escherichia coli* isolated from dairy products in Libya during 2024

DISCUSSION

The isolation, antibiotic susceptibility, and molecular sequencing of foodborne bacteria such as *E. coli* from milk, meat, and their products have been the subject of several studies worldwide. Microbes are becoming an increasingly serious problem, and understanding their transmission, resistance mechanisms, and genetic relationships requires a holistic approach to public health. However, there has been a dearth of studies into the prevalence of *E. coli*-resistant strains in animal-origin foods such as milk and meat in Libya. Thus, the present study investigated the incidence of *E. coli* in milk, meat, and their associated products, in addition to examining their antibiotic susceptibility. It should be mentioned that this investigation is part of a series of studies carried out in Libya (Garbaj et al., 2016, 2017; Naas et al., 2017, 2019; Azwai et al., 2016, 2024) on locally isolated microorganisms, probably for the first time, aiming to establish the Libyan Integrated Program for Antimicrobial Resistance Surveillance.

The association of *E. coli* contamination between milk, meat, and their products is primarily due to shared sources and similar handling practices throughout the food production chain (Oliver et al., 2005). Since *E. coli* is a common inhabitant of the intestinal tract of animals, contamination can easily occur during milking or slaughter if fecal matter comes into contact with the product (Callaway et al., 2003). Cross-contamination is also common during processing, especially when hygiene practices are poor or equipment is shared between raw products. Environmental factors such as contaminated water, feed, or farm surroundings, along with inadequate refrigeration and storage, can further promote bacterial growth and spread (Wang et al., 2017). In many cases, small-scale or backyard farming systems where animals for both milk and meat are kept together increase the risk of widespread contamination. Altogether, these factors contribute to the frequent detection of *E. coli* in both dairy and meat products (Omar et al., 2024).

Escherichia coli is commonly used as an indicator of human fecal contamination due to its constant presence in human waste. Its presence in food can result from animal, environmental, or human sources throughout the farm-to-table chain, potentially leading to foodborne illnesses and outbreaks in both humans and animals.

As shown in Table 3 and Figure 1, *E. coli* isolates exhibited the highest resistance to penicillin and cloxacillin (100%), followed by neomycin (93.4%), rifampicin (86.9%), and methicillin (75%). This may reflect the widespread antimicrobial use in the country. All 92 isolates (100%) were MDR, particularly those from lben and chicken, which demonstrated resistance to nine antibiotic classes. No extensively XDR or PDR strains were detected. As the first study in this region, no previous data are available for comparison.

Several studies have examined antimicrobial resistance in *E. coli* from animal-derived food in neighboring countries (Faour-Klingbeil and Todd, 2019). Jouini et al. (2009) analyzed 98 isolates of *E. coli* from 40 food samples in Tunisia using disk diffusion and agar dilution methods. High resistance was observed in over 43% of isolates to antibiotics such as tetracycline, sulfonamides, nalidixic acid, ampicillin, streptomycin, and trimethoprim-sulfamethoxazole. However, all isolates remained susceptible to cefotaxime, ceftazidime, cefoxitin, aztreonam, and amikacin. The emergence of such resistant strains poses a serious global health threat by limiting treatment options for bacterial infections.

Compared to previous studies in North Africa and the Middle East, the antibiotic resistance rates observed in the present study indicated some variation. In Algeria, *E. coli* from retail chicken meat showed higher resistance to sulfamethoxazole-trimethoprim and tetracycline (96.6%) and amoxicillin (65.5%) than reported here (Laarem et al., 2017). In contrast, findings from Egypt demonstrated similar resistance patterns, with moderate rates in *E. coli* from raw milk and cheese, including tetracycline (27.5%), ampicillin (18.9%), and streptomycin (18.5%). Lower resistance rates were observed for cefotaxime (4.5%) and ciprofloxacin (1.4%). These findings highlight significant antibiotic resistance in the region (Ombarak et al., 2018).

Although the resistance rate to carbapenems among different food samples was very low, the findings of the current study were much higher than those of clinical samples (0.22%-0.64%) reported in China (Li et al., 2023). Carbapenems are highly effective against *E. coli* infections; however, their use should be cautious to prevent the sporadic emergence of resistance.

Antibiotics frequently administered in food animal production for prophylaxis, therapeutic purposes, and growth promotion include sulfamethoxazole, enrofloxacin, benzylpenicillin, tylosin, amoxicillin, trimethoprim, oxytetracycline, ampicillin, and streptopenicillin. In poultry production, agents such as amikacin, neomycin, enrofloxacin, doxycycline, tetracycline, tilmicosin, and colistin sulfate are commonly employed as growth promoters (Chattopadhyay, 2014). Antibiotics and growth promoters are often applied indiscriminately to entire flocks, frequently without the guidance of veterinary professionals or public health authorities. Poultry producers or farmers commonly neglect the recommended antibiotic withdrawal period before selling products like meat, milk, dairy products, eggs, and fish (Khalifa et al., 2024). In broiler chickens, antimicrobials are routinely added to feed and water for disease prevention, leading to residues in meat and other products, posing significant public health risks. Antibiotics, including doxycycline, colistin sulfate, neomycin, tetracycline, enrofloxacin, ciprofloxacin, and amikacin, are commonly used in food animals for treatment, prophylaxis, and growth promotion. However, residues of these antibiotics can enter the human food chain, particularly if poor record-keeping leads to the sale of animal products before the required withdrawal periods are observed (Roess et al., 2013).

All isolates from different food samples were highly resistant to commonly used antibiotics, particularly the medicines belonging to the penicillin class (penicillin, methicillin, and cloxacillin). Likewise, these present results were close to the findings of Shrestha et al. (2019), who concluded that *E. coli* isolates from clinical samples exhibited high resistance against penicillin (100%). In China, the prevalence of resistant *E. coli* in both animals and foods has notably increased; studies indicate that ampicillin has documented the highest resistance rates compared to other antibiotics (60.5%; Sun et al., 2021; Liu et al., 2024). It is not surprising that the penicillin class is the most common and extensively used antibiotic, not only in humans but also in food-producing animals.

During the present study, *E. coli* isolates indicated lower resistance to amoxicillin and ampicillin (20.6%-26%). However, this contrasted with Rafiq et al. (2024), who reported 100% resistance to amoxicillin in *E. coli* from chicken meat. Similarly, Al Azad et al. (2019) and Islam et al. (2024) found high ampicillin resistance (94%-100%) in *E. coli* from broiler chickens.

The current results demonstrated that all *E. coli* isolates were sensitive to nitrofurantoin and carbapenems (imipenem, meropenem, and ertapenem), with only 3.2% showing resistance to carbapenems. These findings align with Moglad (2020), who reported low resistance (1.4%) in clinical isolates, and are consistent with results from Zanzibar, Tanzania, reported by Omar et al. (2024).

In the present study, the MAR index of *E. coli* ranged from 0.09 to 0.6. An MAR index above 0.2 suggested a high risk of MDR infection. Notably, *E. coli* from chicken meat showed resistance to 9 of 12 tested antibiotic classes, likely due to the overuse of antimicrobials by Libyan poultry farmers.

Out of all *E. coli* isolates, 43 were confirmed as ESBL producers, with a higher prevalence in raw milk than in meat products. This contrasted with the findings of Omar et al. (2024), who reported more ESBL *E. coli* in broiler chicken organs, but aligns with the findings of Mgaya et al. (2021) from Tanzania. Differences may stem from differences in farm hygiene and biosecurity practices (Zhang et al., 2015). The high prevalence of ESBL-producing *E. coli* observed in the present study may be attributed not only to the widespread use of antibiotics as prophylactics in food animals but also to horizontal gene transfer mechanisms. The routine use of antibiotics such as beta-lactams for disease prevention in livestock creates selective pressure that favors the survival and proliferation of resistant strains (Chattopadhyay, 2014). Additionally, ESBL genes commonly carried on mobile genetic elements such as plasmids, integrons, and transposons can be rapidly transferred between bacterial populations via conjugation, transformation, or transduction, facilitating the spread of resistance even across different species and environments (Carattoli, 2013; Rozwandowicz et al., 2018). This dual mechanism of antibiotic pressure and genetic exchange accelerates the dissemination of ESBL *E. coli* in both animal and human reservoirs, particularly in settings with limited biosecurity and antimicrobial regulation.

The current study revealed different antibiotic resistance rates across different sample types. Chicken meat isolates showed higher resistance than processed meat isolates, which was linked to a greater prevalence of ESBL-positive strains and prolonged antibiotic use for poultry treatment. Resistance was lower in dairy product isolates compared with raw milk and lben isolates, and the reason could be related to heat treatment. In addition, the presence of *E. coli* in meat, combined with low sanitary management, highlighted potential health risks for consumers. While cooking generally destroys *E. coli*, risks arise from undercooking, poor hygiene, cross-contamination, and improper handling.

An alternative explanation for the high *E. coli* contamination in raw and fermented milk was the poor hygiene practices during milking, inadequate equipment sanitation, and a lack of pasteurization. *E. coli* is commonly present in bovine feces, and contamination can occur when udders, hands, or milking tools are not adequately cleaned (Oliver et al., 2005). In many traditional dairy settings, raw milk is handled without refrigeration, allowing bacteria to multiply rapidly, especially in warm climates (FAO/WHO, 2006). Additionally, water used to clean equipment or animals may be contaminated, serving as another source of *E. coli* introduction. While fermentation is often perceived to reduce microbial load, it does not ensure safety if initial milk quality is poor or if fermentation conditions (time, temperature, starter cultures) are not properly controlled (Beukes et al., 2001). In regions where fermented milk products such as lben are traditionally consumed, cross-contamination and storage in unsterilized containers further increase the risk (Gran et al., 2003). Moreover, the absence of the pasteurization process that effectively kills *E. coli* makes both raw and fermented milk products more susceptible to contamination (Chye et al., 2004).

The food chain serves as the primary pathway for the transmission of antibiotic-resistant bacteria between animals and humans. Traditional fermented dairy products, which are not heat-treated before consumption, act as a vehicle for antibiotic-resistant bacteria, directly connecting the animals' natural microbiota to the human gastrointestinal tract (Hao et al., 2014).

To mitigate the emergence and spread of ESBL-producing *E. coli*, a One Health approach is essential, recognizing the interconnectedness of human, animal, and environmental health. This integrated strategy involves coordinated efforts among veterinarians, medical professionals, farmers, environmentalists, and policymakers to reduce antimicrobial misuse, improve surveillance, and strengthen biosecurity practices across sectors. Promoting responsible antibiotic stewardship in livestock production, improving food hygiene, and implementing routine screening of animal-derived foods for resistant pathogens are critical components. Additionally, strengthening laboratory capacities and data-sharing frameworks across human and veterinary health systems can improve early detection and response to antimicrobial resistance threats. Adoption of the One Health framework is particularly important in regions with high resistance burdens and limited regulatory enforcement, such as North Africa and the Middle East, to safeguard both public health and food safety (FAO, OIE, WHO, 2022).

CONCLUSION

The present study highlighted the significant presence of multidrug-resistant (MDR) *E. coli* in food products of animal origin, particularly raw milk, fermented milk, and chicken meat in Libya. All isolates indicated resistance to at least three antibiotic classes, with some demonstrating resistance to up to nine, indicating a high prevalence of MDR. Notably, 100% of isolates exhibited resistance to penicillin and cloxacillin, while 43 isolates were phenotypically confirmed as ESBL producers, with higher prevalence in raw milk samples. However, no extensively drug-resistant or pan-drug-resistant *E. coli* were detected, possibly due to limited exposure to last-resort antibiotics such as carbapenems and nitrofurantoin, to which most isolates remained susceptible. The high multiple antibiotic resistance index values further suggested intense antibiotic selection pressure, likely due to unregulated and prophylactic use of antimicrobials in animal farming. The present findings underscore the need for stricter control measures on antibiotic use in agriculture, proper hygiene practices across the food production chain, and enhanced public awareness about antimicrobial resistance. Implementing the One Health approach integrating animal, human, and environmental health, is critical for curbing the spread of resistant *E. coli* and safeguarding food safety and public health. Future restudies should employ genomic analysis to characterize the resistance genes and mobile genetic elements responsible for the observed MDR in Libyan food products.

DECLARATIONS

Authors' contributions

Salah Mohamed Azwai, Aboubaker Mohamed Garbaj, Allaaeddin Ali El Salabi, and Ibrahim Mohamed Eldaghayes designed and planned the study. Salah Mohamed Azwai, Aboubaker Mohamed Garbaj, Jihan Ali Sherif, Samira Abd Farag, Salem Farhat Abureema, and Fatim Taher Gammoudi did the laboratory work. Salah Mohamed Azwai, Aboubaker Mohamed Garbaj, Ibrahim Mohamed Eldaghayes, Jihan Ali Sherif, Samira Abd Farag, Salem Farhat Abureema, and Fatim Taher Gammoudi contributed equally in the preparation of the manuscript. All authors contributed to the revision of the final manuscript.

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Competing interests

The authors declare that there is no conflict of interest.

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Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

Availability of data and materials

All data generated during the study are relevant and included in this published article and will be available from the corresponding author upon reasonable request.

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