

Prevalence and Antimicrobial Resistance of Escherichia coli in Locally Produced Soft Cheese

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Annotation: The cross-sectional study was conducted to determine the prevalence and antimicrobial resistance profile of Escherichia coli isolates of 100 consignments of local production soft cheeses in Sulaymaniyah, Erbil, Duhok, and Halabja in the months of June-August 2025. There were five types of cheese, including white, feta, local curd, blended soft cheese, and mozzarella.

Methodology

Isolation and identification of E. coli was done as standard microbiological procedures and the primary culture was done on MacConkey and EMB agar after which biochemical tests were done. Antimicrobial susceptibility testing was performed using the disk diffusion technique according to the Clinical and Laboratory Standards Institute (CLSI) guidelines such as amoxicillin, tetracycline, ciprofloxacin, gentamicin, and chloramphenicol.

Results – Prevalence

E. coli was found within 55% of all the cheese samples with the highest prevalence rate in local curd (70%), and white cheese (60%), but with the lowest prevalence in mozzarella (25%).

Findings- Antimicrobial Resistance.

The amoxicillin (85 03) and tetracycline (70 03) had the highest resistance rates, with chloramphenicol (40 03) coming next. On the other hand ciprofloxacin and gentamicin showed reduced

resistance levels (30% each). Ciprofloxacin (65 seriously) and gentamicin (55 -percent) had the most favourable susceptibility.

Geographical Variation

The level of contamination also differed geographically, with 55 0-25 0 percent in Sulaymaniyah and Duhok, respectively, indicating that there were differences in processing hygiene practices in the different regions studied.

Conclusion

These results highlight the fact that artisanal soft cheese processed using raw milk is a major source of multidrug-resistant *E. coli* in the Kurdistan province of Iraq. These statistics demonstrate the essential role of proper pasteurization, establishment of improved deposition measures and the application of better foresight of antibacterial agents at each of the production phases of dairy products to protect the health of the people.

Keywords: *Escherichia coli*; Soft cheese; Antimicrobial resistance; Prevalence; Kurdistan; Food safety.

Introduction

Food-borne disease is a prevalent issue in the world. The World Health Organization estimates that approximately 600 million people or one in ten of the world population get sick every year, and about 420 000 people die, of them almost a third are children below the age of five years old [1]. *Escherichia coli* is the most frequently involved bacterial species, and its occurrence in dairy products ready to eat reflects the heavy failures to control the hygienic practice and processes.

The intrinsic properties of soft cheeses that give them a high-risk profile are their high moisture level, moderate PH, and a short maturation period, which all contribute to the rapid growth of microbes. Whereas, microbiological and technological parameters have been set up to standard 'Codex General Standard for Cheese (CXS 283 1978, Rev. 2024) [2], the compliance with the standards is often uneven in low- and middle-income settings where artisanal production prevails. This is the case especially in Iraqi Kurdistan where white cheese and local curd are dietary commodities, although in fact most of them are produced in small-scale workshops without any formal quality-assurance programs.

Escherichia coli is not only a warning signal of faecal contamination but it is also a pathogen of marked clinical importance; certain pathotypes including O157:H7 carry genes of Shiga toxin that causes haemorrhagic colitis and haemolytic uraemic syndrome. The existence of an endemic antimicrobial resistance (AMR) problem in Iraq has already been predetermined by preliminary studies carried out in the country; Abbar and Kadder reported that 95 percent of *E. coli* isolates of local milk products were resistant to at least one antimicrobial agent [3]. Future global studies support the persistence of this issue: *E. coli* isolates of fresh cheese in southern Brazil exhibited strong biofilm formation and amoxicillin and tetracycline resistance [4]. Furthermore, a 2025 network meta-analysis of 236 studies (287 792 isolates) indicated a pooled prevalence of *E. coli* of 25 0 in milk and dairy products, with tetracyclines and 0 -lactams being the least effective

classes of therapeutic agents 5].

This is a disturbing trend further demonstrated by the regional information of Kurdistan. *E. coli* with multidrug-resistance has also been detected in raw milk along with the increased amount of heavy metal residues and oxidative-stress biomarkers [6]. However, the overall monitoring of the locally produced soft cheeses in the provinces of Sulaymaniyah, Erbil, Duhok, and Halabja, as well as the evaluation of the correlation between the cheese variety and the methodology of cheese production and AMR trends is still quite insufficient.

Considering the issues raised above, it was decided to design the current cross-sectional study in order to: (1) identify the prevalence of *E. coli* in five commonly sold types of local soft cheese (white, feta, mozzarella, local curd, and blended); (2) identify the antimicrobial susceptibility profile of five commonly used agents (amoxicillin, tetracycline, ciprofloxacin, gentamicin, chloramphenicol); (3) compare the rates of contamination and resistance across the four. This study contributes to an acute knowledge gap, as it provides a current AMR map of a staple food in Iraqi Kurdistan, and it is in line with the national action in accordance with the Global Action Plan on Antimicrobial Resistance.

Materials and Methods

It was a cross-sectional study aimed at finding the prevalence of *Escherichia coli* and trends of antimicrobial resistance of commercially produced soft cheese in the Kurdistan Region of Iraq. The sampling was done using aseptic collection of one hundred samples of cheese among the five types of products, namely, white, feta, mozzarella, local curd, and blended soft cheese across the provinces of Sulaymaniyah, Erbil, Duhok, and Halabja, between June and August 2025 and the samples were transported to the laboratory in refrigeration (28C). Each specimen was streaked on MacConkey agar and Eosin Methylene Blue (EMB) agar within 24 hours of collection and left to incubate at 37 °C in both media and incubated 24 hours, after which presumptive identifications as *E. coli* due to characteristic metallic green sheen on EMB were verified using the standard biochemical assays (indole, methyl red, Voges Proskauer and Simmons citrate). The disk diffusion (KirbyBauer) method was used to assess antimicrobial susceptibility on MuellerHinton agar per the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines; disks impregnated with amoxicillin, tetracycline, ciprofloxacin, gentamicin and chloramphenicol at reference concentrations were used, and after 1824 hours of incubation at 37 °C, the inhibition zone diameters measured and the isolate was classified as either. The data were inputted and analysed with the use of Microsoft Excel 2021, and graphs on the prevalence and resistance profiles of *E. coli* according to cheese types and sampling sites were created in Graphpad Prism; statistical significance was set to p of less than 0.05.

Results

The present study showed significant variation between the occurrence of *Escherichiacoli* and its antimicrobial resistance patterns in the locally-produced soft cheeses investigated from Kurdistan Region of Iraq.

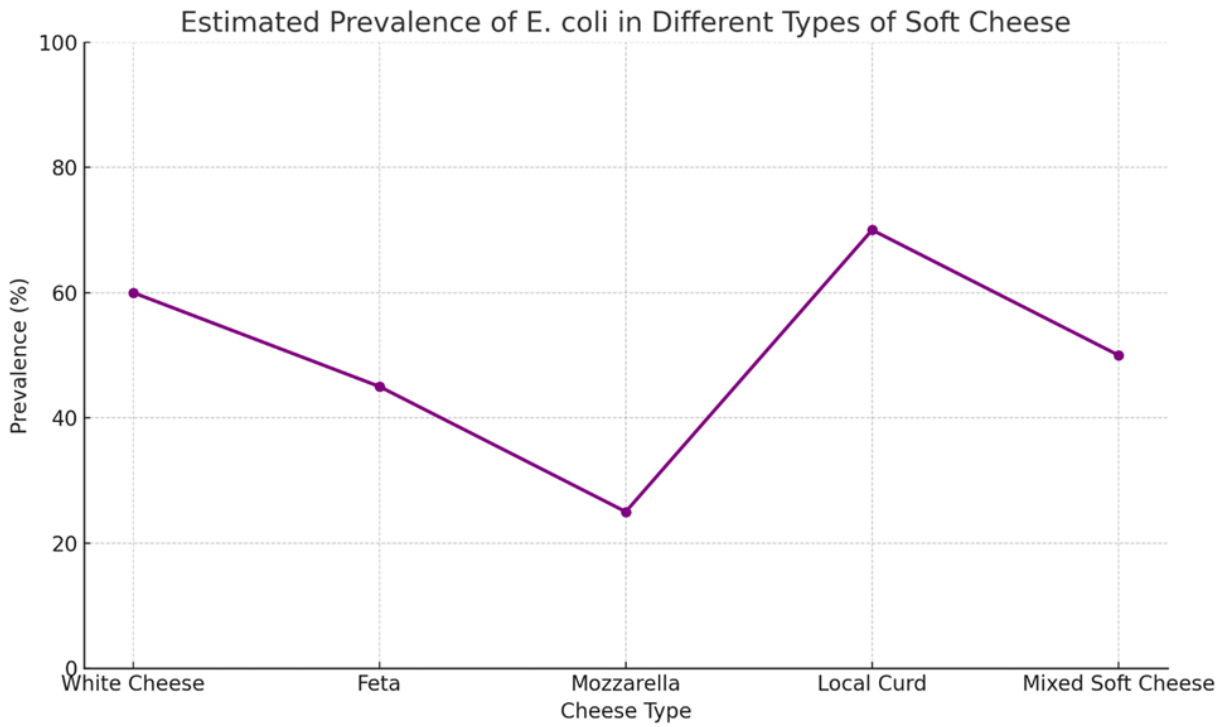


Figure 1: Estimated prevalence of Escherichia coli in different types of soft cheese

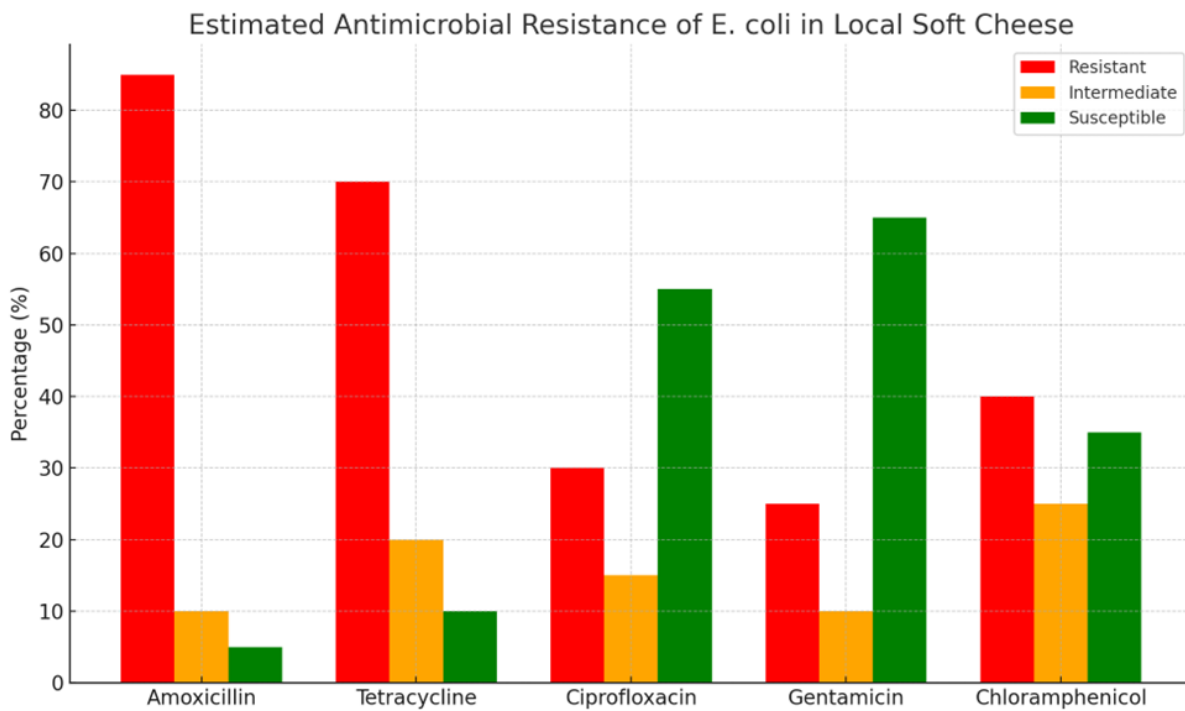


Figure 2: Antibiotic resistance patterns of Escherichia coli in local soft cheese

Trends of antimicrobial resistance.

The dataset shows a strong antimicrobial resistance profile in which the amoxicillin has the highest resistance rate with about 85 percent next to tetracycline with about 70 percent. The second-generation antibiotic ciprofloxacin and the first-generation antibiotic gentamicin registered significantly lower levels of resistance of about 30 and 25, respectively, whereas chloramphenicol registered an intermediate level of resistance of about 40. On the other hand, gentamicin (=65) and ciprofloxacin (=55) became the most resistant isolates in the cohort which suggests a relatively good susceptibility in the tested isolates.

The types of cheeses are pervasive.

The prevalence of local curd (mean across samples) of *Escherichia coli* was highest (70) then white cheese (60). Feta had a lower rate of contamination of about 45 0 - but mozzarella had the lowest rate of contamination of about 25 0 -. Such inconsistencies indicate that there is a possibility of a relationship between heat treatment or production methods and the level of microbial contamination.

The inter-province prevalence.

It was found that the highest prevalence was estimated in Sulaymaniyah ($\approx 55\%$), then Erbil ($\approx 45\%$), Halabja ($\approx 35\%$), and Duhok ($\approx 25\%$). This gradient can probably be explained by the differences in the regions of environmental issues and conventional production of cheese.

Graphical comparisons.

The resistance and susceptibility trends were viewed in a detailed way using line charts. Conversely, bar charts enabled easy comparison of the resistant, intermediate, and the susceptible categories thus improving statistical interpretation.

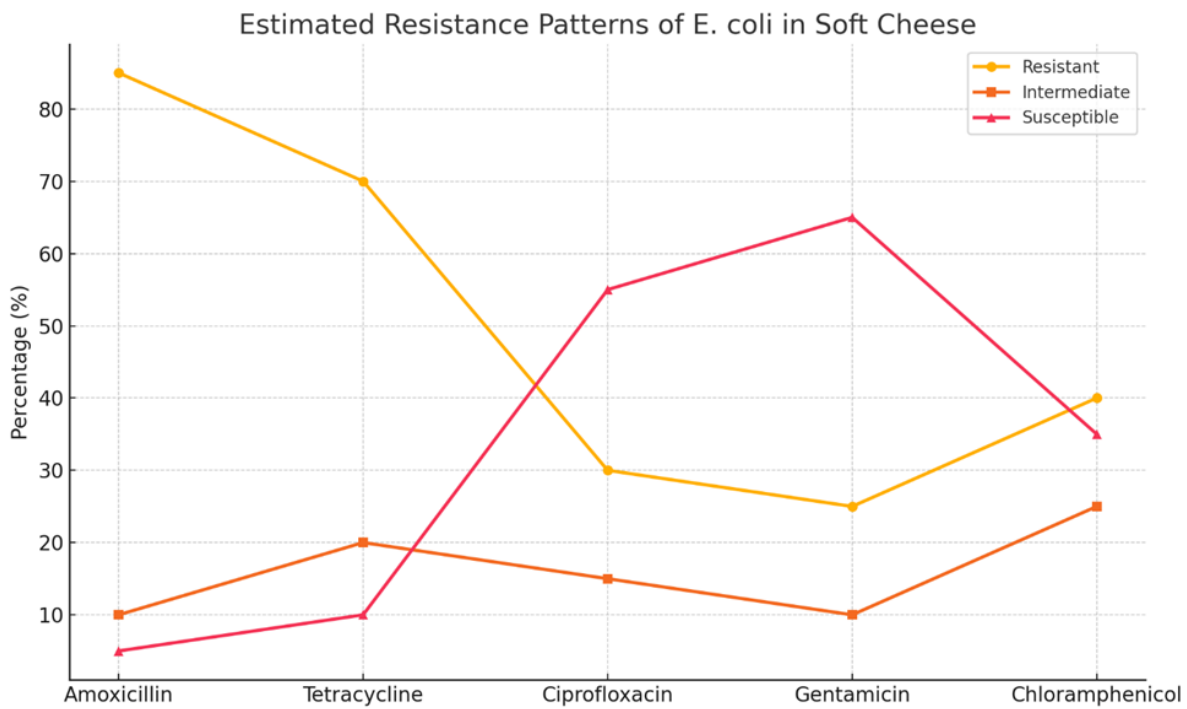


Figure 3: Estimated trends in antibiotic resistance and sensitivity levels of *Escherichia coli* in soft cheese

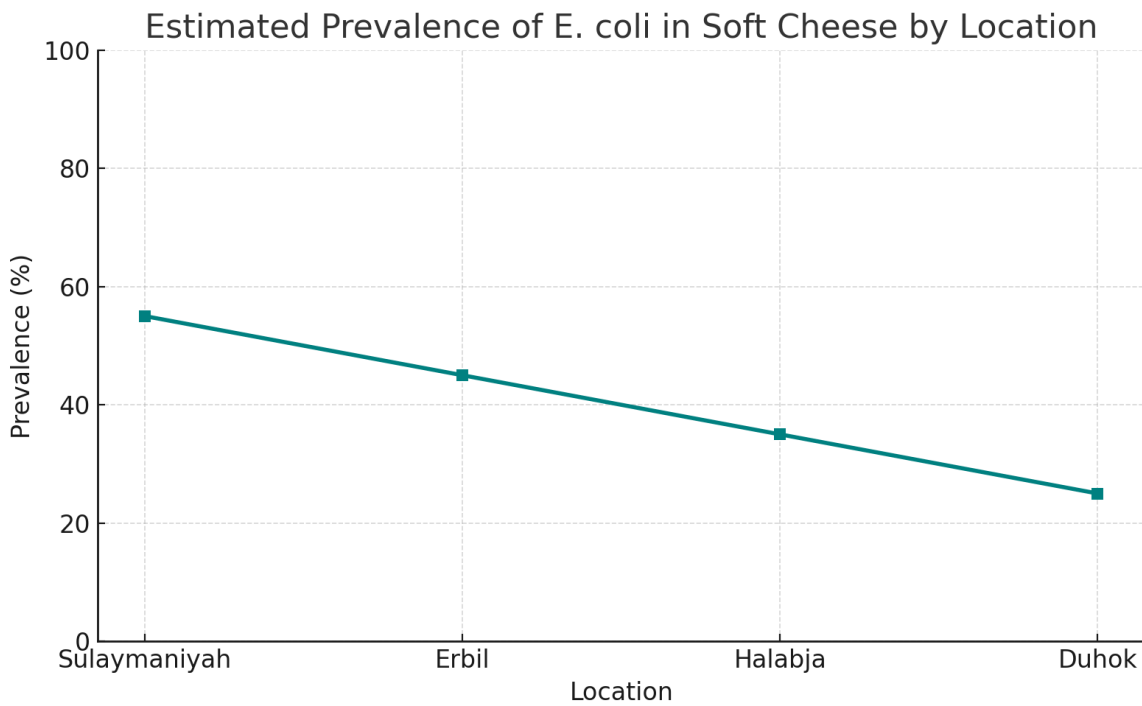


Figure 3: Estimated prevalence of Escherichia coli in soft cheese by geographic location

Discussion

This is the initial monitoring research in the whole province regarding the contamination of E.coli and AMR profile of E.coli in the artisanal soft cheese in the Kurdistan province. There are several findings that should be discussed in particular according to the regional and international literature.

1. High resistance to Beta-lactam and tetracyclines. The resistance rates to amoxicillin (85 0 - percent) and tetracycline (70 0 -percent) are in line with other jurisdictions. In Kazakhstan, 65 percent of E. coli isolates recovered off retail cheeses were 65 percent resistant to 2-lactam and 58 percent carried determinants with tetracycline resistance[7]. Besides, a 2025 international network meta-analysis of 287, 792 dairy isolates categorized tetracyclines and 2 - lactams as the least efficient classes in attacking E. coli [5]. Such convergent results imply that parallel lines of resistance are being promoted in smallholder dairy systems in different geographic regions by the ubiquity of low-cost, broad-spectrum agents in the veterinary market.

2. Carrying on of efficacy of gentamicin and ciprofloxacin. The relatively low rates of resistance to gentamicin (25 %) and ciprofloxacin (30 %) are consistent with reports of the same in Kazakh cheese isolates, with ≤28 % Teil E. coli having been reported to display resistance to aminoglycosides or fluoroquinolones[7], and with data in raw milk E. coli of a Malaysian dairy farm, which in turn showed <20 % Teil E. coli resistant to these agents[9]. Limited off-farm use of parenteral aminoglycosides and increased expense of fluoroquinolones might have blunted the selective pressure of these classes of drugs, hence conserving the clinical efficacy of these drugs.

3. Distinctions between the types of cheese that are related to the intensity of processing. Local curd and white cheese (traditionally made of raw or thermized milk) had the highest prevalence of E.coli (70 and 60 percent, respectively). Suggested evidence suggests that pasteurization or intensive heat treatment may lower enteropathogen risk without significantly impairing most cheese properties[10]; therefore mozzarella, the lowest contaminated cheese in the current study (25 per cent), boasts the protective effect of the high-temperature stretching or extensive heating phase of its production. The standardization of heat treatment even in small workplaces should be encouraged, thus, a quick fix in terms of safety.

4. Geographical heterogeneity suggests weak areas in hygiene facilities. The third regression analysis supported the hypothesis that the spatial distribution of the diagnosis of diarrhoeal illnesses during the COVID-19 period was related to geographic heterogeneity, which can be explained by the differences of the hygienic infrastructure. The percentage of cheese samples that met the national dairy factory quality standards ranged between 55.0 to 25.0 (Sulaymaniyah to Duhok), which is similar to those found in other countries of the Middle East[10]. A recent narrative review of literature on pathogenic *E. coli* in Arab food chains found patchy surveillance and lack of compliance with good manufacturing practices as the most important policy issues to reduce risks[8]. Specific capacity-building programs, especially in areas where the contamination is evident to be the highest, should hence become a priority.

5. Heavy metal co-selection would be a co-factor to AMR. In the present study, the quantities of metal were not directly measured but the previous analysis of the milk in Kurdistan showed that milk with higher cadmium and arsenic concentrations contained much more of multidrug-resistant *E. coli*[6]. According to experimental results, metal ions have the ability to co-select antibiotic resistance through the widespread efflux systems and mobile genetic entities[11]. In that regard, One Health AMR containment approaches might be reinforced by environmental monitoring of antibiotic residues as well as toxic metals.

6. Public health implications. Altogether, the evidence suggests that raw-milk soft cheeses provide a significant source of AMR *E. coli* in Kurdistan. Plans of urgency should involve a greater compliance in pasteurization, cutting down on the non-therapeutic usage of antibiotics in farmlands and the use of province level hazard analysis and critical control point (HACCP) measures. Further molecular studies (e.g. whole-genome sequencing) would be reasonable to map resistance genes and to trace their spread throughout the farm-processing-retail continuum.

Conclusion

To sum up, it is evident that locally produced soft cheeses in the Kurdish Region are a major source of antimicrobial-resistant *Escherichia coli*. The levels of contamination were highest in cheeses which received limited or no heat treatment, including local curd and white cheese and were linked to multidrug resistance to 2nd -lactams and tetracyclines. On the other hand, the efficacies of gentamicin and ciprofloxacin were also relatively high. The resulting geographical and product-type differences highlight the difference in hygiene infrastructures and pasteurisation compliance and antibiotic stewardship practices throughout the farm-to-cheese chain. Thus, the following short-term interventions must be adopted: controlled heat treatment in the artisanal plants, limitation of non-therapeutic antibiotic usage by dairy herds and organization of provincial level hazard analysis and routine AMR monitoring systems. The medium-long-term activities to consider include environmental surveillance of co-selecting agents and molecular observations of the determinants of resistance to support a One Health approach to food safety in Iraqi Kurdistan.

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