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Original Article

Public health risks of *Escherichia coli* and *Staphylococcus aureus* in raw bovine milk sold in informal markets in Egypt

Walid Elmonir[1], Etab M. Abo-Remela[2,3], Azza Sobeih[4]

[1] Hygiene and Preventive Medicine (Zoonoses) Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt
[2] Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt
[3] Biology Department, Faculty of Sciences, Taibah University, Al-Madina Al-Munawara, Saudi Arabia
[4] Food control (Milk Hygiene) Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

Abstract

Introduction: Milk is an important food in Egypt and most of it is sold as raw milk in informal markets.
Methodology: This study was conducted to investigate the public health risks of *Escherichia coli* and *Staphylococcus aureus* in milk sold in informal markets in Egypt. A total of 121 milk samples were analyzed for occurrence, virulence genes and antibiotic resistance of *E. coli* and *S. aureus*.
Results: A total of 35/121 (28.9%) of milk samples were contaminated with 16/121 (13.2%) *E. coli*, 22/121 (18.2%) *S. aureus*, and 3/121 (2.5%) both isolates. Shiga-toxin producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC) and Enterotoxigenic *S. aureus* were detected in 5/121 (4.1%), 2/121 (1.7%) and 8/121 (6.6%) of the examined milk samples, respectively. Multiple drug resistances (MDRs) were showed by 14/16 (87.5%) and 21/22 (95.5%) of *E. coli* and *S. aureus* isolates, respectively. *E. coli* isolates showed high resistance for cephalothin (87.5%), ampicillin (68.8%) and tetracycline (68.8%), but were sensitive for gentamicin and chloramphenicol. Resistance phenotypes *of E. coli* were diverse; however, STEC isolates were significantly associated with co-resistance to cephalothin, ampicillin and tetracycline ($P< 0.05$). Two (9.1%) of *S. aureus* isolates were methicillin-resistant (MRSA) but sensitive to gentamicin (GS-MRSA). Five (22.7%) of *S. aureus* isolates were gentamicin-resistant methicillin-sensitive *S. aureus* (GR-MSSA). *S. aureus* isolates also showed high resistance for ampicillin (100%), tetracycline (90.1%) and sulfamethoxazole-trimethoprim (90.1%).
Conclusion: These findings highlighted the potential public health hazards of *E. coli* and *S. aureus* pathogens in raw milk sold in informal markets in Egypt.

Key words: Bovine milk; *E. coli*; *S. aureus*; virulence genes; antibiotic resistance.

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Introduction

Raw milk consumption by consumers may be attributed to the lack of awareness of foodborne pathogens in raw milk [1]. Another segment of the population may consume dairy products manufactured from raw milk. In a study in Egypt, over 80% of the farmers would process dairy products from raw milk [2]. In developed countries, up to 5% of food-borne infections were related to the consumption of milk and dairy products [3]. The case scenario could be worse for developing countries where high rates of milk contamination associated with unhygienic milk production and lack of efficient preservation [4].

Escherichia coli pathogens are often used as indicator of fecal contamination of milk and may impose presence of pathogenic serotypes for humans [4,3]. Whereas, *Staphylococcus aureus* contamination of milk either associated with milkers or milk handlers, especially those with poor hygienic habits as coughing or sneezing during milking or milk handling [5], or with infected cows as reservoirs of *S. aureus* infection [6]. In addition, *E. coli* and *S. aureus* are major causes of subclinical and clinical mastitis [6,7]. In subclinical mastitis, *E. coli* and *S. aureus* are shed in milk without abnormalities in milk consistency or udder shape; hence, humans may impact their health by consuming or processing milk from these cases.

Shiga-toxin producing *E. coli* (STEC) and Enterotoxigenic *E. coli* (ETEC) were associated with several life-threatening food-borne outbreaks worldwide [8,9]. STEC produces cytotoxins encoded by *stx*1 and *stx*2 genes. These cytotoxins are associated

with serious human illnesses as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) that usually end up with fatal consequences [9]. STEC causes around 3 million cases of acute illness and over 200 deaths each year [9]. ETEC causes diarrhea via production of heat-stable and heat-labile enterotoxins encoded by *ST* and *LT* genes, respectively. ETEC isolates are the most common cause of endemic diarrhea in children in developing countries [8,10]. In addition, it is the most frequent etiology of travelers' diarrhea [8].

S. aureus is one of the most common pathogens of food poisoning. *S. aureus* pathogens in milk, under favorable conditions, produce heat-stable enterotoxins which cause vomiting, and abdominal cramping with or without diarrhea [5]. The disease is self-limiting; however, few cases especially in infants and elderly may suffer acute illness and death [5]. Staphylococcal enterotoxins (SEs) are encoded by several genes; however, *sea*, *seb*, *sec*, *sed* and *see* were the most common genes implicated in cases of food poisoning worldwide [5]. The seriousness of *E. coli* and *S. aureus* milk-borne food poisoning was exaggerated with the emergence of Multi drug resistant (MDR) isolates worldwide, which may pose an additional threat to human health [11,12].

Egypt is considered the largest producer of bovine milk in Africa with an annual production of more than 5 million tons [13]. However, around 70% of milk is produced by Smallholder and sold in informal markets in Egypt [2]. Standard hygienic measures are rarely applied in this kind of production, therefore this study aimed to investigate the prevalence of *E. coli* and *S. aureus* as important food-borne pathogens in raw milk collected from informal markets in Kafrelsheikh governorate. The potential zoonotic hazards of these pathogens in term of virulence and antibiotic resistance were also studied.

Methodology

Study area and design

Kafrelsheikh is the largest governorate located in the middle of Nile-Delta of Egypt (31°06′42″N 30°56′45″E). A cross-sectional study was conducted between June and December 2014 to determine the prevalence, virulence genes and antibiotic resistance of *E. coli* and *S. aureus* as food-borne pathogens in raw milk sold in informal market for residents of Kafrelsheikh governorate. In informal milk markets, consumers purchased raw milk directly from individual farmers or milk collectors. The official authorities do not supervise informal markets. Majority of farmers who sell their milk in informal markets are smallholders of 3 to 5 household reared animals [2]. Farmers either sell their milk directly to consumers or sell it to milk collectors, who in turn sell the milk in the market. Milk in informal markets is sold without any heat treatment.

Sampling

A total of 121 raw milk samples were collected from informal markets in 10 villages in Kafrelsheikh governorate. Milk samples were collected from 10 to 15 individual farmers or milk collectors in each village. All collected milk samples were of normal colour, odour and without any flakes. Collected milk samples were immediately transported in icebox to lab for further analysis. On Arrival to Lab, milk samples were tested for possible heat treatment by peroxidase (storch) test [14].

Microbial isolation and identification

Pre-enrichment: all milk samples were diluted at rate of 1:9 in nutrient broth (LabM, Heywood, U.K.) and incubated at 37°C for 6 hours.

Isolation and identification of E. coli[15]: 1 mL of broth culture was mixed with 9 mL of EC broth (Oxoid, Hampshire, U.K.) and incubated at 44°C for 20 hours. An inoculum from EC broth with gas production was streaked on Levine's Eosin Methylene Blue agar (Oxoid, Hampshire, U.K.) and MacConkey agars (Oxoid) and incubated at 35°C for 20 hours. Suspected colonies were purified by sub-culturing on new selective agar plates and preserved on in nutrient agar slants for further analysis. For isolation of *E. coli* O157, the milk samples were selectively enriched in Tryptone Soy broth supplemented with 20mg/L Novobiocin (Oxoid, Hampshire, U.K.) and incubated for 6 hours at 37°C. A loopful from the broth was streaked on Sorbitol MacConkey agar (Oxoid, Hampshire, U.K.) supplemented with Cefixime -Tellurite supplement (Oxoid, Hampshire, U.K.) and incubated at 35°C for 20 hours. All suspected *E. coli* colonies were confirmed by biochemical tests using API-20E (bioMérieux, Marcy-l'Etoile, France) and PCR according to [16]. Confirmed isolates were sent for serotyping at Animal Health Research institute, Ministry of Agriculture of Egypt.

Isolation and identification S. Aureus[17]: A loopful from each pre-enrichment culture was streaked on Baired Parker Agar (Oxoid, Hampshire, U.K.) supplemented with Egg Yolk Tellurite (50mL/L) (Oxoid, Hampshire, U.K.) and incubated at 37°C for 24-48 hours. Suspected *S. aureus* colonies were identified by PCR detection of Staphylococcal *16s RNA* gene [18], biochemical tests using API Staph system

(bioMérieux, Marcy-l'Etoile, France) and tube coagulase test. Tube coagulase test was conducted according to the guidelines of FDA [17]. In brief, 0.3 mL of overnight culture was transferred into a sterile tube containing 0.5 mL of rabbit plasma with EDTA. The tubes were incubated at 37°C and examined periodically for 6 hours. Only tubes with complete and firm coagulation were considered positive.

DNA extraction and Molecular analysis

DNA extraction: DNA extraction from overnight Broth culture was performed using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Briefly, 200 µL of the sample was incubated with 10 µL of proteinase K and 200 µL of lysis buffer at 56ºC for 10 minutes. After incubation, 200 µL of 100% ethanol was added to the lysate. The sample was then washed and centrifuged. Nucleic acid was eluted with 100 µL of elution buffer provided in the kit.

Molecular analysis: PCR amplification of the targeted virulence genes was conducted by serious of multiplex PCR reactions as follow; for *Stx*1 and *Stx*2 genes [19], for *Sta* and *LT* genes [20] and for SEs genes [21] using the following mixture; 25µLEmeraldAmp Max PCR Master mix (Takara Bio, Kusatsu, Japan), 1µL(20 pmol) of each primer (Metabion, Steinkirchen, Germany), 10µL of DNA template and water added up to 50µL reaction volume. For *mec*A gene [22],a 25- µL reaction volume containing 12.5 µL of EmeraldAmp Max PCR Master Mix (Takara Bio, Kusatsu, Japan), 1 µL(20 pmol) of each primer, 4.5 µL of water, and 6 µL of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler and the PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Darmstadt, Germany) before examination. Primers sequences, target genes, amplicon sizes and annealing temperature are listed in Table 1.

Antibiotic sensitivity test

Standard disk diffusion assay was conducted using Muller-Hinton agar and broth culture equivalent to 0.5 McFarland standards as recommended by the Clinical and Laboratory Standards Institute [23]. Antibiotic disks were chosen based on commonly used antibiotics for animal and human therapy in the study region and the guidelines of CLSI [23]. A total of 15 antibiotic disks (Oxoid, Hampshire, U.K.) were used (14 antibiotics for *E. coli* isolates and 10 antibiotics for *S. aureus*) as showed in Table 2. *S. aureus* isolates that showed phenotypic resistance for cefoxitin was confirmed as MRSA by molecular detection of *mec*A gene [22].

Statistical analysis

Association between virulence genes and antibiotic resistance profile in the isolated *E. coli* and *S. aureus* was calculated using Fisher's exact test on SPSS v19 (SPSS Inc. 2010). Significant association was considered at $P<0.05$.

Table 1. Primers used for detection of virulence genes in *E. coli* and *S. aureus* pathogens isolated from milk in this study.

Pathogen	Target gene	Primers sequences (5' – 3')	Amplicon (bp)	Annealing temperature
E. coli	*Stx1*	F: ACACTGGATGATCTCAGTGG R: CTGAATCCCCCTCCATTATG	614	58˚C
	Stx2	F: CCATGACAACGGACAGCAGTT R: CCTGTCAACTGAGCAGCACTTTG	779	
	STa	F: GAAACAACATGACGGGAGGT R: GCACAGGCAGGATTACAACA	229	57˚C
	LT	F: GGTTTCTGCGTTAGGTGGAA R: GGGACTTCGACCTGAAATGT	605	
S. aureus	*sea*	F: GGTTATCAATGTGCGGGTGG R: CGGCACTTTTTTCTCTTCGG	102	50˚C
	seb	F: GTATGGTGGTGTAACTGAGC R: CCAAATAGTGACGAGTTAGG	164	
	sec	F: AGATGAAGTAGTTGATGTGTATGG R: CACACTTTTAGAATCAACCG	451	
	sed	F: CCAATAATAGGAGAAAATAAAAG R: ATTGGTATTTTTTTTCGTTC	278	
	see	F: AGGTTTTTTCACAGGTCATCC R: CTTTTTTTTCTTCGGTCAATC	209	
	*mec*A	F: GTA GAA ATG ACT GAA CGT CCG ATA A R: CCA ATT CCA CAT TGT TTC GGT CTA A	310	50˚C

Results

A cross-sectional study was conducted to assess the potential public health risks associated with consumption or dairy manufacture of informally marketed raw bovine milk in Kafrelsheikh governorate, Egypt.

A total of 16 (13.2%) *E. coli* isolates and 22 (18.2%) *S. aureus* isolates were detected in 35/121 (28.9%) of examined milk samples. Three milk samples (2.5%) showed the co-existence of *E. coli* and *S. aureus* pathogens. Fifteen *E. coli* isolates belonged to six O-serogroups (O125, O126, O157, O158, O146, and O167), and one isolate was untypable (OUT).

Results in Table 3 and Figures (1, 2) showed that investigated virulence genes were detected in 43.8% and 45.5% of the *E. coli* and *S. aureus* isolates, respectively. *Stx*2 (18.8%) and *STa* (12.5%) were the predominant virulence genes in STEC and ETEC isolates, respectively. In *S. aureus*, *sec* gene was the most prevalent gene (27.3%), while lowest detection rates were for *sea* and *seb* genes (4.5% for each). None of the *E. coli* and *S. aureus* isolates showed the existence of *LT* or *Sed* genes, respectively (Figures 1, 2).

Combined serotyping and virulence genes results showed that Toxigenic *E. coli* isolates were detected in 7/121 (5.8%) of milk samples as follows: 5/121 (4.1%) STEC and 2/121 (1.7%) ETEC. STEC were form five different serogroups: 1/121 (0.8%) O157-STEC, and 4/121 (3.3%) non-O157 STEC. Non-O157 STEC included 1/121 (0.8%) of milk samples for each of O158, O146, O126, and O125 serogroups. The 2 ETEC isolates were from O167 and O126 serogroups (0.8% for each). Enterotoxigenic *S. aureus* isolates were detected in 8/121 (6.6%) of the milk samples.

Table 2. Antibiotic resistance phenotypes of *E. coli* and *S. aureus* pathogens isolated from milk in this study.

Antibiotic	Concentration (µg)	Uses	*E. coli* (n = 16)		*S. aureus* (n = 22)	
			Bp	R (%)	Bp	R (%)
Ampicillin (AMP)	10	Clinic/Vet	13	11 (68.8)	28	22 (100)
Amoxicillin-Clavulanic Acid (AMC)	20	Clinic	13	1 (6.3)	NR	-
Cefoxitin (FOX)	30	Clinic/Vet	14	4 (25)	21	2 (9.1)
Cephalothin (KF)	30	Clinic/Vet	14	14 (87.5)	NR	-
Cefotaxime (CTX)	30	Clinic	22	4 (25)	NR	-
Gentamicin (CN)	10	Clinic/Vet	12	0 (0)	12	5 (22.7)
Amikacin (AK)	30	Clinic/Vet	14	10 (62.5)	14	13 (59.1)
Kanamycin (K)	30	Clinic/Vet	13	3 (18.8)	13	14 (63.6)
Streptomycin (S)	10	Clinic	11	5 (31.3)	NR	-
Nalidixic Acid (NA)	30	Clinic	13	9 (56.3)	NR	-
Erythromycin (E)	15	Clinic/Vet	NR	-	13	17 (77.3)
Chloramphenicol (C)	30	Clinic/Vet	12	0 (0)	12	16 (72.7)
Nitrofurantoin (F)	300	Clinic	14	10 (62.5)	14	11 (50)
Tetracycline (TE)	30	Clinic/Vet	11	11 (68.8)	14	20 (90.1)
Sulfamethoxazole-Trimethoprim (SXT)	25	Clinic	10	3 (18.3)	10	20 (90.1)

Bp: Break-point; R: Resistance; NR: Not recorded.

Table 3. Frequency distribution of virulence genes in *E. coli* and *S. aureus* isolated from milk in this study.

Isolate	Pathotype	Virulence gene	positive	%
E. coli (n = 16)	STEC	*Stx*1	2	12.5
		*Stx*2	3	18.8
		Total	5	31.3
	ETEC	*Sta*	2	12.5
		Lt	0	0
		Total	2	12.5
	Total		**7**	**43.8**
S. aureus (n = 22)	Enterotoxigenic	*sea*	1	4.5
		seb	1	4.5
		sec	6	27.3
		sed	0	0
		see	2	9.1
	Total		**10**	**45.5**

Majority of *E. coli* isolates (87.5%) showed multiple drug resistances (MDR) to three or more classes of antibiotics. The highest resistance rates were for cephalothin (87.5%) followed by ampicillin (68.8%) and tetracycline (68.8%), while all isolates (100%) were sensitive for gentamicin and chloramphenicol (Table 2). Resistance phenotype profiles were diverse among *E. coli* isolates giving up to 16 different patterns (Table 4).

MDR was also recorded by 95.5% of the *S. aureus* isolates. All *S. aureus* isolates were resistant to ampicillin (100%) and 90.1% of the isolates showed resistance for tetracycline and sulfamethoxazole-Trimethoprim (Table 2). Two MRSA isolates (9.1%) were identified by resistance to Cefoxitin and PCR detection of *mec*A gene in these isolates (Table 2, 4). The MRSA isolates were resistant to 9 different antibiotics however they retained sensitivity for gentamicin (GS-MRSA). In addition, 5 (22.7%) of *S. aureus* isolates were gentamicin-resistant methicillin-sensitive *S. aureus* (GR-MSSA). Resistance phenotypes similarities were more common in *S. aureus* than *E. coli* isolates as more than half of the isolates (12/22, 54.5%) can be grouped in only 5 resistance profiles (Table 4). Moreover, co-resistances to ampicillin, amikacin, kanamycin, erythromycin chloramphenicol, nitrofurantoin, tetracycline and sulfamethoxazole-trimethoprim were recorded in 4 (18.2%) of *S. aureus* isolates.

E. coli isolates with either *Stx*1 or *Stx*2 genes were significantly associated with co-resistance to cephalothin, ampicillin and tetracycline (P= 0.03),

Figure 1. Molecular detection of virulence genes in *E. coli* isolated from milk in this study. (A) Shiga-toxin genes (*Stx*1 and *Stx*2). (B) Enterotoxigenic genes (*STa* and *LT*). P: positive control. N: negative control. L: ladder. 1-16: *E. coli* isolates.

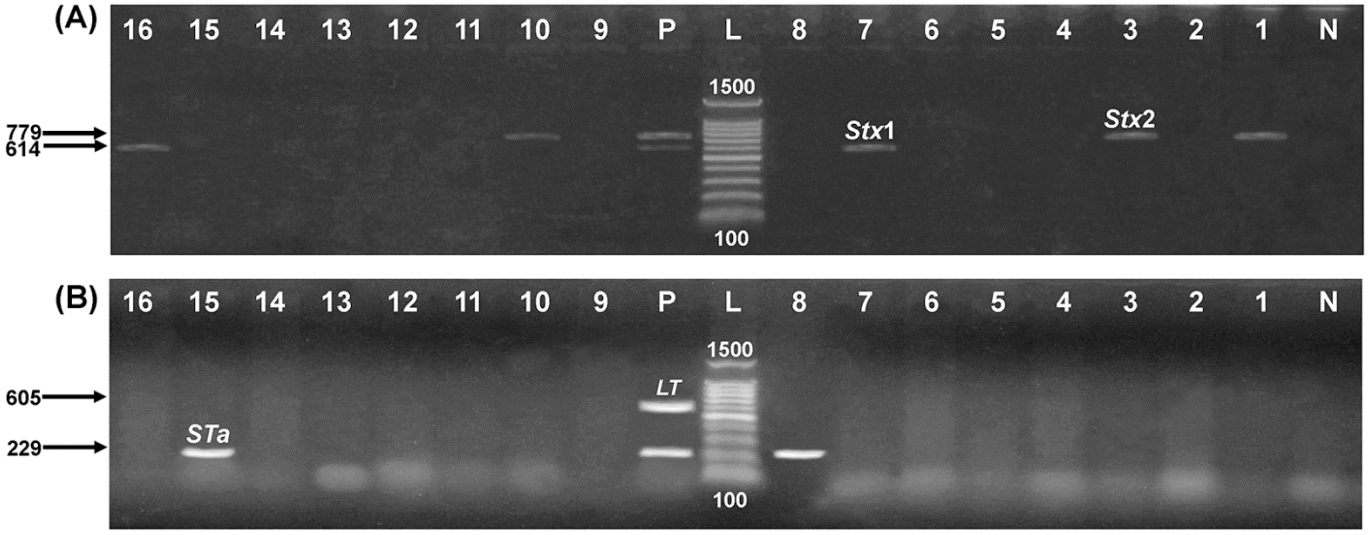
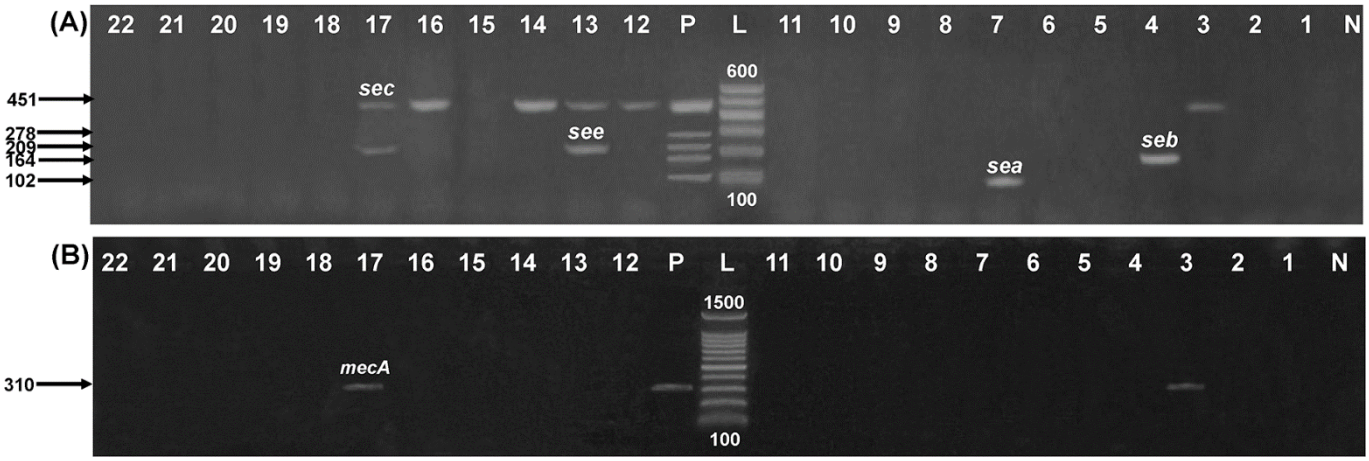


Figure 2. Molecular detection of Staphylococcal enterotoxins and Methicillin resistance genes in *S. aureus* isolated from milk in this study. (A) Staphylococcal enterotoxins genes (*sea*, *seb*, *sec*, *sed* and *see*). (B) Methicillin resistance gene (*mecA*). P: positive control. N: negative control. L: ladder. 1-22: *S. aureus* isolates.



however there was no clear association between virulence genes and resistance phenotype in the isolated *S. aureus* (Table 4).

Discussion

A cross-sectional study was conducted to assess the potential public health risks associated with consumption or dairy manufacture of informally marketed raw bovine milk in Kafrelsheikh governorate. Almost one third (28.9%) of the examined bovine milk in this study showed the existence of either or both *E. coli* or *S. aureus* pathogens. *E. coli* was detected in 13.2% of the examined milk samples, which was comparable with previous report (15.9%) in Saudi Arabia [24]. Higher prevalence was recorded in Ethiopia (29.6%) by Garedew *et al*. [4].

STEC were recorded in 4.1% of the milk samples, which was lower than another report (17.4%) in Iran

Table 4. Association between antibiotic resistance phenotypes and virulence genes in *E. coli* and *S. aureus* pathogens isolated from milk in this study.

Pathogens	Isolate ID	Antibiotic resistance	Virulence
E. coli			
O125	E7	**AMP**, FOX, **KF**, AK, NA, F, **TE**, SXT	***Stx1***
O126	E3	**AMP**, **KF**, AK, K, NA, F, **TE**	***Stx2***
O126	E12	AMP, AMC, KF, S, NA, F, TE	
O126	E15	AMP, KF, AK, K, F, TE, SXT	*Sta*
O157	E1	**AMP**, **KF**, CTX, NA, F, **TE**	***Stx2***
O125	E4	AMP, KF, CTX, AK, NA, TE	
O125	E5	AMP, FOX, KF, CTX, AK, S	
O125	E6	AMP, AK, NA, F, TE, SXT	
O126	E9	AMP, AK, S, NA, F, TE	
O158	E16	**AMP**, FOX, **KF**, F, **TE**	***Stx1***
O146	E10	**AMP**, **KF**, AK, S, **TE**	***Stx2***
O125	E11	KF, AK, K, NA, F	
O126	E14	KF, NA, F, TE	
O125	E2	KF, AK, S	
OUT	E13	FOX, KF, CTX	
O167	E8	KF	*Sta*
S. aureus	S3	AMP, FOX, AK, K, E, C, F, TE, SXT	*mecA, Sec*
	S17		*mecA, Sec, See*
	S9	AMP, CN, AK, K, E, C, F, TE, SXT	
	S11		
	S1	AMP, AK, K, E, C, F, TE, SXT	
	S2		
	S7		*Sea*
	S18		
	S20	AMP, CN, AK, K, E, C, TE, SXT	
	S13	AMP, CN, K, E, C, TE, SXT	*Sec, See*
	S4	AMP, E, C, F, TE, SXT	*Seb*
	S5		
	S8	AMP, CN, AK, E, TE, SXT	
	S19	AMP, AK, K, E, C, TE	
	S22	AMP, K, E, C, F, SXT	
	S12	AMP, AK, K, TE, SXT	*Sec*
	S14		*Sec*
	S6	AMP, E, C, TE, SXT	
	S15	AMP, C, TE, SXT	
	S21	AMP, E, TE, SXT	
	S10	AMP, TE, SXT	
	S16	AMP	*Sec*

AMP: Ampicillin; AMC:Amoxicillin-Clavulanic Acid; FOX: Cefoxitin; KF: Cephalothin; CTX: Cefotaxime; CN: Gentamicin; AK: Amikacin; K: Kanamycin; S: Streptomycin; NA: Nalidixic Acid; E: Erythromycin; C: Chloramphenicol; F: Nitrofurantoin;TE: Tetracycline;SXT: Sulfamethoxazole-Trimethoprim; OUT: Untypable O-serogroup; Bold: highlight the significant association between phenotypic co-resistance to cephalothin, ampicillin and tetracycline with *Stx* genes ($P < 0.05$).

[25] and higher than prevalence (2.4%) reported in USA [1]. O157-STEC were detected in 0.8% of the milk samples which was lower than other studies in Egypt (2.3%) [3], and in Saudi Arabia (4.8%) [24]. However, Selim *et al*. [11] failed to detect any O157-STEC in milk samples in another study in Egypt. Non O157-STEC isolates were predominant in this study (3.3%). Four different Non O157-STEC O serogroups were detected (O158, O146, O125 and O126), which indicates high diversity of *E. coli* serotypes circulation among cattle in the study regions. This diversity of STEC O serogroups may also implies high rate of *Stx* genes transfer between different serogroups. Particularly, when the predominant O125 and O126 serogroups, classical enteropathogenic (EPEC), gained *Stx* genes probably from mixing with other STEC O serogroups in same host as previously reported [26]. O158-STEC and O125-STEC were previously reported from cattle samples in Egypt [11] and elsewhere [27]. All detected STEC serogroups detected in this study were implicated in human diseases as follow; O157-STEC in cases of Diarrhea in Egypt [11], O126-STEC, O157-STEC and O158-STEC in urinary tract infection cases in Egypt [11,28], while O125-STEC and O146-STEC were detected in human diarrhea cases from elsewhere [29]. ETEC were isolated from 1.7% of milk samples. O126-ETEC and O167-ETEC were the reported serogroups in this study. O126-ETEC and O167-ETEC were associated with cases of human diarrhea in Egypt [10] and worldwide [8]. Moreover, O126-ETEC was isolated from more than 30% of human urine samples in Egypt [28].

The relatively high rate of *E. coli* prevalence in examined milk samples may indicate high rate of fecal contamination, which correlate with inadequate udder hygiene. Hygienic milking practices as the use of teat dipping, udder washing, use of disinfectant in animal's house cleaning are not applied by most of smallholders in many developing countries including Egypt [4].

Virulence genes were detected in 43.8% of *E. coli* isolates. In STEC, *Stx*2 was prevalent than *Stx*1, which agreed with several studies [24,25]. *LT* gene was not detected in ETEC, yet both *LT* and *ST* genes were previously reported in human isolates from cases with diarrhea in Egypt [10].

S. aureus was recorded in 18.2% of the milk samples, which was higher than that reported in Brazil (7.3%) [30], and lower than another report in Czech Republic (32.2%) [31]. Enterotoxigenic *S. aureus* isolates were detected in 6.6% of milk samples, which was lower than another study (25%) in Hungary [32]. Raw milk sold in informal market usually combine unhygienic production with lack of any preservation before or during marketing, which could be the reasons for the relative high *S. aureus* prevalence in milk samples in this study.

SEs genes were detected in 45.5% of the isolated *S. aureus*, which was higher than those reported in Brazil (22.2%) [30], and in Hungary (27.1%) [32]. The *sec* gene was the prevalent SE gene in this study. The *sec* gene was also the predominant SE gene in *S. aureus* isolated from cases of mastitis in Egypt [5]. The *sea*, *seb* and *see* genes were also detected but *sed* gene was absent from all *S. aureus* isolates in this study. Enterotoxins of *sea*, *sec* and to lesser extent *see* genes were implicated in several Staphylococcal food poisoning outbreaks in USA, France, Japan, Taiwan and UK [5].

MDRs were reported at high rates for *E. coli* (87.5%) and *S. aureus* (95.5%) isolates in this study. In accordance with our findings, high MDR rates of these two pathogens in milk and dairy products were reported in several countries including Egypt [11,12,25,28,31]. The highest resistance rates of *E. coli* isolates were for cephalothin, which agreed with findings of Paneto *et al*. [27]. In addition, *E. coli* isolates showed high resistance for ampicillin and tetracycline, which was in line with several reports worldwide[11,25,27]. Same high resistance against ampicillin and tetracycline were also reported in *E. coli* isolates from human cases in Egypt [11,33]. This may indicate high rates of resistance transmission for these antibiotics between animal and human isolates in Egypt. Similar findings were reported by Cho *et al*. [34], who detected a significant association for cephalothin and gentamicin resistance between *E. coli* isolates from animals and humans in contact with them.

Despite diversity of phenotypic resistance patterns among *E. coli* isolates in this study, there was a significant association ($P< 0.01$) between isolates with *Stx* genes (STEC) and phenotypic co-resistance to cephalothin, ampicillin and tetracycline. This finding was in line with Rehman *et al*. [35], who observed significant positive associations between resistance phenotypes and some virulence genes in *E. coli* isolates from ruminants in China.

All *S. aureus* isolates showed high resistance for ampicillin (100%) and tetracycline (90.1%), which agreed with other reports in Czech Republic [31] and in China [12]. MRSA isolates represented 9.1% of the *S. aureus* isolates in this study. This finding was lower than another report (51%) in Czech Republic [31]. The MRSA isolates showed resistance to all antibiotics except for gentamicin (GS-MRSA), however five of the

MSSA isolates were resistant to this antibiotic (GR-MSSA). Both GS-MRSA and GR-MSSA were implicated in nosocomial and community acquired human outbreaks worldwide [36,37].

Unlike high diversity of resistance profiles of *E. coli* isolates, 54.5% of the *S. aureus* isolates can be grouped in 5 resistance profiles. The major profile (18.2% of the isolates) showed co-resistance to 8 antibiotics including ampicillin, kanamycin, and tetracycline which agreed with Li *et al*. [12] findings, who reported the predominance of co-resistance to ampicillin, kanamycin and tetracycline in *S. aureus* isolates from milk samples in China. This high degree of similarities between *S. aureus* isolates in this study may indicate genetic relatedness and a common source of infection.

Conclusions

Around one third of the examined samples were contaminated with *E. coli* and /or *S. aureus*. Over 40% of these pathogens were toxigenic and some of these toxigenic isolates are highly pathogenic to human even in low infectious doses (i.e. STEC). MDRs was reported in a very high rate by majority of *S. aureus* and *E. coli* isolates and transmission of the antibiotic resistance of theses pathogens to humans via foods may complicate the treatment options for clinical cases. These findings indicate that raw milk sold in informal markets in Egypt may possess a potential public health hazards for consumers. Restriction of informal raw milk marketing and public awareness about its zoonotic hazards may be a good strategy for minimizing the risks of milk-borne food poisoning in Egypt.

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Corresponding author

Walid Elmonir, Ph.D.,
Hygiene and Preventive Medicine (Zoonoses) Department, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish Street, 33516 Kafrelsheikh, Egypt.
Phone/Fax: +20-473231311
E-mail: walid.elmonir@gmail.com

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