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Prevalence and Characterization of *Escherichia coli* in Raw Milk and Some Dairy Products at Mansoura City

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Abstract

The present study aimed to detect the prevalence and antibiotic sensitivity pattern of *Escherichia coli* in raw milk and some dairy products (white soft cheese, yoghurt, and Laban rayeb) in Mansoura city, Egypt. A total of 200 samples, obtained equally from raw milk (farm and market milk), white soft cheese (Kareish and Domiati), yoghurt (small scale and large scale), and Laban rayeb (small scale and large scale) were examined for the presence of *E. coli* by using eosin methylene blue agar (EMB). Suspected *E. coli* isolates were confirmed by biochemical tests and then selected numbers of *E. coli* strains were identified serologically. Furthermore, serologically identified strains were subjected to antibiotic sensitivity testing. In total, the prevalence of *E. coli* recovered from the examined raw milk and dairy products samples was 28% (56/200). The highest prevalence was detected in raw market milk (52%), followed by Kareish cheese (48%) while, the lowest prevalence was obtained in large-scale yoghurt and large-scale Laban rayeb samples (8%). The selected numbers of *E. coli* strains subjected to serologic examination showed variable somatic and H antigens. About 58.8% of *E. coli* strains showed multi-antibiotic resistance (MAR) criteria at least to one antimicrobial in three different classes of antibiotics. The highest resistance was obtained from erythromycin (100%) then oxacillin (94%), cefepime (82%), penicillin G (76.5%), and ampicillin (58.5%), nalidixic acid (52.9%) and cefazolin (47.1%). the obtained results show the great hazard proposed to public health, therefore, the application of hygienic measures in all practices concerning dairy industry from farm to fork is not just advice but a necessity to maintain human health.

KEYWORDS
Antibiotic resistance, Dairy products, E. coli, Raw milk.

INTRODUCTION

Milk is the complete ideal natural undisputed food for newborn animals all over the world obtained from milking of dairy animals and still consumed raw by several populations who is believing that heat treatment destroys its content of beneficial nutrients (Angulo *et al.*, 2009). These priceless content of essential nutrients; protein, carbohydrates, fat, vitamins, minerals and water make milk and dairy products an ideal food medium also for bacteria that contaminate them from different sources (Yohannes, 2018).

Here in Egypt, there is usual consumption of raw milk and dairy products like yoghurt, raw milk cheese, and Laban rayeb which all may be manufactured and handled under unhygienic practices, especially in rural areas (Al-Ashmawy *et al.*, 2016). All these products are the most traditional popular products which could be made at a small scale level with deficient hygiene during milking, absence of heat treatment, storage, loose covering, and no refrigeration make these products may be exposed to microbial contaminants proposing health risk to consumers (Sánchez-Gamboa *et al.*, 2018).

The microbial community of raw milk and raw milk-based dairy products may introduce foodborne diseases or outbreaks to the public due to the pathogenicity and toxigenicity of these bacteria especially when present in high counts enhanced by poor hygienic practices during manufacturing (Khalifa and Nossair, 2016). This could be more complicated when the causative bacteria harbored multi-antibiotic resistance (MAR) feature as this protects it from the action of several classes of antibiotics that used against it leaving no therapy for infected patients (WHO, 2019).

E. coli is a common inhabitant of the animals' intestinal tract (Tark *et al.*, 2017) and it is one of the most common raw milk contaminants with several routes but mainly from animal manure. *E. coli* is the consistent indicator of fecal contamination and the presence of other enteric pathogens in raw milk constituting public health hazard to consumers (Soomro *et al.*, 2002). It is one of the potential pathogenic foodborne pathogens and several outbreaks attributed to *E. coli* contaminated milk have been recorded (Ombarak *et al.*, 2016). Besides that it is used as a control parameter to evaluate both hygienic practices and the pasteurization process in the dairy industry; its presence indicates poor hygiene during manufacturing of dairy products mainly due to fecal-oral route contamination and failure of the pasteurization

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process due to its sensitivity to heat (Yohannes, 2018).

E. coli is considered a main cause of diarrhea in humans and according to virulence factors it is classified into several serotypes such as enterohaemorrhagic *E. coli* (EHEC) which belongs to Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enter invasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC) (Jafari *et al*., 2012). STEC strains can produce Shiga toxins (*Stx1*, *Stx2*) and intimin (*eae*) which are the most important virulence factors in *E. coli* strains in milk and dairy products and many studies revealed that STEC also harbored MAR feature (Momtaz *et al*. 2012). It causes severe syndromes like bloody diarrhea, hemolytic-uremic syndrome leading to kidney failure, thrombotic thrombocytopenic purpura, and hemorrhagic colitis (Kuyucuoglu *et al*., 2012) through consumption of food like raw milk and dairy products because those are the main sources of *E. coli* to humans.

Unnecessary overuse of antibiotics in dairy animals for treatments, feed additives, uncalculated doses, and preventive therapy leads to bacteria developing a characteristic protective antibiotic resistance which encoded genetically and this is a great problem for human health worldwide (Hassani *et al*. 2022). *E. coli* is a member of Enterobacteriaceae; according to the World Health Organization (WHO) is one of the most critical pathogens that can cause harmful and even lethal infections such as bloodstream infections which could be more hazardous by being resistant to several different classes of antibiotics, especially carbapenems and third-generation cephalosporins – the best existing antibiotics used in the treatment of MAR bacteria (Shrivastava *et al*., 2018). *E. coli* resistant strains in food can transmit antimicrobial resistance and its encoded genes to other bacteria and humans via raw milk or raw milk products (Rasheed *et al*., 2014; Yoon and Lee, 2022).

E. coli harbored resistant genes by mutation, transposons, or plasmids and one of the major resistant routes is developing Extended-spectrum β-lactamases which are enzymes that hydrolyze the β-lactam ring, and ESBL-producing *E. coli* has a serious hazard for humans (Khoshbakht *et al*., 2014) being able to resist β-lactam antibiotics by mutation or horizontal gene transfer (Ali *et al*., 2016) . Studies confirmed that beta-lactamase emergence threats human health because MAR is transferred to humans and may compromise the effect of infection treatment (Kaesbohrer *et al*., 2012).

Consequently, *E. coli* antibiotic resistance profile must be taken into consideration, especially with the frequent occurrence as a food contaminant. The current study was accompanied to add recent data concerning the prevalence and antibiotic susceptibility profiles of *E. coli* stains isolated from raw milk and some dairy products sold and consumed in and around Mansoura city hoping to provide detailed information about the presence of MAR *E. coli* in such samples which proposed as a major concern to human health.

MATERIALS AND METHODS

Sampling

A total of 200 samples of raw milk and some dairy products including 50 raw milk (farm and market milk, 25 each), 50 white soft cheese (Kareish and Domiati, 25 each), 50 yoghurt (small scale and large scale, 25 each) and 50 Laban rayeb (small scale and large scale, 25 each) were collected randomly from dairy shops, local markets and supermarkets from different localities at Mansoura city, Egypt. All dairy product samples were within the expiry date printed on the product label. They were placed in an icebox (2-5°C) and transferred as quickly as possible to the lab for examination.

Counting of E. coli

All samples were prepared aseptically before the examination as stated in the previous methodology (FDA, 2020). Briefly, 25 g and/or mL of the sample was mixed with 225 mL of Buffered Peptone Water (BPW, Oxoid, Hampshire, UK) to obtain the first dilution, and further tenfold serial dilution was done using one mL of first dilution step to 9 mL BPW up to dilution step 10^6. From the selected dilutions 0.1 mL was transferred and evenly distributed using a sterile glass spreader onto the surface of Eosin Methylene Blue agar (EMB, HiMedia, Maharashtra, India) in duplicate. The inoculated plates were incubated aerobically at 37°C for 24–48 h. Presumptive colonies characterized by dark centered and flat, with or without green metallic sheen were considered E. coli. Two consecutive plates with colonies between 30-300 were recorded and the calculation was based on colony-forming units (CFU) per g and/or mL. From each sample, 3-5 suspected *E. coli* isolates were selected for further identification ISO 4833-2 (2013).

Identification of E. coli

Suspected isolates of *E. coli* were identified according to MacFaddin (2000) using the microscopical examination of Gram-stained smears, motility test, indole test, methyl red test, Voges–Proskauer test, citrate utilization, urea hydrolysis test, Triple Sugar Iron test and nitrate reduction test. According to the results of these chosen tests, all isolates could be differentiated and identified whether it is *E. coli* or other *Coliform* (*Klebsiella, Citrobacter, Enterobacter, and Serratia*).

Serological identification of E. coli

The isolates were serologically identified according to Kok *et al*. (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic *E. coli* strains. All procedures were performed according to manufacturer instructions. Two separate drops of saline (NaCl or ringers) were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension. One drop of saline was added to the first suspension, mixed, and considered as a control. To the other suspension, one drop of the undiluted antiserum was added and titled back and forward for one minute. Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum (DENKA SEIKEN Co., Japan), further testing with mono-valent sera to identify the O-antigen (8 antisera sets) and H- sera (13 antisera) were performed. Then according to the serotypes, identified pathotypes could be determined; each serotype could be easily pathotyped.

Antimicrobial susceptibility testing

The antimicrobial sensitivity phenotypes of the serologically identified isolates were determined by the Kirby-Bauer disc diffusion method according to Al-Kharousi *et al*. (2019) using discs with variable concentrations of antibiotics (Oxoid Limited, Basingstoke, Hampshire, UK). The tested antibiotic includes Nalidixic acid (NA) (30 ug); Ciprofloxacin (CP) (5 ug); Tetracycline (T) (30 ug); Penicillin G (P) (10 IU); Cefoxitin (C) (30 ug); Erythromycin (E) (15 ug); Oxacillin (OX) (1 ug); Imipenem (IPM)(10 ug); Levoflox-

acin (L) (5 ug); Ceftriaxone (CRO) (30 ug); Cefotaxime (CTX) (30 ug); Cefepime (FEP) (30 ug); Ampicillin (AM) (10 ug); Amikacin (AK) (30 ug); Gentamicin (G) (10 ug); Meropenem (M) (10 ug); Cefazolin (CZ) (30 ug) and Sulphamethoxazol (SXT) (25 ug).

Antimicrobial susceptibility test were evaluated according to the guidelines by National Committee for Clinical Laboratory Standards "NCCLS" (2001). The tested strains were evaluated as susceptible, intermediate, and resistant. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by Singh *et al*. (2010) as follows: MAR index= No. of antibiotics the isolate resistant to (isolates classified as intermediate were considered sensitive for MAR index)/ Total No. of tested antibiotics. *E. coli* (ATCC 25922) was used as a positive control of the test.

RESULTS

This study aimed to investigate the prevalence of *E. coli* in raw milk and some dairy products sold in different localities of Mansoura city. A total of 200 samples of raw milk and some dairy products including were microbiologically examined; out of them, *E. coli* strains could be isolated from 56 samples (28%). Raw market milk scored the highest prevalence with 13 samples (52%) contaminated with E. coli, followed by 12 Kareish cheese samples (48%), farm milk and small-scale yoghurt had equal prevalence 8 samples 32%), 6 samples of small scale Laban rayeb 24%), 5 samples of Domiati cheese (5 20%) and the lowest prevalence was in large scale yoghurt and large scale Laban rayeb samples only 2 samples each, (8%). *E. coli* had mean counts of $3.88\times10^4\pm1.7\times10^4$ CFU/mL, $6.3\times10^3\pm9.04\times10^2$ CFU/mL, $2.62\times10^3\pm8.8\times10^2$ CFU/mL, $1.35\times10^3\pm6.5\times10^2$ CFU/mL, $5.6\times10^3\pm2.7\times10^3$ CFU/mL, $2.9\times10^3\pm8.9\times10^2$ CFU/mL, $4.8\times10^3\pm1.3\times10^3$ CFU/mL and $2.5\times10^2\pm1.5\times10^2$ CFU/mL in raw farm milk, raw market milk, small scale yoghurt, large scale yoghurt, Kareish cheese, Domiati cheese, small scale Laban rayeb, and large scale Laban rayeb, respectively (Table 1).

The highest frequency distribution was found to be between 10^3 and 10^4 as the following 50, 84.6, 75, 100, 83.3, 100, and 66.7% for raw farm milk, raw market milk, small scale yoghurt, large scale yoghurt, Kareish cheese, Domiati cheese, and small scale Laban rayeb while for large scale Laban rayeb found all samples (100%) between 10^2 and 10^3 (Table 2). Numerically, the highest number of isolates was recovered from Kareish cheese (65 isolates) followed by raw market milk (52 isolates) while the small and large-scale Laban rayeb represent the lowest number of isolates (24 and 8 isolates, respectively). Based on biochemical identification of the recovered isolates the most revealed species were *E. coli* and Klebsiella spp. 53.8 , 30.8 % of total isolates from market raw milk and Kareish, respectively followed by 10 isolates Citrobacter spp (15.4%) recovered from Kareish cheese and 6 isolates Enterobacter spp 15.0, 9.2% obtained from each farm raw milk and Kareish cheese, respectively (Table 3).

Twenty-five *E. coli* strains (5 raw farm milk, 5 raw market milk, 3 small scale yoghurt, 2 large scale yoghurt, 3 from Kareish cheese, 2 from Domiati cheese, 3 small scale Laban rayeb, and 2 from large scale Laban rayeb) were selected for further serological identification and only 17 isolates could be characterized as 10 different *E. coli* serotype belongs to five different pathotypes; EHEC pathotype found in all products, EPEC in raw market milk and large-scale Laban rayeb, ETEC in raw farm milk and Kareish cheese, and EAEC only in raw farm milk (Table 4). Antibiotic sensitivity testing of serologically identified strains showed that 10 (58.8%) isolates were MAR and resisted at least one antimicrobial of three or more different antibiotic classes which commonly used for the treatment of *E. coli* infections; 17.6%, 29.4%, and 11.8% of the tested isolates resisted three, five, and six antibiotic classes, respectively (Table 5). The highest resistance was found against erythromycin (100%) followed by oxacillin (94%), cefepime (82%),

Table 1. Prevalence of *E. coli* in raw milk and some dairy products (means ± SE)

		No. of samples	No. of positive samples (%)	Minimum	Maximum	Mean ± SE
Raw milk	Farm milk	25	8 (32%)	2.3×10^3	1.33×10^5	$3.88\times10^4\pm1.7\times10^4$
	Market milk	25	13 (52%)	1.4×10^3	1.19×10^4	$6.3\times10^3\pm9.04\times10^2$
white soft Cheese	Kareish cheese	25	12 (48%)	2.0×10^3	1.0×10^4	$5.6\times10^3\pm2.7\times10^3$
	Domiati cheese	25	5 (20%)	1.7×10^3	4.0×10^3	$2.9\times10^3\pm8.9\times10^2$
Yoghurt	Small scale	25	8 (32%)	0.5×10^3	0.8×10^4	$2.62\times10^3\pm8.8\times10^2$
	Large scale	25	2 (8%)	7.0×10^2	2.0×10^3	$1.35\times10^3\pm6.5\times10^2$
Laban rayeb	Small scale	25	6 (24%)	1.6×10^3	1.0×10^4	$4.8\times10^3\pm1.3\times10^3$
	Large scale	25	2 (80%)	1×10^2	4×10^2	$2.5\times10^2\pm1.5\times10^2$

Table 2. Frequency distribution of the *E. coli* count in raw milk and examined dairy products

Samples	Raw milk		Yoghurt		white soft Cheese		Fermented milk Laban (rayeb/ buttermilk)	
Interval	Farm milk	Market milk	Small scale	Large scale	Kareish cheese	Domiati cheese	Small scale	Large scale
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
$10^2<10^3$	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)
$10^3<10^4$	4 (50)	11 (84.6)	6 (75)	2 (100)	10 (83.3)	5 (100)	4 (66.7)	0 (0.0)
$10^4<10^5$	3 (37.5)	2 (15.4)	2 (25)	0 (0.0)	2 (16.7)	0 (0.0)	2 (33.3)	0 (0.0)
$10^5<10^6$	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
$10^6<10^7$	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	8 (100)	13 (100)	8 (100)	2 (100)	12 (100)	5 (100)	6 (100)	2 (100)

Table 3. Frequency distribution of suspected *E. coli* isolates and other *Coliform*s spp in raw milk and examined dairy products on EMB media according to biochemical identification

Isolates		*E. coli*	*Klebsiella* spp	*Citrobacter* spp	*Enterobacter* spp	*Serratia* spp	Total no. of isolates
Samples		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Raw milk	Farm milk	23 (57.5)	7 (17.5)	3 (7.5)	6 (15)	1 (2.5)	40
	Market milk	28 (53.8)	12 (23%)	7 (13.5)	3 (5.8)	2 (3.8)	52
Yoghurt	Small scale	13 (40.6)	9 (28.1)	8 (25)	1 (3.1)	1 (3.1)	32
	Large scale	6 (35.6)	7 (41.2)	3 (17.6)	1 (5.9)	0 (0.0)	17
white soft Cheese	Kareish cheese	27 (41.5)	20 (30.8)	10 (15.4)	6 (9.2)	2 (3.0)	65
	Domiati cheese	15 (53.6)	10 (35.7)	2 (7.1)	1 (3.6)	0 (0.0)	28
Laban Rayeb	Small scale	13 (54.2)	6 (25.0)	3 (12.5)	1 (4.2)	1 (4.2)	24
	Large scale	5 (62.5)	1 (12.5)	1 (12.5)	1 (12.5)	0 (0.0)	8
Total		130 (48.9)	72 (27.1)	37 (13.9)	20 (7.5)	7 (2.6)	266 (100)

Table 4. Serological identification of *E. coli* strains isolated from raw milk and examined dairy products

Pathotypes	Serotype	Raw milk		Yoghurt		White soft cheese		Laban rayeb		Total number
		Farm milk	Market milk	Small scale	Large scale	Kareish cheese	Domiati cheese	Small scale	Large scale	
EAEC	O_{44}: H_{18}	1	-	-	-	-	-	-	-	1
1ETEC	O_{127}:H_6	1	-	-	-	2	-	-	-	3
EHEC	O_{103}:H_2	1	-	-	-	-	-	-	-	1
	O_{26}:H_{11}	1	2	-	-	1	-	-	-	4
	O_{111}:H_2	-	1	-	-	-	-	1	-	2
	O_{91}: H_{21}	-	-	1	-	-	1	-	-	2
	O_{117}: H_4	-	-	-	1	-	-	-	-	1
	O_{86}	-	-	-	-	-	-	-	1	1
EIEC	O_{159}	-	1	-	-	-	-	-	-	1
EPEC	O_{18}: H_7	-	1	-	-	-	-	-	-	1
Total number		4	5	1	1	3	1	1	1	17

EAEC: enteroaggregative E. coli; ETEC: enterotoxigenic E. coli; EHEC: enterohemorrhagic E. coli; EIEC: enteroinvasive E. coli; EPEC: enteropathogenic E. coli

Table 5. Antibiogram of *E. coli* strains isolated from raw milk and examined dairy products

No	Isolate ID	Dairy product type	*E. coli* serotypes	Antimicrobial resistance profile	No antibiotic classes	MAR index
1	1	Farm milk	O_{44} : H_{18}	E, OX, FEP, P,CRO,CTX	2	0.333
2	2		O_{127} : H_6	E, OX, FEP, P, CRO, CTX, AM, NA, CZ, T, SXT	5	0.611
3	4		O_{103} : H_2	E, OX, FEP, P,CRO,CTX , AM, NA, CZ	3	0.5
4	5		O_{26} : H_{11}	E, OX, FEP, P,CRO,CTX , AM, NA	3	0.444
5	6	Market milk	O_{26}: H_{11}	E, OX, FEP, P, CRO, AM, NA, CZ, T, SXT, CP	5	0.611
6	7		O_{159}	E	1	0.055
7	8		O_{111} : H_2	E, OX, FEP, P, AM, NA, CZ, T, SXT, CP, C	5	0.611
8	9		O_{18} : H_7	E, OX, FEP, P, CRO, CTX, AM, NA, CZ, T, SXT	5	0.611
9	10		O_{26} : H_{11}	E, OX, FEP	2	0.166
10	13	small scale yoghurt	O_{91} : H_{21}	E, OX, FEP, P ,CRO	2	0.277
11	14	large scale yoghurt	O_{117} : H_4	E, OX, FEP, P,CRO,CTX , AM, NA, CZ	3	0.5
12	16	Small scale rayeb	O_{111} : H_2	E, OX	2	0.111
13	19	Large scale rayeb	O_{86}	E, OX, FEP, P, CRO, CTX, AM	2	0.388
14	21	Kareish cheese	O_{127} : H_6	E, OX, FEP, P, CRO, CTX, AM, NA, CZ, T, SXT, CP, C, L, AK, M	6	0.888
15	22		O_{26} : H_{11}	E, OX, FEP, P, CRO, CTX, AM, NA, CZ, T, SXT, CP, C, L, AK, M, G, IPM	6	1
16	23		O_{127} : H_6	E, OX	2	0.111
17	25	Domiati cheese	O_{91} : H_{21}	E, OX, FEP, P, CRO,CTX , AM, NA, CZ, T, SXT, CP, C, L	5	0.777

MAR index: No. of antibiotics the isolate resistant to (isolates classified as intermediate were considered sensitive for MAR index)/Total No. of tested antibiotics.
E: Erythromycin; OX: Oxacillin; FEP: Cefepime; P: Penicillin G; AM: Ampicillin; NA: Nalidixic acid; CZ: Cefazolin; T: tetracycline; CP: Ciprofloxacin; C: Cefoxitin; L: Levofloxacin; CRO: Ceftriaxone; CTX: Cefotaxime; AK: Amikacin; G: Gentamicin; M: Meropenem; IMP: Ipipenem; SXT: Sulphamethoxazol.

penicillin G (76.5%), ceftriaxone (70.5%), both ampicillin and cefotaxime (58.5%), nalidixic acid (52.9%), cefazolin (47.1%) and both tetracycline and Sulphamethoxazol (41.2%) (Table 6).

DISCUSSION

In this study, *E. coli* was detected in 28% (56/200) of examined samples. As shown in Table 1; *E. coli* prevalence in examined raw farm milk was 32% and its count ranged from 2.3×10^3 to 1.33×10^5 CFU/mL with mean counts of $3.88\times10^4\pm1.7\times10^4$ CFU/mL, while in raw market milk 52% and ranged from 1.4×10^3 to 1.19×10^4 CFU/mL with mean counts of $6.3\times10^3\pm9.04\times10^2$ CFU/mL. These findings resembled those of Rahman *et al*. (2017) who mentioned a prevalence of 29.6% of raw milk.

A higher prevalence was observed by Chye *et al*. (2004) who recorded that 65% of raw milk had *E. coli* positive with mean counts from 10^3 to 10^4 CFU /mL. Ali and Abdelgadir (2011) also reported higher *E. coli* between 51.6 and 80% of raw farm milk and raw market milk, respectively with the highest mean counts of 3.93±0.01, and 3.9±0.03 $\log_{10}$/ml, respectively. Furthermore, Gundogan and Avci (2014) mentioned that 74% of raw milk is contaminated with *E. coli* with a count range from 2.5×10^4 to 1.6×10^6 CFU/L. In addition, Ombarak *et al*. (2016) and Megawer *et al*. (2021) recorded higher E.coli prevalence of 76.4% and 75% in raw market milk and raw milk, respectively.

However, a lower prevalence was reported by Sudda *et al*. (2016) with only 16.7% of raw milk contaminated with *E. coli* with a mean count of 2 CFU/mL. Additionally, Disassa *et al*. (2017) recorded 28.1% *E. coli* positive samples from farmers' milk with a mean count of 3.93±0.01 $\log_{10}$/mL while the samples from vendors represent 39.1% positive *E. coli* with mean counts of 4.978±0.180 $\log_{10}$/ml. Meanwhile, Elmonir *et al*. (2018) reported only 13.2% of market milk had E. coli, while, Ribeiro Júnior *et al*. (2019) demonstrated lower *E. coli* counts of 2.4×10^3 CFU/mL in raw milk.

As shown in Table 1, the examined traditional white soft cheeses; Kareish and Domiati had the following *E. coli* prevalence was 48%, 20%, respectively with counts ranging from 2×10^3 to 1×10^4 CFU/mL, and 1.7×10^3 to 4×10^3 CFU/mL, and mean counts of $5.6\times10^3\pm2.7\times10^3$ CFU/mL, and $2.9\times10^3\pm8.9\times10^2$ CFU/mL for Kareish, and Domiati cheese, respectively.

Many studies have recorded *E. coli* contaminating white soft cheeses with different prevalence; 19%, 45%, 60% (1×10^1 to 1.2×10^4 CFU/g), 64% ($4.56\times10^7\pm0.14\times10^7$ CFU/g), 74.5%, and 80% by De Campos *et al*. (2018); Kamal *et al*. (2017); Gundogan and Avci (2014); Farhat *et al*. (2017); Ombarak *et al*. (2016) and Ranjbar *et al*. (2018), respectively.

For Yoghurt samples, the prevalence was 32%, and 8% for small scale, and large-scale yoghurt, respectively with a count range from 5.0×10^2 to 8.0×10^3 CFU/g (mean $2.62\times10^3\pm8.8\times10^2$ CFU/mL), and 7×10^2 to 2×10^3 CFU/g (mean $1.35\times10^3\pm6.5\times10^2$ CFU/mL) for small, and large scale yoghurt samples, respectively.

Yoghurt showed a variable *E. coli* prevalence before as 8.3%, 25%, 44.4%, and 73.3% by Ranjbar *et al*. (2018); Megawer *et al*. (2021); Sobeih *et al*. (2020) and Aman *et al*. (2021).

For examined Laban rayeb, *E. coli* was isolated from 24%, and 8% of small, and large-scale Laban rayeb, respectively with counts ranging from 1.6×10^3 to 1×10^4 CFU/g (mean $4.8\times10^3\pm1.3\times10^3$ CFU/mL), and 1×10^2 to 4×10^2 CFU/g (mean $2.5\times10^2\pm1.5\times10^2$ CFU/g) in small, and large scale Laban rayeb, respectively. Similarly, El-Leboudy *et al*. (2017) detected *E. coli* in 30% of Baladi rayeb while Abd-Alla *et al*. (2020) isolated it from 55% of examined rayeb samples.

According to Egyptian Standards, cheese and fermented dairy products must be free from *E. coli* contamination Egyptian Standards (2005) revealed that positive *E. coli* examined samples in our study were unfit for human consumption.

Table 4, showed that 10 different *E. coli* serotypes belong to five different pathotypes that could be identified serologically; EHEC pathotype found in all products, EPEC in raw market milk and large-scale Laban rayeb, ETEC in raw farm milk, and Kareish cheese, and EAEC only in raw farm milk. Serotypes detected were; O44:H18, O127:H6 and O103:H2 (farm milk), O26:H11, O111:H2, O159 and O18:H7 (market milk), O91:H21 (small scale yogurt), O117:H4 (large scale yogurt), O111:H2 (small scale Laban rayeb), O86 (large scale Laban rayeb), O127:H6, O26:H11 (Kareish cheese), and O91:H21 (Domiati cheese).

In agreement with this study; Neher *et al*. (2015); Garbaj *et al*. (2016) and Sethulekshmi and Latha (2016) isolated STEC from milk samples. Furthermore, Vanitha *et al*. (2018) detected EHEC in raw milk, however, Ribeiro *et al*. (2019) detected EPEC, STEC, and

Table 6. Antimicrobial susceptibility profile of *E. coli* strains (n=17)

Antimicrobials		Sensitive	Intermediate	Resistant
		No (%)	No (%)	No (%)
Aminoglycosides	Amikacin (AK)	14 (82.4)	1(5.9)	2 (11.8)
	Gentamicin (G)	15 (88.2)	1(5.9)	1 (5.9)
Beta-lactams (Penicillins)	Oxacillin (OX)	-	1 (5.9)	16 (94.1)
	Penicillin G (P)	2 (11.8)	2(11.2)	13 (76.5)
	Ampicillin (AM)	5 (29.4)	2 (11.8)	10 (58.8)
Beta-lactams (1st generation Cephalosporins)	Cefazolin (CZ)	7 (41.2)	2 (11.8)	8 (47.1)
Beta-lactams (2nd generation Cephalosporins)	Cefoxitin (C)	11 (64.7)	2 (11.8)	4 (23.5)
Beta-lactams (3rd generation Cephalosporins	Ceftriaxone (CRO)		5 (29.4)	12 (70.5)
	Cefotaxime (CTX)	1 (5.9)	6 (35.3)	10 (58.8)
Beta-lactam (4th generation Cephalosporins)	Cefepime (FEP)	-	3 (17.6)	14 (82.4)
Beta-lactam (Carbapenems)	Meropenem (M)	15 (88.2)	-	2 (11.8)
	Ipipenem (IPM)	16 (94.1)	-	1 (5.9)
Macrolides	Erythromycin (E)	-	-	17 (100)
Quinolones	Nalidixic acid (NA)	5 (29.4)	3 (17.6)	9 (52.9)
	Ciprofloxacin (CP)	9 (52.9)	3 (17.6)	5 (29.4)
	Levofloxacin (L)	12 (70.6)	2 (11.8)	3 (17.6)
Sulfonamides	Sulphamethoxazol (SXT)	9 (52.9)	1(5.9)	7 (41.2)
Tetracyclines	Tetracycline (T)	6 (35.3)	4 (23.5)	7 (41.2)

EHEC in raw milk but were unable to isolate EAEC. Also, (Kasem *et al*., 2021) isolated EHEC, EETEC, EIEC, and EPEC as the main serotype from raw milk cheese. As presented in Table 5; 10 (58.8%) of identified strains (17) exhibited multi-antibiotic resistance (MAR) criteria having the ability to resist at least one antimicrobial of three or more different classes of antibiotics. It was found that 17.6 (3/17), 29.4 (5/17), and 11.8% (2/17) of the tested isolates resisted three, five, and six antibiotic classes, respectively. Similarly, Tark *et al*. (2017) determined that 50% of *E. coli* isolated from raw milk samples were MAR. Higher results were reported by Shecho *et al*. (2017) who showed that 92.3% of *E. coli* isolates were AMR, Elmonir *et al*. (2018) demonstrated MARs showed by 87.5% of *E. coli* isolated from raw milk. In addition, Elafify *et al*. (2020) stated that MAR was detected in 86.11% of STEC isolated from dairy products. Recently, Joseph and Kalyanikutty (2022) revealed that all STEC isolates were AMR, Kasem *et al*. (2021) also reported that 82.4% of *E. coli* isolated showed AMR. On the other hand, lower results of antibiotic resistance were reported by Rahman *et al*. (2017) who mentioned that 28.13% of *E. coli* isolates from milk were MAR. Moreover, Ombarak *et al*. (2018) revealed that 29.7% of *E. coli* isolated from Kareish cheese was AMR.Puig-Peña *et al*. (2020) revealed that 30.1% of *E. coli* were AMR, and more recently, Hammad e al. (2022) revealed that 7.8% of *E. coli* strains from Kareish cheese samples were AMR.

The highest resistance was found against Erythromycin (100%) followed by Oxacillin (94%), Cefepime (82%), Penicillin G (76.5%), ceftriaxone (70.5%), both Ampicillin and Cefotaxime (58.5%), Nalidixic acid (52.9%), Cefazolin (47.1%) and both Tetracycline and Sulphamethoxazol (41.2%) (Table 6). Antibiotic resistance results from the subsequent, repeated, unneeded, and overuse of antibiotics in animal and human treatments from infections Yohannes (2018).

Similar recorded studies were recorded, Elmonir *et al*. (2018) found that STEC isolates resisted ampicillin and tetracycline, Jamali *et al*. (2018) showed that *E. coli* isolated from milk samples were highly resistant to tetracycline. Additionally, El Bagoury *et al*. (2019) reported that all *E. coli* isolated from white soft cheeses were mainly resistant to erythromycin. Furthermore, Alsayeqh *et al*. (2021) in their review on antimicrobial-resistant pathogens from foods revealed that *E. coli* showed the highest resistance against Erythromycin, Tetracycline, Ampicillin, Amoxicillin, Vancomycin, Oxacillin, and Penicillin G; followed by resistance against Nalidixic acid, Ceftriaxone, Ciprofloxacin, Gentamicin, Sulfamethoxazole, Cefotaxime, and Cefoxitin. Also, Kasem *et al*. (2021) reported higher antibiotic resistance in *E. coli* against Erythromycin, Nalidixic acid, Cefotaxime, and Penicillin G.

A higher resistant profile was mentioned by Ababu *et al*. (2020) who stated that all *E. coli* isolated from dairy products resisted Cefoxitin, and Sulphamethoxazole by percentages of 72.73% and 54.54%, respectively.

But lower resistance was also obtained by Ombarak *et al*. (2018) who reported that dairy products E coli Strains were resistant to tetracycline, ampicillin, and sulfamethoxazole-trimethoprim at the following percentage; 18.9%, 18.5%, and 11.3%, respectively. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* can hydrolyze different kinds of cephalosporins including, cefotaxime, ceftazidime, ceftriaxone, and cefepime, thus, enabling *E. coli* to resist such antibiotic (Lee *et al*., 2020). ESBL-*E. coli* have emerged recently as the main cause that enables the distribution of antibiotic resistance worldwide (Ahmed *et al*., 2021).

Antibiogram by disc diffusion method in this study showed that some of the isolated strains were phenotypically ESBL-producing *E. coli* (Batabyal *et al*., 2018) as 70.5% and 58.5% of isolates resisted ceftriaxone, and cefotaxime (Table 6). The danger of MAR *E. coli* is that it could transmit this drug resistance to humans as stated by Yoon and Lee (2022) besides causing its known infections and syndrome.

Different percentages of ESBL were recorded by Ahmed *et al*. (2021) who found that 84.61% of ESBL from dairy products were E. coli, and Joseph and Kalyanikutty (2022) who reported that ESBL producers found in 26.75% of STEC isolates World health organization (WHO) designated a list named as the global priority pathogens list of antibiotic-resistant bacteria that pose the greatest hazard to human health divided into three main priorities based on the urgency and requisite for new antibiotics; carbapenem-resistant and ESBL-producing Enterobacteriaceae belong to priority 1 (critical list) (Shrivastava *et al*., 2018).

CONCLUSION

Higher *E. coli* counts detected in this study may be caused by contamination of raw milk from different environmental sources, traditional manufacturing of dairy products that lack heat treatment and hygienic practices, and for large-scale products, it may originate from post-processing contamination. There is a great need for discovering new techniques for fighting antibiotic-resistant microbes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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