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Prevalence of multidrug resistant *E. coli* strains in dairy animals in relation to seasonal variation at Beheira Governorate

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ABSTRACT

Escherichia coli extensive distribution, quick spread throughout the dairy herds and significant financial losses and thought to be the primary environmental factor responsible for mastitis in lactating dairy farms. A total of 1568 raw milk samples collected from lactating dairy animals (cattle, sheep, and goats) in order to identify and assess the antimicrobial resistance in *E. coli* isolates as well as its correlation with climate change, this study was carried out. Between January 2023 and December 2024, two annual cycles were conducted to collect the samples in three locations of the Beheira governorate: Badr-South El-Tahrir, Itay El Barud, and Rosetta. Bacteriological isolation and identification followed by antimicrobial susceptibility testing against 7 antimicrobial agents was determined. The climate temperature and humidity recorded throughout 2023-2024. The results recorded that the overall prevalence of *E. coli* was 23.8% (373/1568) of all studied dairy farms. The prevalence of *E. coli* in raw milk samples of cattle, sheep, and goats were 29.1%, 22.2%, and 19.1%, respectively, with detailed prevalence in cattle (27.6% in 2023; 30.9% in 2024), in Sheep (21.6% in 2023; 22.8% in 2024) and in Goats (19% in 2023; 19.26% in 2024) during two annual cycles (2023 and 2024) across three sampling locations in Beheira Governorate The prevalence of *E. coli* reported to be increased in summer season in warmer environment from April-June and from July-September due to increase in temperature and temperature-humidity index. Resistance was highest for Penicillin, followed by Enrofloxacin and cefotaxime. A high percentage of *E. coli* isolates from milk were shown to be multidrug-resistant by antibiogram analysis.

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The study concluded that Climatic changes to hot and more humid increasing the prevalence and enhance the release of multidrug resistant. *E. coli* which poses a health risk to consumers. So that further studies to state on and monitoring climate effect on the release of multidrug resistance (MDR) *E. coli* and its impact on people through the food chain is required.

INTRODUCTION

One of major causes of economic losses in dairy farms is mastitis that raises the rates of culling among lactating dairy farms in addition to lowering milk output and quality. A common environmental infection that contributes significantly to mastitis is *E. coli* (**Goulart and Mellata, 2022; Asfaw et al. 2023**).

Globally, the climate has been shifting, and in the near future, it is expected to fluctuate very dynamically (**Astuti et al. 2024**). These changes pose significant threats to milk production, including predicted reductions in milk yield and quality, alongside heightened vulnerability of dairy animals to heat-related microbial diseases **(North et al. 2023)**. climate change is anticipated to increase milk contamination risks **(Feliciano et al. 2020)**, creating complex challenges for food safety and animal health.

These climate-induced stressors significantly elevate risks for conditions like mastitis, a prevalent and economically devastating inflammation of the mammary glands caused by bacterial, fungal, or viral infections. Mastitis is primarily transmitted on dairy farms through unsanitary conditions exacerbated by environmental pressures (**Zigo et al. 2022**). A diverse range of pathogens can cause mastitis, broadly categorized into Gram-positive cocci – notably *Staphylococcus spp.* and *Streptococcus spp.* – and Gram-negative bacilli such *as Escherichia coli* (*E. coli*), *Proteus mirabilis*, *Citrobacter spp.*, and *Klebsiella pneumoniae* (**Sierra et al. 2023; Tong et al. 2025**). Critically, the treatment of this climate-aggravated disease often involves antibiotics, which may inadvertently worsen antimicrobial resistance (AMR) as mentioned by **Al-harbi et al. (2021)**.

Among these mastitis pathogens, E. coli demands particular attention due to its complex biology and significant threat. While E. coli is a frequent resident of the digestive tract in both humans and animals, maintaining a complex relationship with commensal microbiota **(Biswas et al. 2024)**, certain pathotypes are potent pathogens. These pathotypes cause a wide spectrum of intestinal and extra-intestinal infections **(Mora-Coto et al. 2025**), with their pathogenicity directly linked to the acquisition of specific virulence genes. These genes facilitate critical processes like adhesion, invasion, deployment of secretion systems, toxin generation, evasion of immunological regulation, biofilm formation, and adaptation of metabolic components **(Pakbin et al. 2021)**. This inherent virulence potential, combined with its prevalence in mastitis cases, positions E. coli at the heart of a critical challenge.

In the contemporary animal industry, antibiotics are frequently used to treat, prevent, and manage diseases like mastitis, aiming to lower animal morbidity and mortalit **(Kasimanickam et al. 2021).** However, the overuse or misuse of antibiotics in dairy production has profound and far-reaching consequences. This practice can result in the release of active antibiotics into the environment through the disposal of contaminated farm waste **(Polianciuc et al. 2020)** and, more critically, drives the development of antibiotic-resistant organisms. The resulting AMR decreases the effectiveness of essential antibiotics, making it increasingly difficult to treat diseases once readily curable by pathogens like E. coli. Antimicrobial resistance (AMR) represents a global health emergency **(Salam et al. 2023)**, leading the World Health Organisation (WHO) to designate E. coli as a "critical priority" pathogen requiring urgent attention **(Shoaib et al. 2024)**. Consequently, investigating AMR in E. coli, especially strains derived from mastitis, is paramount.

To combat this growing AMR problem, the WHO promotes the "One Health" concept as a global *strategy*. This approach is fundamentally grounded in recognizing the inextricable links between human, animal, and environmental health **(Sandu *e*., 2025)**. Integrating climate considerations into this framework is vital. By taking into consideration how climate change may affect food safety risks, such as increased pathogen prevalence and contamination, the food industry can develop strategies to reduce the probability of contaminated food reaching consumers. This necessitates implementing mitigation strategies explicitly created and grounded in an objective assessment of anticipated climate changes and their multifaceted effects **(Katsini et al. 2024)**. Yet, a significant research gap hinders progress: despite well-established modelling studies projecting decreased milk output due to climate-induced heat stress **(Guzmán-Luna et al. 2022)**, similar predictive models for forecasting climate-driven milk contamination dynamics, including pathogen proliferation and AMR spread, remain scarce.

To confront the critical nexus, the objectives of this study were (1) to determine the prevalence of mastitis in dairy cattle, sheep and goats in three locations in El-Beheira governorate on a seasonally basis for two annual cycles and (2) to determine the antibiotic resistance patterns of *E. coli* isolates against commonly used antibiotics in the area. (3) to study if there is a relation between climatic changes and increased release of MDR *E. coli.*

MATERIALS AND METHODS

Study Setting and Sampling

In Beheira governorate of Egypt, a cross-sectional survey was carried out between January 2023 and December 2024. The study included dairy livestock (cattle, sheep and goat) reared in three localities (Badr-South El-Tahrir, Itay El Barud, and Rosetta) in the Beheira governorate on a few small farms (**Figure 1**). After udder disinfection eliminating the first two to three squirts of milk, a total of 1568 raw milk samples were aseptically collected into sterile universal bottles from each quarter or udder half. During the milking process, samples of milk were taken either early in the morning or late in the afternoon. At the time of sample collection, the study's chosen animals were clinically inspected for any obvious abnormalities on each udder quarter or udder half, such as udder soreness, swelling, or changes in the physical characteristics of the milk. **Table 1** displays the sampling strategy according to the animal type, sample collecting site, and climate season. The collected milk samples were kept in ice box and transported without bacteriological investigation delay to Damanhur Animal Health Research Institute Lab (AHRI).

The study protocol was deemed exempt from ethical review, as it involved solely non-invasive milk sampling. No procedures causing animal harm, distress, or experimentation such as blood collection or administration of substances were performed.

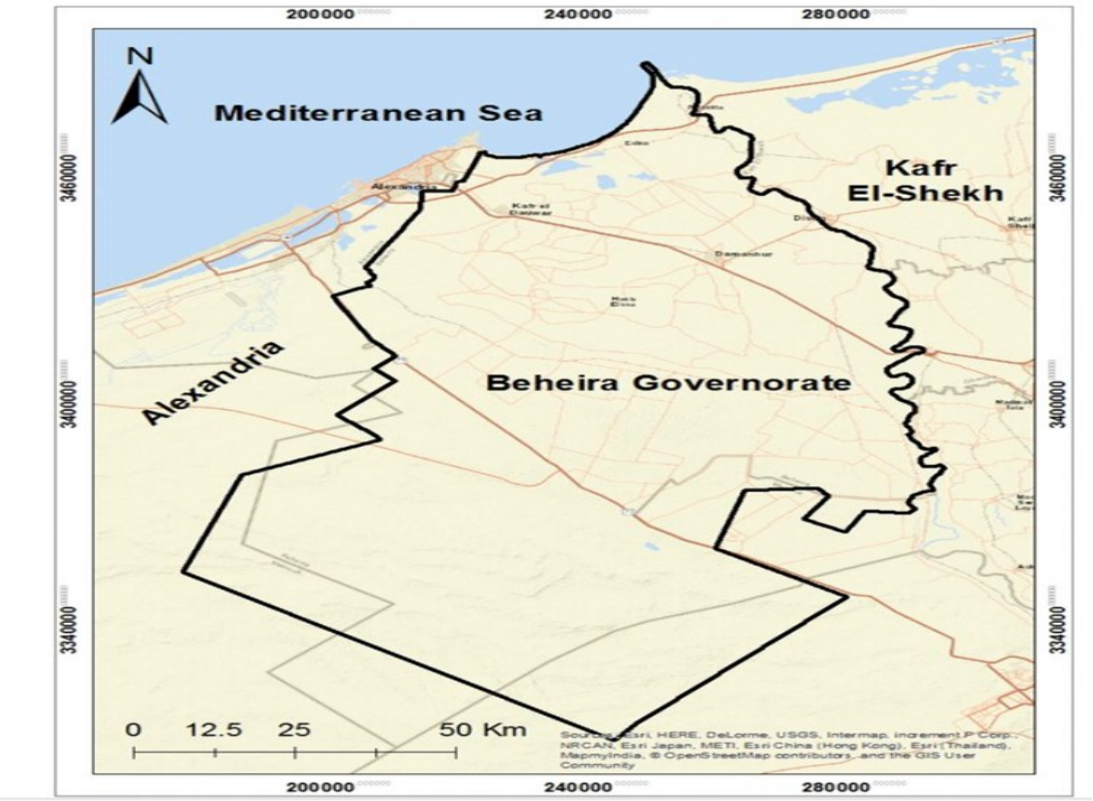

Figure 1: Location of the study area

Table 1. Number of Samples, animal species, location and climatic season of sample collection and the year:

Location	Months	Cattle		Sheep		Goat	
		2023	2024	2023	2024	2023	2024
		Total	Total	Total	Total	Total	Total
Rosetta	January- March	25	22	19	15	18	20
	April - June	25	22	19	15	18	20
	July-September	25	22	19	15	18	20
	October- December	25	22	19	15	18	20
Etay El Barod	January- March	29	27	22	20	21	19
	April - June	29	27	22	20	21	19
	July-September	29	27	22	20	21	19
	October- December	29	27	22	20	21	19
Badr-South El Tahreer	January- March	23	19	25	23	23	22
	April - June	23	19	25	23	23	22
	July-September	23	19	25	23	23	22
	October- December	23	19	25	23	23	22
Total (1568 samples)		308	272	264	232	248	244

Bacteriological Culture, Isolation, and Identification of Bacteria

A total of 1568 milk samples were subjected to bacterial culture, isolation, and identification of the target causal agent (*E. coli*)**. Gemeda *et al.* (2023)** devised protocols for the isolation and identification of *E. coli* was followed. To summarise, the milk sample was centrifuged for 10 minutes at 3000 rpm, the sediment was combined with 3 mL of nutrient broth medium, and it was then incubated for 3–5 hours at 37° C. Next, a loop-full of pre-enriched cultures was taken, inoculated onto MacConkey agar plates, and incubated aerobically for 24 hours at 37°C. The suspected colonies were picked up then purified cultured onto eosin-methylene -blue (EMB) agar plates and incubated aerobically for 24 hours at 37°C. On incubated EMB agar, colonies that had a metallic sheen were determined to be *E. coli*- positive. After that, the samples were cultivated onto nutrient agar plates for additional confirmation utilising Voges-Proskauer, citrate, urease, indole, methyl red, and triple sugar iron agar assays, among other biochemical tests.

Susceptibility testing of *E. coli* isolates against a panel of antibiotics

Using the Kirby-Bauer disc diffusion method, antimicrobial susceptibility was assessed on Mueller-Hinton agar in accordance with the (CLSI) Clinical and Laboratory Standard Institute's recommendations (**Nassar et al. 2019; Weinstein and Lewis, 2020**). The WHO and World Organisation for Animal Health (WOAH) guidelines for antimicrobial use in humans and animals that produce food were followed when choosing antibiotics (**Kasimanickam et al. 2021; Theodoridou Oxinou et al. 2025**). This choice was in line with Indonesia's comprehensive approach to AMR surveillance. Amoxicillin-Clavulanic (AMC) 20/10 µg, Penicillin G (P) (10 units), gentamicin (GEN) (10 µg, cefotaxime (CTX) (30 µg), Enrofloxacin (ENR) (10 µg), Oxytetracycline(O) (10 µg), and Sulphamethoxazole (COT) (25 µg) were among the seven antibiotic discs of the panel. The isolates were classified as extensively drug-resistant (non-susceptible to ≥ 1 antibiotic in all but ≤ 2 antibiotic classes), multidrug-resistant (non-susceptible to ≥ 1 antibiotic in ≥ 3 antibiotic classes), and single drug-resistant (non-susceptible to 1 antibiotic) based on their reactions to the chosen antibiotic classes (**Magiorakos et al. 2012**). The number of antibiotic classes to which a particular isolate shown resistance was divided by the total number of antibiotics exposed to the isolate to determine the Multiple Antibiotic Resistance (MAR) index (**Tilahun, 2022**).

Climatic parameters

The local weather data (temperature, relative humidity) for the study area across four seasons (2023–2024) were obtained from the Central Laboratory for Agricultural Climate, Agricultural Research Center. Daily temperature-humidity index (THI) was calculated using temperature and relative humidity measurements according to the formula established by National Research Council **NRC (1971)**.

THI= (1.8xT+32)-[(0.55-0.0055xRH) x (1.8xT -26)]

Where T = Ambient temperature (°C) and RH = Relative humidity (%).

Statistical Analysis

Descriptions of the study animals or samples were given as the number and percentage.

RESULTS

Prevalence of *E.coli* in Dairy Cattle, sheep, and Goats

In this study, 1568 milk samples including 580 cattle, 496 sheep, and 492 goat samples. The prevalence of *E. coli* in milk samples were 29.1%, 22.2%, and 19.1% respectively, with detailed prevalence in cattle (27.6% in 2023; 30.9% in 2024), in Sheep (21.6% in 2023; 22.8% in 2024) and in Goats (19% in 2023; 19.26% in 2024) during two annual cycles (2023 and 2024) across three sampling locations in Beheira Governorate as details in (**Table 2**).

Table 2. Prevalence of *E. coli* isolated from 1568 raw milk samples collected during four annual seasons of two years 2023 – 2024.

Location	Months	Cattle				Sheep				Goat			
		2023		2024		2023		2024		2023		2024	
		Total	No (%)	Total	No (%)	Total	No (%)	Total	No (%)	Total	No (%)	Total	No (%)
Rosetta	January-March	25	3(12.0%)	22	4 (18.2%)	19	2(10.5%)	15	2 (13.3%)	18	3 (15.8%)	20	4 (20.0%)
	April-June	25	6(24.0%)	22	7 (31.2%)	19	6(31.6%)	15	5 (33.3%)	18	4 (22.2%)	20	5 (25.0%)
	July-September	25	13 (52.0%)	22	15 (68.2%)	19	7(36.8%)	15	7 (46.6%)	18	3 (16.6%)	20	4 (20.0%)
	October-December	25	9(36.0%)	22	7 (31.8%)	19	6(31.6%)	15	4 (26.6%)	18	4 (22.2%)	20	4 (20.0%)
Etay El Barod	January-March	29	4(13.8%)	27	6 (22.2%)	22	3(13.6%)	20	4 (20.0%)	21	4 (19.0%)	19	5 (26.3%)
	April - June	29	8(27.6%)	27	9 (33.33%)	22	6(27.3%)	20	6 (30.0%)	21	3 (14.3%)	19	3 (15.8%)
	July- September	29	8(27.6%)	27	6 (22.2%)	22	5(22.7%)	20	4 (20.0%)	21	5 (23.8%)	19	4 (21.1%)
	October-December	29	9(31.0%)	27	8 (29.6%)	22	6(27.3%)	20	5 (25.0%)	21	5 (23.8%)	19	4 (21.1%)
Badr-South El Tahreer	January -March	23	3(13.0%)	19	3 (15.8%)	25	4(16.0%)	23	5 (21.7%)	23	5 (21.7%)	22	5 (22.7%)
	April-June	23	11 (47.8%)	19	12 (63.1%)	25	3(12.0%)	23	4 (17.4%)	23	4 (17.4%)	22	4 (18.2%)
	July-September	23	6(26.1%)	19	4 (21.1%)	25	4(16.0%)	23	3 (13.0%)	23	3 (13.0%)	22	2 (9.1%)
	October - December	23	5(21.7%)	19	3 (15.8%)	25	5(20.0%)	23	4 (17.4%)	23	4 (17.4%)	22	3 (13.6%)
Prevalence		308	85 (27.6%)	272	84 (30.9%)	264	57 (21.6%)	232	53 (22.8%)	248	47(19%)	244	47 (19.26)
			29.1%				22.2%				19.1%		

Calculated according to the number of samples examined %

Climatic conditions variability

Temperature, relative humidity (%), and temperature-humidity index (THI) values for each region across two annual cycles are presented in **Table 3**. In El-Beheira Governorate, seasonal variations were observed during the two-year period. The April–June and July–September temperatures were slightly higher in the Itay El-Barud and Badr–South El-Tahrir regions compared to other areas. Conversely, relative humidity levels were marginally lower in these same regions over both annual cycles. Accordingly, the highest temperature-humidity index (THI) value (79.1) was recorded in Itay El-Barud during the July-September period of 2023.

Table 3. Climatic Conditions of the Investigated Areas in 2023 and 2024.

Location	Months	Year					
		2023			2024		
		T	RH	THI	T	RH	THI
Rosetta	January - March	16.4	69.3	60.9	16.7	69.1	61.4
	April - June	22.3	66.3	69.5	23.6	66.5	71.5
	July - September	28.5	66.0	78.6	28.5	65.3	78.5
	October - December	22.5	67.3	69.9	21.0	65.2	67.5
Etay El Barod	January - March	15.2	68.2	59.2	15.6	67.0	59.7
	April - June	24.3	53.1	71.1	25.8	52.4	73.1
	July - September	30.2	54.0	79.1	30.0	54.0	78.9
	October - December	21.7	63.8	68.5	20.3	60.5	66.2
Badr-South El Tahreer	January - March	15.0	64.2	58.8	15.3	62.85	59.3
	April - June	24.8	48.2	71.3	26.3	47.8	73.2
	July - September	30.6	49.3	79.0	30.5	49.7	78.9
	October - December	21.3	62.9	67.8	20.0	58.4	65.7

T: Temperature (°C); RH: Relative Humidity (%); THI: Temperature-Humidity Index, calculated based on the formula provided by the NRC (1971).

Antibiotic Susceptibility of *E. coli* isolates from collected milk samples

The results demonstrated that *E. coli* isolates from raw milk in dairy farms had a variable degree of resistance to the tested antimicrobials during two annual cycle (**Table 4 A , B and C**). In 2023, *E. coli* isolates were resistant in cattle (table 4A) during Jan-March, April-June, July-Sept and Oct-December (30.0%, 32.6%, 38.6% and 34.8% respectively), sheep (table 4B) (42.9%, 39.6%, 41.1% and 44.5% respectively) and goats (table 4C) (32.1%, 39.9%, 42.9% and 31.9% respectively). The *E. coli* isolates resistance in 2023 showed highest prevalence in cattle in July-Sept, in sheep all seasons slightly similar except April-June and in goats the highest resistance prevalence was reported in seasons April-June and July-Sept. In 2024, *E. coli* isolates were resistant in cattle during Jan-March, April-June, July-Sept and Oct-December (38.5%, 39.3%, 24.6% and 24.6% respectively), sheep (48.1%, 42.8%, 36.7% and 40.7%) and goats (37.7%, 46.3%, 37.1% and 26.0% respectively). The *E. coli* isolates resistance in 2024 showed highest prevalence in cattle in seasons Jan-March and April-June, in sheep the highest resistance prevalence was reported in Jan-March and other seasons showed similar prevalence, and in goats the highest resistance prevalence was reported in season April-June.

Table 4A: Antibiotic sensitivity of *E. coli* isolated from dairy cattle at Beheira governorate during different annual seasons in 2023 and 2024.

Cattle		2023				2024			
		Jan-March	April-June	July-Sept	Oct-December	Jan-March	April-June	July-Sept	Oct-December
		10 isolates (%)	25 isolates (%)	27 isolates (%)	23 isolates (%)	13 isolates (%)	28 isolates (%)	25 isolates (%)	18 isolates (%)
AMC	R	1 (10%)	3 (12%)	4 (14.8%)	3 (13%)	2 (15.4%)	5 (17.9%)	3 (12.0%)	2 (11.1%)
	I	2 (20%)	5 (20%)	3 (11.1%)	2 (8.7%)	3 (23.1%)	3 (10.7%)	3 (12.0%)	3 (16.7%)
	S	7 (70%)	17 (68%)	20 (74.1%)	18 (78.3%)	8 (61.5%)	20 (71.4%)	19 (76.0%)	13 (72.2%)
GEN	R	2 (20%)	7 (28%)	8 (29.6%)	5 (21.7%)	3 (23.1%)	9 (32.1%)	6 (24.0%)	2 (11.1%)
	I	2(20%)	5 (20%)	6 (22.2%)	3 (13%)	3 (23.1%)	4 (14.3%)	7 (28.0%)	4 (22.2%)
	S	6 (60%)	13 (52%)	13 (48.2%)	15 (65.2%)	7 (53.8%)	15 (53.6%)	12 (48.0%)	12 (66.7%)
CTX	R	3 (30%)	9 (36%)	18 (66.7%)	13 (56.5%)	5 (38.5%)	12 (42.9%)	6 (24.0%)	5 (27.8%)
	I	4 (40%)	8 (32%)	2 (7.4%)	3 (13%)	3 (23.1%)	6 (21.4%)	10 (40.0%)	5 (27.8%)
	S	3 (30%)	8 (32%)	7 (25.9%)	7 (30.5%)	5 (38.5%)	10 (35.7%)	9 (36.0%)	8 (44.0%)
ENR	R	2 (20%)	5 (20%)	7 (25.9%)	8 (34.8%)	5 (38.5%)	8 (28.6%)	5 (20.0%)	6 (33.3%)
	I	3 (30%)	9 (36%)	9 (33.3%)	3 (13%)	2 (15.4%)	5 (17.9%)	9 (36.0%)	2 (11.1%)
	S	5 (50%)	11 (44%)	11 (40.7%)	12 (52.2%)	6 (46.2%)	15 (53.6%)	11 (44.0%)	10 (55.6%)
COT	R	2 (20%)	5 (20%)	7 (25.9%)	4 (17.4%)	3 (23.1%)	7 (25.0%)	4 (16.0%)	2 (11.1%)
	I	4 (40%)	8 (32%)	8 (29.6%)	6 (26.1%)	4 (30.8%)	7 (25.0%)	9 (39.0%)	5 (27.8%)
	S	4 (40%)	12 (48%)	12 (44.5%)	13 (56.5%)	6 (46.2%)	14 (50.0%)	12 (48.0%)	11 (61.1%)
O	R	4 (40%)	13 (52%)	10 (37%)	6 (26.1%)	8 (61.5%)	16 (57.2%)	6 (24.0%)	4 (22.2%)
	I	3 (30%)	8 (32%)	7 (25.9%)	7 (30.5%)	3 (23.1%)	5 (17.9%)	8 (32.0%)	4 (22.2%)
	S	3 (30%)	4 (16%)	10 (37%)	10 (43.4%)	2 (15.4%)	7 (25.0%)	11 (44.0%)	10 (55.6%)
P	R	7 (70%)	15 (60%)	19 (70.4%)	17 (73.9%)	9 (69.2%)	20 (71.4%)	13 (52.0%)	10 (55.6%)
	I	2 (20%)	6 (24%)	5 (18.5%)	3 (13%)	2 (15.4%)	5 (17.9%)	6 (24.0%)	5 (27.8%)
	S	1 (10%)	4 (16%)	3 (11.1%)	3 (13%)	2 (15.4%)	3 (10.7%)	6 (24.0%)	3 (16.7%)
R mean (%)		30 .0%	32.6%	38.6%	34.8%	38.5%	39.3%	24.6%	24.6%

AMC = Amoxycillin +Clavulinic acid GEN= Gentamicin ENR= Enrofloxacine COT= Cotri-moxazole (sulfa. + trimethoprim) O = Oxytetracyclin P=Penicillin
Resistance reported as susceptible (S), resistant (R), or intermediate (I).

Table 4B. Antibiotic sensitivity of *E. coli* isolated from sheep at Beheira governorate during different annual seasons in 2023 and 2024

Sheep		9 Isolates (%)	13 Isolates (%)	16 isolates (%)	17 isolates (%)	11 isolates (%)	15 isolates (%)	14 isolates (%)	13 isolates (%)
AMC	S	3 (33.3%)	5 (38.5%)	4 (25.0%)	4 (23.5%)	4 (36.4%)	6 (40.0%)	3 (21.4%)	3 (23.1%)
	I	1 (11.1%)	3 (23.1%)	3 (18.8%)	5 (29.4%)	1 (9.1%)	2 (13.3%)	2 (14.3%)	4 (30.8%)
	R	5 (55.6%)	5 (38.5%)	9 (56.3%)	8 (47.1%)	6 (54.5%)	7 (46.7%)	9 (64.3%)	6 (46.2%)
GEN	R	4 (44.4%)	3 (23.1%)	5 (31.3%)	6 (35.3%)	5(45.5%)	4 (26.7%)	4 (28.6%)	4 (30.8%)
	I	1 (11.1%)	4 (30.8%)	3 (18.8%)	3 (17.7%)	2 (18.2%)	3 (20.0%)	3 (21.4%)	3 (23.1%)
	S	4 (44.5%)	6 (46.2%)	8 (50.0%)	8 (47.1%)	4 (36.4%)	6 (54.6%)	7 (50.0%)	6 (46.15)
CTX	R	5 (55.6%)	7 (53.8%)	8 (50.0%)	9 (52.9%)	7 (63.6%)	9 (60.0%)	6 (42.9%)	6 (46.2%)
	I	1 (11.1%)	2 (15.4%)	3 (18.8%)	4 (23.5%)	1 (9.1%)	2 (13.3%)	3 (21.4%)	4 (30.8%)
	S	3 (33.3%)	4 (30.8%)	5 (31.3%)	4 (23.5%)	3 (27.3%)	4 (26.7%)	5 (35.7%)	4 (30.8%)
COT	R	2 (22.2%)	4 (30.8%)	6 (37.5%)	7 (41.2%)	4 (36.4%)	5 (33.3%)	5 (35.7%)	5 (38.5%)
	I	3 (33.3%)	3 (23.1%)	5 (31.3%)	4 (23.5%)	1 (9.1%)	3 (20.0%)	2 (14.3%)	2 (15.4%)
	S	4 (44.5%)	6 (46.2%)	5 (31.3%)	6 (53.3%)	6 (54.5%)	7 (46.7%)	7 (38.9%)	6 (46.2%)
ENR	R	3 (33.3%)	4 (30.8%)	6 (37.5%)	6 (35.3%)	4 (36.4%)	5 (33.3%)	5 (35.7%)	4 (30.8%)
	I	1 (11.1%)	5 (38.5%)	4 (25.0%)	4 (23.5%)	0 (0.0%)	3 (20.0%)	4 (28.6%)	3 (23.1%)
	S	5 (55.6%)	4 (30.8%)	6 (37.5%)	7 (41.2%)	7 (63.6%)	7 (46.7%)	5 (35.7%)	6 (46.2%)
O	R	4 (44.5%)	7 (53.8%)	8 (50.0%)	10 (58.8%)	6 (54.6%)	8 (53.3%)	6 (42.9%)	7 (53.8%)
	I	2 (22.2%)	0 (0.0%)	3 (18.8%)	2 (11.8%)	3 (27.3%)	2 (13.3%)	3 (21.4%)	1 (7.7%)
	S	3 (33.3%)	6 (46.2%)	5 (31.3%)	5 (29.4%)	2 (18.1%)	5 (33.3%)	5 (35.7%)	5 (38.5%)
P	R	6 (66.7%)	6 (46.2%)	9 (56.3%)	11 (64.7%)	7 (63.6%)	8 (53.3%)	7 (50.0%)	8 (61.5%)
	I	1(11.1%)	2 (15.4%)	3 (18.8%)	2 (11.8%)	2 (18.2%)	3 (20.0%)	3 (21.4%)	2 (15.4%)
	S	2 (22.2%)	5 (38.5%)	4 (25.0%)	4 (23.5%)	2 (18.2%)	4 (26.7%)	4 (28.6%)	3 (23.1%)
R mean (%)		42.9%	39.6%	41.1%	44.5%	48.1%	42.8%	36.7%	40.7%

AMC = Amoxycillin +Clavulinic acid GEN= Gentamicin ENR= Enrofloxacine COT= Cotrimoxazole (sulfa. + trimethoprim) O = Oxytetracyclin P=Penicillin
Resistance reported as susceptible (S), resistant (R), or intermediate (I).

Table 4C. Antibiotic sensitivity of *E. coli* isolated from goats at Beheira governorate during different annual seasons in 2023 and 2024.

Goat		12 isolates (%)	11 isolates (%)	11 isolates (%)	13 isolates (%)	14 isolates (%)	13 isolates (%)	10 isolates (%)	11 isolates (%)
AMC	R	1 (8.3%)	2 (18.2%)	3 (27.27)	3 (23.1%)	2 (14.3%)	3 (23.1%)	2 (20.0%)	2 (18.2%)
	I	2 (16.7%)	3 (27.3%)	0 (0.0%)	2 (15.4%)	3 (21.4%)	2 (15.4%)	1 (10.0%)	1 (9.1%)
	S	9 (75.0%)	6 (54.5%)	8 (72.72)	8 (61.5%)	9 (64.3%)	8 (61.5%)	7 (70.0%)	8 (72.7%)
GEN	R	2 (16.7%)	4 (36.4%)	4 (36.36)	2 (15.4%)	3 (21.4%)	5 (38.5%)	3 (30.0%)	1 (9.1%)
	I	3 (25.0%)	3 (27.3%)	2 (18.2%)	5 (38.5%)	3 (21.4%)	3 (23.1%)	1 (10.0%)	3 (27.3%)
	S	7 (58.3%)	4 (36.4%)	7 (63.6%)	6 (46.2%)	8 (57.1%)	5 (38.5%)	6 (60.0%)	7 (63.6%)
CTX	R	4 (33.3%)	5 (41.7%)	6 (54.6%)	5 (38.5%)	5 (41.7%)	6 (46.2%)	5 (50.0%)	4 (36.4%)
	I	2 (16.7%)	3 (25.0%)	3 (27.3%)	3 (23.1%)	3 (21.4%)	3 (23.1%)	3 (30.0%)	3 (27.3%)
	S	6 (50.0%)	4 (33.3%)	2 (18.2%)	5 (38.5%)	6 (42.9%)	4 (30.8%)	2 (20.0%)	4 (36.4%)
COT	R	3 (25.0%)	5 (41.7%)	3 (27.3%)	3 (23.1%)	5 (35.7%)	6 (46.2%)	2 (20.0%)	2 (18.2%)
	I	4 (33.3%)	3 (25.0%)	2 (18.2%)	4 (30.8%)	3 (21.4%)	2 (15.4%)	2 (20.0%)	3 (27.3%)
	S	5 (41.7%)	4 (33.3%)	6 (54.6%)	6 (46.2%)	6 (42.9%)	5 (38.5%)	6 (60.0%)	6 (54.5%)
ENR	R	3 (25.0%)	4 (33.3%)	4 (36.4%)	3 (23.1%)	4 (28.6%)	5 (38.5%)	3 (30.0%)	2 (18.2%)
	I	2 (16.7%)	3 (25.0%)	1 (9.1%)	5 (38.5%)	2 (14.3%)	3 (23.1%)	1 (10.0%)	4 (36.4%)
	S	7 (58.3%)	5 (41.7%)	6 (54.6%)	5 (38.5%)	8 (57.1%)	5 (38.5%)	6 (60.0%)	5 (45.5%)
O	R	6 (50.0%)	7 (58.3%)	6 (54.6%)	5 (38.5%)	8 (57.1%)	8 (62.5%)	5 (50.0%)	3 (27.3%)
	I	3 (25.0%)	3 (25.0%)	2 (18.2%)	4 (30.8%)	3 (21.4%)	2 (15.4%)	2 (20.0%)	4 (36.4%)
	S	3 (25.0%)	2 (16.7%)	3 (27.3%)	4 (30.8%)	3 (21.4%)	3 (23.1%)	3 (30.0%)	4 (36.4%)
P	R	8 (66.7%)	6 (50.0%)	7 (63.6%)	8 (61.5%)	10 (71.4%)	9 (69.2%)	6 (60.0%)	6 (54.5%)
	I	1 (8.3%)	5 (41.7%)	1 (9.1%)	2(15.4%)	1 (7.1%)	3 (23.1%)	2 (20.0%)	3 (27.3%)
	S	3 (25.0%)	1 (8.3%)	3 (27.3%)	3 (23.1%)	3 (21.4%)	1 (7.7%)	2 (20.0%)	2 (18.2%)
R mean (%)		32.1%	39.9%	42.9%	31.9%	37.7%	46.3%	37.1%	26.0%

AMC = Amoxycillin +Clavulinic acid GEN= Gentamicin ENR= Enrofloxacine
COT= Cotrimoxazole (sulfa. + trimethoprim) O = Oxytetracyclin
P=Penicillin
Resistance reported as susceptible (S), resistant (R), or intermediate (I). % calculated according to the number of tested isolates. R mean = Sum of R/ 7 (Number of antibiotics used).

In table 5 the MAR index (multidrug resistance index) calculated by dividing the number of antibiotics resisted by each *E. coli* strains on the total number of antibiotics used in the test (7 antibiotics) so that resistance to more than one antibiotic increasing the MAR index. Resistance to 3 antibiotics or more means the *E. coli* strain is a multidrug resistant strain with high MAR index. Table 5 show that the number of MDR *E. coli* strains increased in 2024 (Jan. -March and April – June) than in 2023. While decreased in 2024 (July –Sept. And Oct. - Dec.) these results is the same happen in Cattle, Sheep and Goats. More hot and humid climate increasing the chance of release mor MDR *E. coli* Strains.

Table 5. The multiple antibiotic resistant (MAR) strains index isolated from (cattle, sheep and goats during 2023 and 2024).

Animal species	Season	Year		Strains resist 1 antibiotic	Strains resist 2 antibiotics	Strains resist 3 antibiotics	Strains resist 4 antibiotics	Strains resist 5 antibiotics	Strains resist 6 antibiotics	No. of MDR isolates
Cattle			MAR index	0.14	0.28	0.42	0.57	0.7	0.85	
	Jan-March	2023	No. of isolates	2	3	2	1	0	0	3
		2024	No. of isolates	3	3	3	1	1	0	5
	Apr- June	2023	No. of isolates	9	8	2	2	0	1	5
		2024	No. of isolates	9	8	3	2	1	1	7
	July- Sept	2023	No. of isolates	13	6	2	2	1	1	6
		2024	No. of isolates	10	6	2	3	0	0	5
	Oct - Dec	2023	No. of isolates	9	4	3	1	1	0	5
		2024	No. of isolates	7	5	2	1	1	0	4
Sheep	Season	Year	Number of antibi-otics	1	2	3	4	5	6	No. of MDR isolates
			MAR index	0.14	0.28	0.42	0.57	0.7	0.85	
	Jan-March	2023	No. of isolates	2	3	1	1	0	0	2
		2024	No. of isolates	3	3	2	1	1	0	4
	Apr- June	2023	No. of isolates	4	3	1	2	0	0	3
		2024	No. of isolates	5	4	1	1	1	1	4
	July- Sept	2023	No. of isolates	6	3	2	2	1	0	5
		2024	No. of isolates	7	1	2	1	0	0	3
	Oct - Dec	2023	No. of isolates	6	4	3	1	1	0	5
		2024	No. of isolates	3	5	2	1	0	0	3
Goat	Season	Year	Number of antibi-otics	1	2	3	4	5	6	No. of MDR isolates
			MAR index	0.14	0.28	0.42	0.57	0.7	0.85	
	Jan-March	2023	No. of isolates	3	4	1	2	1	0	4
		2024	No. of isolates	1	3	2	1	1	1	5
	Apr- June	2023	No. of isolates	3	2	2	1	1	1	5
		2024	No. of isolates	2	3	2	1	2	1	6
	July- Sept	2023	No. of isolates	1	4	3	2	0	1	6
		2024	No. of isolates	2	3	2	1	1	0	4
	Oct - Dec	2023	No. of isolates	3	2	3	1	1	1	6
		2024	No. of isolates	4	3	1	1	0	1	3

The MAR index calculated by dividing the number of antibiotics which resisted by *E. coli* on the total number of antibiotics used in the test.

In table 5 hot and humid seasons enhanced the release of MDR isolates with high MAR index. Decreased temperature and humidity decreased the chance of MDR strains release. Results reported that in 2024 hot and humid seasons had higher number of MDR *E. Coli* strains than in 2023. While, in 2024 less temperature and low humidity resulted in decreased number of MDR *E. coli* strains with lower MAR index

DISCUSSION

A zoonotic pathogen with a high capacity for acquiring AMR is *E. coli*, which could have detrimental effects on human health and animal food safety (**Aflakian et al. 2023**). According to **Zhang et al. (2024)**, Determining the seasonal prevalence of mastitis in dairy cattle, sheep, and goats across three locations in the El-Beheira governorate over two annual cycles was the objective of this study. Additionally, we sought to ascertain the antibiotic resistance patterns of *E. coli* isolates against commonly used antibiotics in the region.

Results improve knowledge of the frequency of *E. coli* in collected milk samples from dairy farms that raise goats, sheep, and cattle throughout the course of two annual cycles. Over the course of two annual cycles, a total of 373/1568 (23.8%) *E. coli* isolates were recovered. This isolation rate was in line **with Ibrahim et al. (2022)** findings in that, 32% of the raw farm milk under investigation harbourd *E. coli*. According to **Gundogan and Avci (2014)**, E. coli was found in 74% of raw milk, indicating a higher prevalence. Furthermore, **Megawer et al. (2021)** found that raw milk had a 75% *E. coli* prevalence. Conversely, **Kareem et al. (2023) and Ombarak et al. (2023)** observed low recovery rates of 4.6% and 9.3%, respectively, indicating low prevalence of *E. coli*.

The study results prove*d that E. coli* was found in milk samples collected from cattle, sheep, and goats at three distinct locations in the Beheira governorate and its prevalence was 29.1%, 22.2%, and 19.1% respectively, more over detailed prevalence in cattle (27.6% in 2023; 30.9% in 2024), in Sheep (21.6% in 2023; 22.8% in 2024) and in Goats (19% in 2023; 19.26% in 2024) during two annual cycles (2023 and 2024) The difference between 2023 and 2024 in *E. coli* prevalence revealed that in goats (19.0% vs. 19.3%, P=0.942), sheep (18.5% vs. 21.7%, P=0.491), and cattle (27.6% vs. 30.9%, P=0.520). Since $P > 0.05$, these differences are not statistically significant.

E. coli prevalence demonstrated significant variation across host species, geographical locations, and seasonal periods (Table 2). Among cattle, the highest infection rates occurred in Rosetta during July-September (52.0% in 2023; 68.2% in 2024), followed by Badr-South El-Tahrir in April-June (47.8% in 2023; 63.1% in 2024). Itay El-Barud exhibited peak prevalence during October-December 2023 (31.0%) and April-June 2024 (33.3%). For sheep, maximum contamination was observed in Rosetta during July-September (36.8% in 2023; 46.6% in 2024). Itay El-Barud showed elevated prevalence in both April-June (27.3% in 2023; 30.0% in 2024) and October-December (27.3% in 2023; 25.0% in 2024). Badr-South El-Tahrir recorded peak rates during October-December 2023 (20.0%) and January-March 2024 (21.7%). Regarding goats, Itay El-Barud demonstrated the highest prevalence during July-September 2023 (23.8%) and January-March 2024 (26.3%). Rosetta showed elevated contamination in April-June (22.2% in 2023; 25.0% in 2024) and October-December 2023 (22.2%), while Badr-South El-Tahrir peaked during January-March (21.7% in 2023; 22.7% in 2024). Spatiotemporal analysis revealed distinct geographical patterns: Rosetta consistently showed the highest *E. coli* prevalence during April-June and July-September. Itay El-Barud exhibited peak contamination in October-December, whereas Badr-South El-Tahrir recorded maximal rates during April-June followed by January-March and October-December. These results support those of **Iqbal et al. (2017)**, who found that greater temperatures resulted in higher concentrations of *E. coli*, underscoring the importance of temperature-driven bacterial growth on a global scale. *E. coli* grows best at 37 °C, warmer temperatures not only promote bacterial growth but also increase its pathogenic potential (**Chatreman et al. 2020**).

Mastitis continues to be a serious problem in the dairy industry, affecting animal welfare worldwide and resulting in large financial losses. mastitis infection are all correlated with temperature and humidity (**Vitali et al. 2020; Krebs et al. 2023**). Clinical mastitis is more likely to occur when the temperature-humidity index (THI) rises, although there was no discernible pattern with average rainfall. According to **Vitali et al. (2016)** and **Vitali et al. (2020)**, hot weather, particularly temperatures above 24 °C, has also been connected to higher milk Somatic cell count (SCC), increased microorganisms, decreased dry matter intake, and low immunity, which results in a negative energy balance and increases the susceptibility of dairy cattle to diseases. Seasonal protocols for managing mastitis are outlined by the seasonal dynamics of mastitis incidence in dairy cows and buffaloes kept on sub-tropical farms (**Gayathri et al. 2024a, Gayathri et al. 2024b**). In both annual cycles, July through September were the season with the highest recorded temperatures and THI. A common metric for assessing heat stress in dairy cows is the THI (**Hamel et al. 2021**). Relative humidity is the limiting element of heat stress in humid areas (**Baldwin et al. 2023**). In comparison to low THI, **Dawod (2022)** found that daily milk production and milk composition decreased at high THI. **Tamami et al. (2018)** and **Nasr and El-Tarabany (2017)** observed higher SCC values, decreased daily milk output, and composition in high THI levels as opposed to low THI levels.

Antimicrobial therapy is typically used on dairy farms to treat and prevent mastitis, and the widespread and improper use of antibiotics to treat this condition is thought to be the cause of rising antimicrobial resistance (**Singh et al. 2018; Drugea et al. 2025**). As a result, over the past ten years, a number of *E. Coli* bacterial strains that were isolated from dairy animals have progressively developed resistance to various antimicrobial agents. Data regarding the rising levels of acquired resistance to cefoxitin, sulphamethoxazole, cloxacillin, b-lactamase, tetracycline, quinolone, cephalosporin, amoxicillin, penicillin, cephalexin, trimethoprim, and chloramphenicol have been reported in several publications (**Ababu et al. 2020; Elias et al. 2020; Hassani et al. 2022; Widodo et al. 2022**). As shown in Table 4(A, B and C).

E. coli isolates from collected milk samples of cattle, sheep, and goats, during two annual cycles demonstrated an elevated rate of penicillin resistance followed by resistance to ofloxacin and cefotaxime. According to **İşnel and Kırkan (2012)**, who obtained *E. coli* isolates from goats with subclinical mastitis that shown resistance to cefotaxime and penicillin, these results were in agreement with this study findings. Since there is a negative correlation between pH and biofilm formation, **Kundukad et al. (2017)** reported that administering penicillin intramammary increases the formation of biofilms. Therefore, chemical agents such as sodium bicarbonates can be used to raise pH, which will reduce infection and biofilm formation. Also, **Mshelia et al. (2014)** reported that *E. coli* isolates from endometritis showed resistance against ofloxacin.

antimicrobial medicines are currently the mainstay of treatment for E. coli infections. The increase of medication resistance has an impact on animal productivity and is a health risk to humans, making treatment procedures more difficult and perhaps increasing medical expenses and treatment failures. Developing efficient infection control methods and encouraging the responsible use of antibiotics require ongoing surveillance and thorough study on MDR *E. coli* (**Xu et al. 2023**).

Magiorakos et al. (2012) defined multidrug resistance (MDR) as phenotypic resistance to three or more distinct antibiotic classes. The study found that the seasons of July–September and April–June had the highest rates of MDR. Summertime isolates of *E. coli* displayed the highest levels of phylogenetic group diversity and antibiotic resistance gene carriage. Temperature is one of the favourable and survival-supporting environmental elements that may be responsible for the somewhat higher incidence of antibiotic-resistant E. coli in the summer. The mean temperature in the summer is above 30 °C, which is ideal for bacterial growth. The study indicated a tendency towards summer and showed

seasonal fluctuation in organism detection (**Yasmin et al. 2022**).

CONCLUSION

This study provides critical insights into the prevalence, antimicrobial resistance patterns, and climate-associated dynamics of *E. coli* contamination in raw milk from dairy farms across El-Beheira Governorate. Key findings reveal an overall *E. coli* contamination rate of 23.8% in raw milk samples, indicating substantial risk of zoonotic transmission through unpasteurized dairy products. The elevated frequency of contamination during warmer seasons aligns with peak temperature-humidity indices, underscoring the influence of climatic factors on pathogen prevalence.

Notably, widespread MDR observed among isolates represents a serious public health concern. The persistence of MDR *E. coli* strains in the dairy supply chain necessitates urgent interventions to mitigate transmission risks through human food pathways. These findings highlight the emerging threat of climate-amplified antimicrobial resistance in food systems.

Consequently, Recommended that enhanced surveillance of antimicrobial use in livestock production and implementation of climate-adaptive food safety protocols. Sustained monitoring of resistance patterns and targeted stewardship programs are essential to curb the proliferation of drug-resistant pathogens in dairy ecosystems under changing climatic conditions.

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