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Occurrence of shiga-toxigenic and extended-spectrum β-lactamase (ESBL) Producing *E. coli* in some varieties of cheese and its public health hazard.

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ABSTRACT

The capability of pathogenic shiga toxin-producing *E. coli* (STEC) to produce the toxins *Stx1* and *Stx2*, which cause intestinal disorders, makes their presence in dairy products a public health problem. This study major goal was to identify the presence of ESBL-producing *E. coli* and shiga toxins in several cheese varieties that are sold in and around Assiut City. Ninety random samples of white soft cheese, including Kariesh, Domiati, and Tallaga cheese (30 of each), were collected and checked for the presence of *E. coli*, which were 86.7, 0.0, and 16.7%, respectively. A total of 31 *E. coli* isolates (34.4%) were recovered from 90 samples. Twelve *E. coli* isolates were positive for ESBL production and were multidrug-resistant. Ten of which were molecularly examined for the detection of shiga toxins (*Stx*1 and *Stx*2) genes and a *fliC-specific* gene for *E. coli O175:H7*. Two isolates harbored *Stx1*, four isolates harbored *Stx2* virulence gene, and seven isolates were *E. coli O175:H7*. The susceptibility to ten antimicrobial agents was evaluated by the disk diffusion method. The results of antimicrobial susceptibility testing showed high sensitivity to colistin (CL), Ciprofloxacin (CP), trimethoprim-sulfamethoxazole (COT), gentamicin (GEN), and tetracycline (TE) with 90.3, 87.1, 83.8, 80.6, and 58.1%, respectively, while resistance to amoxicillin (AMX), Erythromycin (E), ampicillin (AMP), cefotaxime (CTX), and amoxicillin-clavulanic acid (AMC) with 93.6, 93.6, 77.5,51.5 and 48.4%, respectively. The acquired initial findings revealed sanitary and hygienic deficiencies throughout the production process of Kariesh cheese and showed that these products able to contain multidrug-resistant and virulent strains of *E. coli* and may play a role in the spread of antimicrobial- resistant (AMR) to other microorganisms, posing a risk to public health. This implies that soft cheese needs to be viewed as a medium through which possibly harmful germs can spread. To get thorough findings, more research and analysis of more samples are still required.

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INTRODUCTION

One of the fundamental dietary groups for humans is cheeses. But unpasteurized milk is frequently used to make fresh white cheese, which is then sold in open marketplaces. Contamination with harmful germs may become more likely as a result. It is extremely concerning for public health when multidrug-resistant pathogenic microorganisms are found in food (**De la Rosa-Hernández et al. 2018).**

In the different geographical locations in Egypts, traditional methods are used to manufacturing fresh, soft cheeses. The conventional manufacturing process comprises curd formation, renneting, and preparation for marketing. This cheese is manufactured without starter cultures from raw milk that has typically been heat-treated. Microbial contamination of cheese may originate from various sources during processing **(Temelli et al. 2006),** storage **(Brito et al. 2008)** or by contamination from workers **(Callon et al. 2008).**

In Egypt, white soft cheese varieties such as Domiati, Tallaga, and Kariesh are the most popular local types **(Hegazy et al. 2012).** Cheese is a valuable dairy product and an essential component of a balanced diet, but it has also been a perfect medium for the growth and multiplication of numerous bacteria, including *Salmonella* and *E. coli*, which could pose a risk to the public's health **(Zakary et al. 2011).**

E. coli belongs to the family Enterobacteriaceae, which incriminated in all warm-blooded animals, confirmative detections entail a lack of medical and health management criteria **(Dias et al. 2012).** Cheese is thought to be one of the most prevalent source of enteropathogenic *E.coli* (EPEC) which can result in an elevated incidence of illness and death in both the aging and younger populations **(Kousta et al. 2010).**

Shiga toxigenic *E. coli* (STEC) is a significant foodborne zoonotic pathogen that causes hemorrhagic colitis, hemolytic uremic syndrome, and mild to severe diarrhea, STEC virulence genes including, intimin (eae) and hemolysin (hlyA), as well as shiga toxin genes *stx1* and *stx2* **(Gyles, 2007).**

Shiga toxin-producing *E. coli* (STEC), and verotoxin-producing *E. coli* (VTEC), has drawn significant attention as a global public health concern for a variety of sporadic infections and major outbreaks. This is because there are fewer, or frequently non effective antibiotics available for controlling of infections caused by such bacteria. Additionally, the most well-known serotype of *E. coli,* O157:H7, has become more resistant to drugs in recent years, leading to significant issues in healthcare settings across the globe (**Spano et al. 2017**). One of the major global health concerns of this century is multidrug resistance (MDR), and its global spread is becoming more prevalent. This is especially problematic in developing countries where non compliance with the implementation of regulation over the supply, use, and withdrawal period of antibiotics in veterinary, human, and food animal agriculture **(Adamu et al. 2018).**

Two forms of shiga toxin types (*stx1&2*) as well as subtypes that predominate in the pathogenicity of STEC are recognized, and they are known to have a significant role in determining the severity of sickness (**Cavalcanti et al. 2020**).

Global public health issues are increasingly being caused by *E. coli*, which produces narrow and extended-spectrum β-lactamases (ESBL) (**Feng et al. 2018**). Plasmid-mediated β-lactamase enzymes, known as ESBLs, are highly effective in hydrolyzing monobactams, penicillin, and cephalosporins of the third and fourth generations, with the exception of cephamycin and carbapenem (**Hassuna et al. 2020**).

Cheese may be regarded as a possible cause of foodborne infections. The most significant foodborne pathogen contaminates the cheese during the post-processing stage (**Vrdoljak et al. 2016).** It has been established that animals that can harbor a number of zoonotic infections, such as *E. coli*, which produces beta-lactamases. **(Carattoli, 2008).** Hence, the main objective of the current research was to

determine the occurrence and characteristics of shiga toxin and ESBL-producing *E. coli* from some varieties of soft cheese sold and in and around Assiut City, hoping to provide detailed information about the presence of multiple antidrug resistance (MAR) *E. coli*, which is proposed as a major concern to human health.

MATERIALS and METHODS

Samples collection:

A total of 90 randomized samples of white soft cheese, including 30 each of Kariesh, Domiati, and Tallaga cheese, were gathered from street vendors, dairy shops, and supermarkets in the Assiut governorates and placed in sterile polyethylene bags under aseptic conditions and safety precautions. The samples were promptly delivered to the lab after being stored in an ice box (2-4^{o}C) for isolation and identification of *E coli* .

Preparation of samples *(*APHA, 2004):

Separately, 10 g of each processed cheese sample was added to 90 ml of sterile buffered peptone water (BPW) in a sterile polyethylene stomacher bag. The mixture was then completely homogenized for three minutes using a stomacher (Seward Stomacher 80, BioMaster, England) before the samples were incubated.

Isolation and identification of *E. coli:*

One ml from each BPW was transferred to 5 ml MacConkey broth tube and incubated at 37° C for 24 hrs. A loopful from each incubated broth tube was streaked on MacConkey (MAC) agar (Merck, Germany) and incubated aerobically at 37°C for 24 h. The suspected *E. coli* pink colonies were streaked on Eosin Methylene Blue Agar (EMB), and incubated for 24 h. at 37°. Morphologically typical colonies with metallic sheen were picked up and subculture on nutrient agar slope at 37° C for 24 hrs for morphological and biochemical identification of *E. coli*, according to **Quinn et al. (2011).**

Phenotypic detection of *E. coli* O157: H7:

Sorbitol MacConkey agar medium (Oxoid, England) plates were used to disseminate the isolates of *E. coli.* then the plates were incubated for twenty-four hours at 35 oC. According to **FDA (2002),** the growth of *E. coli* on MacConkey agar supplemented with sorbitol results in colorless, pale-colored, sorbitol-negative colonies (typical *E. coli* 0157: H7).

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing was performed by the Kirby Bauer disc diffusion method according to the Clinical Laboratory Standards Institute guidelines (Sigma-Aldrich, USA) for the isolated *E. coli* strains against 10 antimicrobial agents, and the results were interpreted according to **CLSI (2020).** Briefly, the inoculum concentration was standardized by turbidimetry correspond to 0.5 on the McFarland turbidity standard and then dispersed on Mueller-Hinton agar plates (Himedia) using sterile swabs. The used antimicrobials agents were erythromycin (E), ampicillin (AMP), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), cefotaxime (CTX), colistin (CL), Ciprofloxacin (CP), gentamicin (GEN), tetracycline (TE) and trimethoprim-sulfamethoxazole (COT) . The diameter of the zone of inhibition generated by each antibiotic disc was measured and interpreted **(CLSI, 2020).** Isolates that exhibited resistance to three or more antibiotic classes were categorized as multidrug resistant (MDR) (**Magiorakos et al. 2012).** Isolates in this study with intermediate susceptibility were classified as resistant.

Extended Spectrum b-Lactamases (ESBL) testing :

Using the double-disc synergy test (DDST) diffusion method, ESBL production was detected in all *E. coli* isolates that produced ESBLs. Tests were conducted using cefotaxime (CTX 30 µg) and amoxicillin-clavulanic acid (AMC 20/10 µg). When cefotaxime and cefotaxime-clavulanic acid had a zone diameter greater than 5 mm, it was deemed to be ESBL positive. **(CLSI, 2020**).

Molecular detection of shiga toxins (*Stx*1 and *Stx*2) genes and *fli*C specific gene oF *E. coli* O175:H7 in some *E. coli* isolates by application of PCR assay:

"Application of PCR assay was performed in Reference Lab for veterinary quality control

on poultry production, Animal Health Research Institute, Doki , Giza, Egypt". Ten isolates were selected from ESBL-identified *E. coli and* subjected to PCR (polymerase chain reaction) assays to identify the characteristic *fliC-specific* gene for *E. coli* O175:H7 and the shiga toxins (*Stx*1 and *Stx*2) genes. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. All DNA samples were stored at -20°C until tested for the existence of shiga toxins (*Stx*1 and *Stx*2) genes and fliC-specific *gene* for *E. coli* O175:H7 in *E. coli* isolates using specific primers supplied from Metabion (Germany) as shown in Table (A). PCR amplification and analysis of the PCR products were performed as previously described by **Sadek and Koriem (2022).**

Statistical analysis:

statistical analysis of data was done with the Chi square test to determine whether there was a significant difference between them, and a "P" value of less than 0.05 was deemed statistically significant. Data were analyzed using GraphPad Prism 9.5.1 software **(GraphPad Software Inc., San Diego, CA, USA).**

Table A. Primers sequences, target genes, amplicon sizes and cycling conditions.

			Amplification (35 cycles)					
Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	Reference
*Stx*1	ACACTG-GATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Dipineto et al. 2006
*Stx*2	CCATGACAAC-GGACAGCAGTT CCTGTCAACTGAG-CAGCACTTTG	779						
Escherichia coli O157:H7 fliC	GCGCTGTCGAGTTC-TATCGAGC CAAC-GGTGACTTTATCGCCATTCC	625	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Fratamico et al. 2000

RESULTS

Totally thirty one positive samples (34.4%) out of 90 examined soft cheese samples were *E coli* positive represented 26 (86.7%,) out of 30 kariesh cheese, 5 (16.7%) out of 30 tallaga cheese while domiati cheese were negative for *E coli* (Table 1). Twelve *E. coli* isolates mainly from Kariesh cheese were positive for ESBL-production and were multidrug-resistant. Ten isolates were selected from ESBL-identified *E. coli and* subjected to gene-specific polymerase chain reaction (PCR) assays to identify the

characteristic *fliC-specific* gene for *E. coli* O175:H7 and the shiga toxins (*Stx*1 and *Stx*2) genes.7 out of 10 ESBL-producing *E. coli* were confirmed as *E. coli* O175:H7 by phenotyping which were sorbitol negative and gave pale colonies in MacConkey sorbitol agar and genotyping by *fliC-specific* gene for *E. coli* O175:H7. Two isolates harbored *Stx1*, four isolates harbored *Stx2* virulence genes, and seven isolates were O175:H7 (Table 3).

Table 1. Incidence of *E.coli* in cheese varieties.

Type of Cheese**	No of Samples	Positive Samples		Negtive Samples	
		No	%	No	%
Kariesh	30	26	86.7	4	13.3
Domiati	30	0	0	30	100
Tallaga	30	5	16.7	25	83.3
total	90	31	34.4	59	65.6

** High significance differences between types cheese types (P<0.0001, $\chi 2 = 56.19$)

Table 2. Antimicrobial susceptibility of *E. coli* isolated from cheese samples (n : 31).

antibiotics	sensitive**		resistant**	
	No	%	No	%
Amoxicillin (AMX) 20 µg	2	6.4	29	93.6
ampicillin (AMP)10 µg	7	22.5	24	77.5
amoxicillin-clavulanic acid (AMC)20/10 µg	16	51.6	15	48.4
cefotaxime (CTX)30 µg	15	48.4	16	51.6
tetracycline (TE)30 µg	18	58.1	13	41.9
ciprofloxacin (CP) 5µg	27	87.1	4	12.9
gentamicin (GEN)10 µg	25	80.6	6	19.4
erythromycin (E)15 µg	2	6.4	29	93.6
colistin (CL)10 µg	28	90.3	3	9.7
trimethoprim-sulfamethoxazole (COT) 1.25/23.75 µg	26	83.8	5	16.2

* High significance differences among sensitivity of different types of antibiotics (P<0.0001, $\chi 2 = 119.4$)
** High significance difference among resistant of different types of antibiotics (P<0.0001, $\chi 2 = 119.4$)

Table 3. PCR results for detection of *Stx1, Stx2, fli*C genes in ten isolates of ESBL-producing *E. coli* .

Serial number of tested *E. coli*	ESBL-production	Colony colour on MacConkey sorbitol agar	**Stx1** gene	**Stx2 gene**	**O157:H7** *fli*C gene
1	+	Red colony	-	+	Not tested
2	+	Pale colony	-	+	+
3	+	Pale colony	+	-	+
4	+	Pale colony	-	-	+
5	+	Red colony	-	+	Not tested
6	+	Pale colony	-	-	+
7	+	Red colony	+	-	Not tested
8	+	Pale colony	-	-	+
9	+	Pale colony	-	+	+
10	+	Pale colony	-	-	+

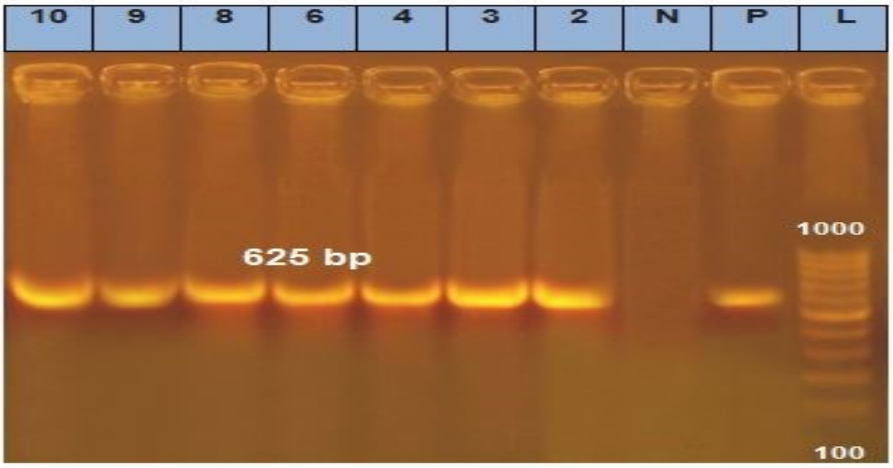

Figure (1): Agarose gel electrophoresis patterns of uniplex PCR amplification products for *E. coli* O157:H7 *fli*C gene. Lane L, DNA ladder marker (100 bp). Lane P, control positive *E. coli* O157:H7 *fli*C gene (625 bp). Lane N, negative control. Lanes 2,3,4,6,8,9 and 10 positive *E. coli* O157:H7 isolates for *fli*C gene.

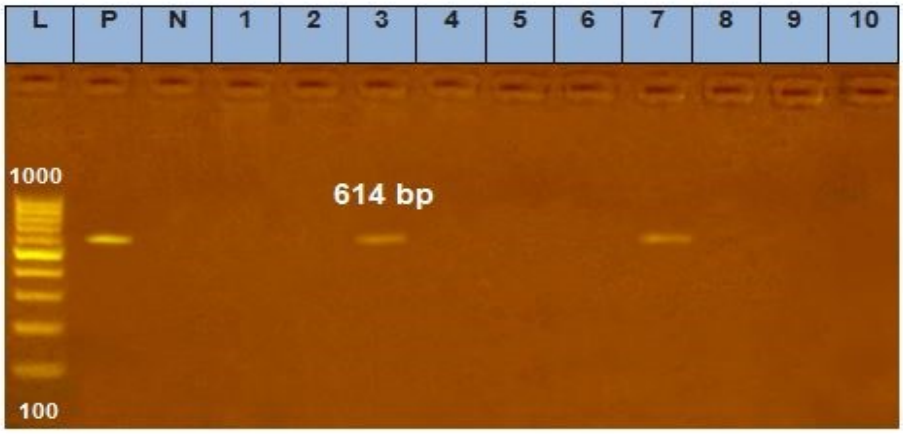

Figure (2): Agarose gel electrophoresis patterns of uniplex PCR amplification products for *E. coli Stx*1 genes. Lane L, DNA ladder marker (100 bp). Lane P, control positive *E. coli Stx*1 (614 bp) genes. Lane N, negative control. Lanes 3 and 7 positive *E. coli* isolates for *Stx*1 gene. Lanes 1,2,4,5,6,8-10, negative *E. coli* isolates for *Stx*1 genes.

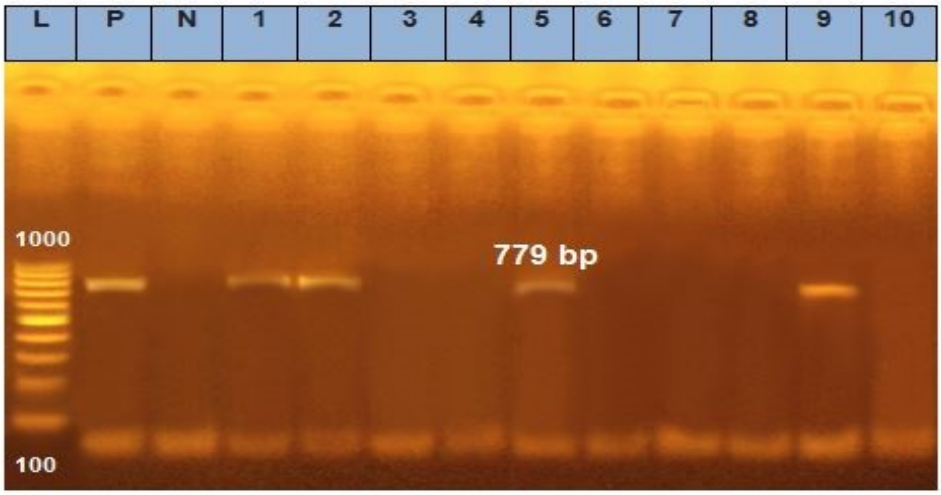

Figure (3): Agarose gel electrophoresis patterns of uniplex PCR amplification products for *E. coli Stx*2 genes. Lane L, DNA ladder marker (100 bp). Lane P, control positive *E. coli Stx*2 (779 bp) genes. Lane N, negative control. Lanes 1,2,5 and 9 positive *E. coli* isolates for *Stx*2 gene. Lanes 3,4, 6,7,8 and 10, negative *E. coli* isolates for *Stx*2 genes

DISCUSSION

E. coli that produces shiga toxins (STEC) are considered zoonotic bacteria that have been identified as a significant cause of foodborne disease, and as a result, have become a major public health concern around the world. The Eropian Union documented 6,378 instances of STEC infections that were confirmed in 2016. **(EFSA, 2017).**

Current outbreaks of foodborne disease have been recorded due to the ingestion of contaminated products infected with pathogenic organisms or their toxins, in particular *E. coli,* which is considered an essential cause of diarrhea in humans. Based on the current research, the total occurrence of *E. coli* species was **34.4%** of the examined cheese samples (Table 1). Lower incidence was reported by **EL-Bagoury et al. (2019)** 13.33% and **Heikal et al. (2014)** 26.7%,, while higher icedience, privately reported by **AbdEL-Tawab et al. (2020)** 42.5 % and **Imre et al. (2022),** 83.8%. The reported results are markedly influenced by the study design, seasonal and geographic variations, the testing methods (including the culture media and growth temperatures), or the differences in hygiene and milking practices **(Metz et al. 2020)**.

Table (1) also revealed that *E. coli* could be isolated from examined Kareish cheese samples with an incidence rate of 86.7%. A nearly similar finding were reported by **Sobeih et al. (2023); Mohamed et al. (2020); Ibrahim et al. (2019)** and **Ombark et al. (2016),** while a lower incidence were reported by **Farag et al. (2023); Omr & Abdeen (2023); Ibrahim et al. (2022); AbdEL-Tawab et al. (2020) ; El Bagoury et al.(2019) ; El-nahas et al. (2015),** and **Awad (2016).** On the contrary, **AboZeed (2014)** could not detect *E. coli* from the examined Kariesh cheese. According to **the Egyptian Standard for Karish cheese (1008/4/2005),** it should be free from *E. coli.* It is advised to follow proper manufacturing practices, as well as distribution and retail storage practices, to ensure cheese's microbiological safety

It is evident from Table (1) that *E. coli* could not be isolated from the examined Damietta cheese samples. The obtained data are in close agreement with **Thabet (2003)**, who reported that *E. coli* could not be isolated from the examined Damietta cheese samples,which may be due to the storage of Damietta cheese in salted whey for more than 8 weeks before marketing, which inhibt the growth of *E. coli*. A higher incidence rate was obtained by **Kur-**

sun et al. (2011), who detected *E. coli* in 40% of the examined samples, while **Ibrahim et al. (2022)** reported a high incidence of *E. coli* 20%. in Domiati cheese .In contrast, a lower incidence of 2.8% was reported by **El Bagoury et al. (2019).**

The result of table (1) declared that the prevalence of *E. coli* in Tallaga cheese was 16.7%. The same result was obtained by **Sobeih et al.** ***(2023).*** On the contrary, **El Bagoury et al. (2019)** could not detect *E. coli* from the examined Tallaga cheese. **AbdEL-Tawab et al. (2020)** reported a high prevalence of 32% of *E. coli* in Tallaga cheese, while a lower prevalence was reported by **Ahmed (2012).**

significant variations between the incidence of *E. coli* in examined cheese samples ($P<0.0001$, $\chi2 = 56.19$). Interestingly, from statistical analysis it is clear that, Damietta cheese is of suprior quality than Kareish cheese and tallaga cheese based on zero prevalence of *E. coli*. Contamination of milk and milk products with *E. coli* is largely due to unhygienic conditions during production, processing, handling, and distribution. *E. coli* is a good indicator of fecal contamination which exists as a normal microflora of the intestinal tract of humans and warmblooded animals **(Ibrahim et al. 2020).**

From a sanitary perspective, inadequate sanitation and using of row or inadequate heat treated milk in cheese manufacturing could be the cause of the high percentage of *E. coli* found in soft cheese samples **(Najand and Ghanbarpour, 2006).** The isolation of *E. coli* represents a serious public health hazard because some strains of *E. coli* may belong to enteropathogenic, toxigenic, or both groups, which cause severe gastrointestinal disturbance. **(Thomas et al. 2016).**

Antimicrobial resistance is recognized as a worldwide problem in human and veterinary practices. The unselective and inaccurate use of antimicrobials as well as their use as growth -promoting agents, has led to increase in antibiotic residues in food products of animal origin. These residues allowing the emerging of resistant bacteria through several encoding antibiotic resistance genes **(Cheng et al. 2020).** Consequently, food of animal origin constitute an ultimate environment for the development of different high pathogenic and resistant bacteria **(Kim et al. 2022).**

Antimicrobial sensitivity testing for all 31 *E. coli* isolates against ten different antibiotics (Table 2) showed high sensitivity to colistin (CL), Ciprofloxacin (CP), trimethoprim-sulfamethoxazole (COT), gentamicin (GEN), tetracycline (TE), and amoxicillin-clavulanic acid (AMC) with 90.3, 87.1, 83.8, 80.6, 58.1, and 51.6%, respectively, while resistance to amoxicillin (AMX), Erythromycin (E), ampicillin (AMP), cefotaxime (CTX), and amoxicillin-clavulanic acid (AMC) with 93.6, 93.6, 77.5, 51.5, and 48.4%, respectively. Current data were nearly in contact with **Omr & Abdeen (2023),** who revealed that *E. coli* strains showed higher sensitivity to gentamicin, ciprofloxacin, and amoxicillin/clavulanic acid, while **Ibrahim et al. (2022)** revealed that 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, and both Tetracycline and Sulphamethoxazol (41.2%). Antibiotic resistance results from the repeated, and overuse of antibiotics in animal and human treatments from infections **(Yohannes, 2018).** Similar studies were recorded, **Elmonir et al. (2018)** found that STEC isolates were resist to ampicillin and tetracycline, and **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 of antimicrobial-resistant pathogens from foods, revealed that *E. coli* showed the highest resistance against erythromycin, tetracycline, ampicillin, amoxicillin, oxacillin, and penicillin G, as well as showed resistance against cefotaxime and cefoxitin. Also, **Kasem et al. (2021)** reported higher antibiotic resistance in *E. coli* against erythromycin, cefotaxime, and penicillin G.

The data analysis revealed that there were

highly statistically significant variations concerning the antimicrobial sensitivity of different antibiotics ($P<0.0001$, $\chi2=119.4$), there were also highly significant variations concerning the antimicrobial resistance of different antibiotics ($P<0.0001$, $\chi2=119.4$). In general the drug resistance rate of ESBL-producing *E. coli* was higher than that of ESBL-negative *E. coli*.

From the public health point of view, 12 (38.7%) out of 31 identified *E. coli* strains, exhibited multi-antibiotic resistance (MAR), having the ability to resist at least one antimicrobial of three or more different classes of antibiotics. A nearly similar finding was 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 the *E. coli* isolated from Kareish cheese was AMR. **Puig-Peña et al. (2020)** revealed that 30.1% of *E. coli* were AMR. lower results of antibiotic resistance were reported by **Hammad et al. (2022),** who revealed that 7.8% of *E. coli* strains from Kareish cheese samples were AMR.

Phenotypic confirmation of ESBL- production showed that 12 isolates of the total of 31 *E. coli* isolates were ESBL- producers. A nearly similar finding was reported by **Joseph and Kalyanikutty (2022),** who reported that ESBL - producers were found in 26.75%, while a higher incidence was recorded by **Ahmed (2021),** who found that 84.61% of ESBL was from dairy products. Enzymes that have evolved from narrow-spectrum parent enzymes, known as ESBLs, are inhibited by β-lactamase inhibitors such as clavulanic acid and have hydrolytic activity against penicillins, aztreonam, and extended-spectrum cephalosporins (cefoxitin or cefotaxime), but not against cephamycins or carbapenems **(Lee et al. 2012).**

The World Health Organization (WHO), established a list of antibiotic-resistant bacteria that represent the biggest threat to human health, known as the global priority pathogens list. This list is separated into three primary categories according to the need for new antibiotics and their urgency. Enterobacteriaceae that produce ESBL and are resistant to carbapenems are classified as critical list priority one **(Shrivastava et al. 2018).**

The molecular assay of the 7 out of the selected 10 phenotypic ESBL-positive *E. coli* isolates which were sorbitol negative and gave pale colonies in MacConkey sorbitol agar were confirmed as *E. coli* O157:H7 isolates for *fli*C gene-specific of *E. coli* O175:H7 (Table 3 and Figure 1). Nearly similar findings were obtained by **Sobeih et al. (2023),** while a lower incidence in the rate range of 7% –12% was detected in previous studies by **Amagliani et al. (2016*)***, **Imre et al. (2022*),*** and **Farag et al. (2023).** In contrast, **Abebe et al. (2023)** and **El-Baz (2019).** could not detect *E. coli* O157:H7 in milk products. According to **Rahimi et al. (2012),** the acidity of the sample determines whether *E. coli* O157:H7 survives in food; the bacteria die when the pH drops below 3.5. Therefore, the acidity and the temperature at which the cheese was processed may be the inhibition cause of the lack of *E. coli O157:H7* in the yogurt and cheese samples. The presence of this pathogen represents a potential health risk to the consumers. Therefore, good hygienic measures should be applied during production. Also, periodic control and examination must be carried out by the health authorities to provide a healthy and safe product for the consumers.

Table 3 of the current study displays two *E. coli* isolates with a 20% presence of the *Stx1* gene and four isolates with a 40% presence of the *Stx2* gene, with a total prevalence rate of 60% for both. **Farag et al. (2023)** found nearly similar results, reporting 40% detection of both *Stx1* and *Stx2* positive *E. coli* isolates in Karish cheese. Conversely, higher results were recorded by **Vanitha et al. (2018), Elafify et al. (2020),** and **Elbehiry et al. (2021).** But **Taha et al. (2019)** detected them at different rates. **Freedman et al. (2016)** stated that *stx2* is more virulent and causes more severe symptoms than *stx1*.

From the public health point of view, the pathogenic group of *E. coli* known as shiga

toxin-producing *E. coli* (STEC) is a major foodborne pathogen that contributes significantly to foodborne infections and outbreaks with symptoms ranging from mild to bloody diarrhea and hemolytic uremic syndrome, which can lead to kidney failure **(FAO/WHO, 2018).** Furthermore, a number of recent studies have shown that all STEC strains (VTEC) are pathogenic, extremely harmful, and can result in serious illness or even death, particularly in young children and the elderly **(Koutsoumanis et al. 2020).** Lastly, the discovery of STEC contamination in samples that met both national and international legal requirements highlights the need for new, more stringent microbiological criteria.

CONCLUSION

The current study results indicated that handling and manufacturing of Kariesh cheese are done with inadequate cleanliness, which in comply with Egyptian standard requirements. The presence of coliforms and *E. coli* in kariesh cheese indicates a potential fecal contamination and enter pathogen, making it a vehicle for the transmission of certain food-poisoning germs to consumers. As a result, it is advised to make kareish cheese using premium non contaminated raw milk. Additionally, every milk used to make kareish cheese needs to be pasteurized or ultraheat treated (UHT). Antibiotics are thought to be the primary treatment for *E. coli,* but since they are overused and misused, multidrug-resistant bacteria have arisen and are now a global threat to public health. For the purpose of preventing STEC infections in human, raw milk must be heat-treated effectively before being consumed or processed. In order to eliminate the public health risk associated with STECs, consideration should also be given to the concepts of hazard analysis and critical control points. Excellent hygiene systems, flawless manufacturing systems, and moreover further studies are needed to assess the prevalence of VTEC in different dairy products in addition to ways to prevent and eleminate these pathogens from dairy products to protect consumers from health hazards.

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