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DOI 10.26873/SVR-785-2019 **Original Research Article**

OCCURRENCE AND MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *Enterobacteriaceae* IN MILK AND SOME DAIRY PRODUCTS

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Abstract: In the present study a total of one hundred samples were randomly purchased from different supermarkets in Damietta city, Egypt, including 25 samples each of raw milk, ice cream, Kareish and Domiati cheese. The collected samples were screened initially for cefotaxime-resistant bacteria using selective enrichment. The suspected colonies were further characterized by analysis of resistance genes in ESBL-producing isolates. A total of 59 ESBLs producers colonies (24 from raw milk, 15 from kareish cheese, 13 from Domiati cheese and 7 from ice cream) were recovered and identified as *Escherichia coli* (n = 29), *Klebsiella pneumonia* (n = 8), *Klebsiella oxytoca* (n = 5), *Enterobacter aerogenes* (n =8), *Citrobacter diversus* (n = 8) and *Serratia liquefaciens* (n = 1). Resistance to two or more antibiotics was observed among the recovered isolates. *E. coli* isolates showed high resistance pattern against cefaclor (100%), cefoxitin (100%), cefalaxim (96.5%), ceftazidime (93%), pencillin (93%) and 79% for Pipercillin/Tazobactam. All the recovered *Klebseilla* spp. isolates were resistant to Cefepime and Pipercillin/Tazobactam (84.6% each) and exhibited 100% resistant to other antimicrobials agents. Meanwhile, all the recovered isolates were sensitive to imipenem and meropenem. Gene encoding *blaCTX-M1* was mostly predominant among screened genes, being present in 13 (48%) dairy samples for *blaTEM* and *blaSHV,* they were detected in 12 (44%) and 4 (14.8%) isolates, respectively. In conclusion, there is clear evidence of circulation of antibiotic-resistant food borne ESBL producing *Enterobacteriaceae* in the examined dairy samples. The concern about increasing the risk of dissemination of such multi-drug resistant pathogens rises with a potential asymptomatic colonization and complication of systemic infection in human subjects. There is also a possible interface for the exchange of resistance genes within and across species and with commensal bacteria of the human and animals.

Key words: dairy products; ESBL producing *Enterobacteriaceae;* Egypt; public health

Introduction

The emergence of antimicrobial resistance foodborne zoonotic bacteria, particularly *Enter-*

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obacteriaceae that carry ESBLs, has been recognized as one of the most important global problems in both veterinary and human medicine (1). As a matter of growing concern, livestock and animal-derived foods especially milk and its products are the most incriminated foods of animal origin that is linked to disease outbreaks around the word and represent important reservoirs for ESBLs-producing *Enterobacteriaceae* (2). *Escherichia coli* and *Klebsiella pneumoniae* are considered the most predominant species of ESBLs producing *Enterobacteriaceae* worldwide and are being the causal of urinary tract infections, pneumonia and sepsis in human patients (3). In most of developing countries involving Egypt, there are no restricted rules for the use of antibiotics for the treatments of dairy ailments particularly third and fourth generations of cephalosporin due to their therapeutic effectiveness or their short withdrawal periods. Hence, massive and indiscriminate use of these antibacterial agents is critically important to dairy farming and could have the potential to the spread of ESBLs-producing bacteria or even multi drug resistance (MDR) pathogens (4). Resistance to β- lactamase in the family *Enterobacteriaceae* has been reported to be linked to the production of class A and C β-lactamase enzymes which able to hydrolyze and inactivate the β-lactam ring and confer different degrees of resistance to various β- lactam classes (5). ESBLs are bacterial enzymes that degrade oxyiminocephalosporins with the most clinically important class A β-lactamase enzymes and plasmid-mediated Temoniera (TEM), sulfhydryl variable (SHV), and Cefotaxime-Munich (*CTX-M*) types are the three main families of ESBLs (6).

Raw milk can be contaminated with ESBLs producing *Enterobacteriaceae* in several entities such as mastitis, directly by animal feces or indirectly during milking (7). Unfortunately, the vast majority of the population in Egypt's, especially in rural families, still consume raw dairy products without pasteurization including traditional Egyptian cheese as Kareish and Domiati cheese (most popular soft white cheese) with a general believe that pasteurization would drastically affect the milk quality (8). To date, very limited information existing regarding ESBLs producing *Enterobacteriaceae* isolated from dairy cattle (3). Therefore, it is of utmost significance to address the potential occurrence of ESBLs producing *Enterobacteriaceae* from raw milk and some dairy products which marketed in different localities in Damietta Governorate, Egypt and to highlight the threats to human health posed by consumption of raw milk and dairy products.

Materials and methods

Sampling and sample preparation

A total of 100 samples (25 each of market raw milk, Kareish cheese, Domiati cheese and small scale ice cream) were randomly purchased from groceries, retail outlets, supermarkets in Damietta city, Egypt in March 2018. All the collected samples were transported immediately in coolers in its original package under aseptic conditions to the laboratory for bacteriological examinations which were started quickly after receiving the samples according to the previously described protocol (9).

Isolation and identification of cefotaxime-resistant bacteria

Twenty five ml or g of each dairy samples were diluted with 225 ml of buffered peptone water (BPW). In case of cheese, samples were homogenized in a stomacher for through mixing. All the prepared samples were immediately plated onto MacConkey agar plates (Oxoid, Basingstoke, UK) supplemented with 1 mg /L cefotaxime (Sigma-Aldrich) and incubated for 24 h at 37 °C. At least three grown colonies were selected and sub-cultured onto MacConkey agar supplemented with 1 mg/L cefotaxime at 37 °C for 24 h to obtain pure cultures for subsequent analysis. Bacterial characterization was performed using Gram staining, and conventional biochemical testing including Oxidase, indole, methyl red, vogus-prauskaur, citrate, and urease.

Serotyping of identified E. coli

Serotyping of biochemically confirmed *E. coli* isolates were performed by agglutination tests by using rapid diagnostic *E .coli* antisera sets according to the previously described protocol (10) (Denka Seiken Co., Japan**)** at Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt.

Antimicrobial susceptibility testing and ESBLs detection

All recovered isolates were screened for susceptibility test using 13 antimicrobial agents on Mueller-Hinton agar using disc diffusion method and evaluated according to CLSI criteria (11). The following antimicrobial discs were applied: Penicillin G. (P 10 µg), Ampicillin (AM10 µg), Imipenem (IPM 10 µg), Meropenem (MEM 10 µg), Cefalaxin (CL 30 µg), Cefaclor (CEC30 µg), Cefoxitin (FOX 30 µg), Ceftazidime (CAZ 30 µg), Cefotaxime (CTX 30 µg), Cefepime (FEP 30 µg), Azteonam (ATM 30 µg), Amoxicillin-clavulinic (AMC 20/10 µg), Piperacillin-tazobactam (Tpz 100/10). Each isolate that exhibited resistant to one or more of the third and fourth-generation cephalosporins were confirmed for ESBL production by Double Disk Synergy Test (DDST) according to CLSI guidelines.

Characterization of β-lactamases

Genomic bacterial DNA was extracted from the identified ESBLs producers *E. coli* (n= 17) and *Klebseilla* spp (n= 10) using the QIAamp® DNA Mini Kit Qiagen according to the manufacturer`s guidelines. The purified DNA was further analyzed by PCR targeting ESBL encoding genes including *blaTEM, blaSHV and blaCTX-M* using specific oligonucleotide primers sequences and PCR conditions as described previously (Table 1).

Results

Out of hundred dairy samples, 30 yielded bacterial growth on MacConkey agar supplemented with cefotaxime : 8 (32%) from raw milk, 8 (32%) from Domiati cheese, 9 (36%) from kareish cheese and 5 (20%) from ice cream. Overall, 59 ESBLs producers colonies (24 came from raw milk, 15 from kareish cheese, 13 from Damietta cheese and 7 from ice cream) and were biochemically identified as *E. coli* (n = 29, 49%), *Klebsiella pneumonia* (n = 8, 13.5%), *Klebsiella oxytoca* (n = 5, 8.5%), *Enterobacter aerogenes* (n =8, 13.5%), *Citrobacter diversus* (n = 8, 13.5%) and *Serratia liquefaciens* (n = 1, 1.7%) (Table 2&3). The most prevalent phenotype was *E.coli* (n = 17, 17%), *Klebsiella pneumonia* (n = 6, 6%), 5 (5%) for each of *Enterobacter aerogenes* and *Citrobacter diversus*, 4 (4%) for *Klebsiella oxytoca* and 1 (1%) for *Serratia liquefaciens* (Table 3).

The pattern of antimicrobial drug susceptibility for the 59 ESBLs producers are presented in Table 4&5. Briefly, *E. coli* isolates showed high resistance pattern against cefaclor (100%), cefoxitin (100%), cefalaxim (96.5%), ceftazidime (93%), pencillin (93%) and 79% for Pipercillin/Tazobactam. All the recovered *Klebseilla* spp. isolates (n =13) were resistant to Pencillin, Ampicillin, Cefalaxim, cefaclor, Cefoxitin and Ceftazidime (100% each) and to Cefepime and Pipercillin/Tazobactam (84.6% each). On the other side, all recovered isolates were sensitive to imipenem and meropenem. Gene encoding *blaCTX-M1* was mostly predominant among screened genes (fig 1&2), being present in 13 (48%) dairy samples. for *blaTEM* and *blaSHV*, they were detected in 12 (44%) and 4 (14.8%) isolates, respectively. Three isolates harbored the three screened β-lactamase genes; *blaCTX-M1* and *blaTEM* were detected in four isolates; while *blaSHV* and *blaCTX-M1* were observed in one isolate.

Table 1: Oligonucleotide primers sequences used for amplification of B-lactamase resistance genes

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
blaCTX-M1 (F)	5′ TTAGGAAGTGTGCCGCTGTA ′3	655	12
blaCTX-M1 (R)	5′ CGGTTTTATCCCCCACAAC′3		
blaSHV (F)	5′ AGCCGCTTGAGCAAATTAAAC ′3		13
blaSHV (R)	5′ ATCCCGCAGATAAATCACCAC ′3	713	
blaTEM (F)	5′ CATTTCCGTGTCGCCCTTATTC ′3		13
blaTEM (R)	5′ CGTTCATCCATAGTTGCCTGAC ′3	800	

Table 2: Distribution of ESBLs producers Enterobacteriacea spp. from the examined dairy samples

Sample number	Origin	*Number of isolated Enterobacteriacea spp.* from the positive samples					
		E.coli	*K. penumonia*	*K. oxytoca*	*Enterobac-ter*	*Citrobacter*	*Serratia*
	Milk						
1	Pos n= 8/25 (32%)	2					
6		3			1		
10		3		1			
11						3	
12					2	1	1
13		1		1			
14					3		
21			2				
	Damietta chesses						
23	Pos n= 8/25 (32%)			1			
27		1	1				
29		2					
31			1				
35					1		
41		1	1				
43		1					
49						1	
	Kariesh cheese						
51	Pos n= 9/25 (36%)			2			
54		1					
58						2	
59		1				1	
61		2					
69		1					
70		3					
74		2					
75			2				
	Ice Cream						
79	Pos n= 5/25 (20%)		1				
80		2					
83		2					
94		1					
97					1		
	Total pos n = 30/100 (30%)	29	8	5	8	8	1

Table 3: Occurrence and distribution of different ESBLs producers *Enterobacteriaceae* spp. in examined dairy samples

Enterobacteriacae spp.	Milk n =25		Kariesh cheese n = 25		Damietta cheese n = 25		Ice Cream n =25		Total Dairy samples	
	Pos samples	N of colonies	Pos samples	N of colonies	Pos samples	N of colonies	Pos samples	N of colonies	Pos samples	N of colonies
E.coli	4 (16%)	9	6 (24%)	10	4 (16%)	5	3 (12%)	5	17 (17%)	29
Klebseilla .pneumoniae	1 (4%)	2	1 (4%)	2	3 (12%)	3	1 (4%)	1	6 (6%)	8
Klebseilla .oxytoca	2 (8%)	2	1 (4%)	2	1 (4%)	1	0	0	4 (4%)	5
Enterobacter aerogenes	3 (12%)	6	0	0	1 (4%)	1	1 (4%)	1	5 (5%)	8
Citrobacter diversus	2 (8%)	4	2 (8%)	3	1 (4%)	1	0	0	5 (5%)	8
Serratia liquefaciens	1 (4%)	1	0	0	0	0	-	0	1 (1%)	1
Total number of ESBLs n=59		24		17		11		7		59

Table 4: Characteristics of ESBL-positive *Enterobacteriaceae* identified from dairy samples

Sample number	Origin	Species	b-lactam antibiotic resistances												
			P	AM	IPM	MEM	CL	CEC	FOX	CAZ	CTX	FEP	ATM	AMC	TPZ
1	Milk	E.col (O128:H2)	R	R	S	S	R	R	R	R	R	S	R	R	R
1	Milk	E.coli (O91:H21)	R	R	S	S	R	R	R	R	R	S	S	R	R
6	Milk	E.coli (O55:H7)	R	S	S	S	R	R	R	S	R	S	S	R	R
6	Milk	E.coli (O121:H7)	R	R	S	S	R	R	R	R	R	R	S	S	R
6	Milk	E.coli (O146:H21)	R	S	S	S	R	R	R	R	R	R	S	S	R
10	Milk	E.coli (O111:H2)	R	R	S	S	R	R	R	R	R	R	S	S	S
10	Milk	E.coli (O111:H2)	R	S	S	S	R	R	R	R	R	S	S	S	R
10	Milk	E.coli (O114:H4)	S	S	S	S	R	R	R	R	R	S	S	S	S
13	Milk	E.coli (O78)	R	S	S	S	R	R	R	R	R	S	S	S	S
27	Domiate cheese	E.coli (O111:H2)	R	R	S	S	R	R	R	R	R	R	R	R	R
29	Domiate cheese	E.coli (O121:H7)	R	R	S	S	R	R	R	R	R	R	R	R	R
29	Domiate cheese	E.coli (O26:H11)	R	S	S	S	R	R	R	R	R	R	S	S	R
41	Domiate cheese	E.coli (O26:H11)	R	S	S	S	R	R	R	R	R	S	S	S	R
43	Domiate cheese	E.coli (O114:H4)	R	R	S	S	R	R	R	R	R	R	S	R	R
54	kareish cheese	E.coli (O124)	R	R	S	S	R	R	R	R	R	R	S	R	R
59	kareish cheese	E.coli (O111:H2)	R	R	S	S	R	R	R	R	R	R	S	R	R
61	kareish cheese	E.coli (O91:H21)	R	S	S	S	R	R	R	R	R	S	S	S	R
61	kareish cheese	E.coli (O121:H7)	R	S	S	S	S	R	R	R	R	S	S	S	S
69	kareish cheese	E.coli (O91:H21)	R	S	S	S	R	R	R	R	R	S	S	S	R
70	kareish cheese	E.coli (O126:H21)	R	S	S	S	R	R	R	R	R	R	S	S	R
70	kareish cheese	E.coli (O128:H2)	R	R	S	S	R	R	R	R	R	S	R	R	R
70	kareish cheese	E.col i (O111:H2)	R	R	S	S	R	R	R	R	R	S	R	R	R
74	kareish cheese	E.coli (O127:H6)	R	R	S	S	R	R	R	R	R	S	R	S	R
74	kareish cheese	E.coli(O146:H21)	R	R	S	S	R	R	R	R	R	R	S	S	S
80	Ice cream	E.coli (O26H11)	R	R	S	S	R	R	R	R	R	R	S	S	S
80	Ice cream	E.coli (O91:H21)	R	R	S	S	R	R	R	R	R	R	S	S	R
83	Ice cream	E.coli (O111:H2)	R	R	S	S	R	R	R	R	R	R	S	R	R
83	Ice cream	E.coli (O121:H7	R	R	S	S	R	R	R	R	R	R	R	S	R
94	Ice cream	E.coli (O26:H11)	R	S	S	S	R	R	R	R	R	R	S	S	S

Table 4: Continuation

Sample number	Origin	Species	b-lactam antibiotic resistances												
			P	AM	IPM	MEM	CL	CEC	FOX	CAZ	CTX	FEP	ATM	AMC	TPZ
21	Milk	*k. pneom*	R	R	S	S	R	R	R	R	R	R	R	S	S
21	Milk	*k. pneom*	R	S	S	S	R	R	R	R	R	S	S	R	R
27	Domiate cheese	*K.pnemo*	R	R	S	S	R	R	R	R	R	R	S	S	R
31	Domiate cheese	*K.pnemo*	R	R	S	S	R	R	R	R	R	S	S	S	S
41	Domiate cheese	*k.pnemo*	R	R	S	S	R	R	R	R	R	R	S	S	R
75	kareish cheese	*k.pnemo*	R	R	S	S	R	R	R	R	R	R	R	S	R
79	Ice cream	*k.pnemo*	R	R	S	S	R	R	R	R	R	R	S	S	S
10	Milk	*k. oxy*	R	R	S	S	R	S	R	R	R	R	S	S	R
13	Milk	*k. oxy*	R	R	S	S	R	S	R	R	R	R	S	R	R
23	Domiate cheese	*K.oxy*	R	R	S	S	R	S	R	R	R	S	S	R	R
51	kareish cheese	*k. oxy*	R	R	S	S	R	R	R	R	R	R	S	S	R
51	kareish cheese	*k.oxy*	R	R	S	S	R	R	R	R	R	R	S	S	R
6	Milk	*Enterobac-ter*	R	R	S	S	R	R	R	R	R	R	R	R	R
12	Milk	*Enterobac-ter*	S	S	S	S	R	R	R	R	R	S	S	S	S
12	Milk	*Enterobac-ter*	R	R	S	S	R	R	R	R	R	S	S	S	R
14	Milk	*Enterobac-ter*	R	R	S	S	R	R	R	R	R	S	S	R	R
14	Milk	*Enterobac-ter*	R	S	S	S	R	R	R	R	R	S	S	S	R
14	Milk	*Enterobac-ter*	R	S	S	S	R	R	R	R	R	S	S	S	R
35	Domiate cheese	*Enterobac-ter*	R	R	S	S	S	S	R	R	R	R	S	S	R
97	Ice cream	*Enterobac-ter*	R	S	S	S	R	R	R	R	R	S	S	S	R
11	Milk	*Citerobac-ter*	R	R	S	S	R	R	R	R	R	R	S	S	R
11	Milk	*Citerobac-ter*	R	R	S	S	R	R	R	R	R	S	S	R	R
11	Milk	*Citerobac-ter*	R	S	S	S	R	R	R	R	R	S	S	S	R
12	Milk	*Citerobac-ter*	R	R	S	S	R	R	R	R	R	S	R	R	R
49	Domiate cheese	*Citerobac-ter*	R	R	S	S	R	R	R	R	R	S	S	R	R
58	kareish cheese	*Citerobac-ter*	R	R	S	S	R	R	R	R	R	R	S	S	R
58	kareish cheese	*Citerobac-ter*	R	R	S	S	R	R	R	R	R	S	R	S	R
59	kareish cheese	*Citerobac-ter*	R	S	S	S	R	S	R	R	R	S	S	S	S
12	Milk	*Serratia*	R	S	S	S	R	R	R	R	R	S	S	R	R

P= Pencillin , AM = Ampicillin, IPM = Impipenem, MEM = Meropenem, CL=Cefalaxim, CEC=Cefaclor, FOX = Cefoxitin, CAZ = Ceftazidime , CTX= Cefotaxime, , FEP = Cefepime, ATM=Azteronam, AMC=Amoxycillin/clavulinic, TPZ = Pipercillin/Tazobactam,

Table 5: Antimicrobial resistance profiles in the identified *Enterobacteriaceae* spp.

Used anti-biotic		Raw milk						Damietta Cheese					Kareish Cheese				Ice cream		
		E.coli	*K. P*	*k.O*	*E*	*C*	*S*	*E.coli*	*K. P*	*k.O*	*E*	*C*	E.coli	*K. P*	*k.O*	*C*	E.coli	*K. P*	*E*
p	**% of Resistance**	88.88 (8/9)	100 (2/2)	100 (2/2)	83.33 (5/6)	100 (4/4)	100 (1/1)	100 (5/5)	100 (3/3	100 (1/1)	100 (1/1)	100 (1/1)	90 (9/10)	100 (2/2)	100 (2/2)	100 (3/3)	100 (5/5)	100 (1/1)	100 (1/1)
AM		66.66 (6/9)	100 (2/2)	100 (2/2)	50 (2/4)	75 (3/4)	0 (0/1)	60 (3/5)	100 (3/3	100 (1/1)	100 (1/1)	100 (1/1)	50 (5/10)	100 (2/2)	100 (2/2)	66.66 (2/3)	80 (4/5)	100 (1/1	0 (0/1)
CL		100 (9/9)	100 (2/2)	100 (2/2)	100 (6/6)	100 (4/4)	100 (1/1)	100 (5/5)	100 (3/3	100 (1/1)	0 (0/1)	100 (1/1)	90 (9/10)	100 (2/2)	100 (2/2)	100 (3/3)	100 (5/5)	100 (1/1)	100 (1/1)
CEC		100 (9/9)	100 (2/2)	0 (0/2)	100 (6/6)	100 (4/4)	100 (1/1)	100 (5/5)	100 (3/3	0 (0/1)	0 (0/1)	100 (1/1)	100 (10/10)	100 (2/2)	100 (2/2)	100 (3/3)	100 (5/5)	100 (1/1)	100 (1/1)
FOX		100 (9/9)	100 (2/2)	100 (2/2)	100 (6/6)	100 (4/4)	100 (1/1)	100 (5/5)	100 (3/3	100 (1/1)	100 (1/1)	100 (1/1)	100 (10/10)	100 (2/2)	100 (2/2)	100 (3/3)	100 (5/5)	100 (1/1)	100 (1/1)
CAZ		77.77 (7/9)	100 (2/2)	100 (2/2)	100 (6/6)	100 (4/4)	100 (1/1)	100 (5/5)	100 (3/3	100 (1/1)	100 (1/1)	100 (1/1)	100 (10/10)	100 (2/2)	100 (2/2)	100 (3/3)	100 (5/5)	100 (1/1)	100 (1/1)
CTX		100 (9/9)	100 (2/2)	100 (2/2)	100 (6/6)	100 (4/4)	100 (1/1)	100 (5/5)	100 (3/3	100 (1/1)	100 (1/1)	100 (1/1)	100 (10/10)	100 (2/2)	100 (2/2)	100 (3/3)	100 (5/5)	100 (1/1)	100 (1/1)
FEP		33.33 (3/9)	100 (2/2)	100 (2/2)	16.66 (1/6)	25 (1/4)	0 (0/1)	80 (4/5)	66.66 (2/3	0 (0/1)	100 (1/1)	0 (0/1)	40 (4/10)	100 (2/2)	100 (2/2)	33.33 (1/3)	100 (5/5)	100 (1/1)	0 (0/1)
ATM		11.11 (1/9)	50 (1/2)	0 (0/2)	16.66 (1/6)	25 (1/4)	0 (0/1)	40 (2/5)	0 (0/3	0 (0/1)	0 (0/1)	0 (0/1)	30 (3/10)	50 (1/2)	0 (0/2)	33.33 (1/3)	20 (1/5)	0 (0/1)	0 (0/1)
AMC		33.33 (3/9)	0 (0/2)	50 (1/2)	33.33 (2/6)	50 (2/4)	100 (1/1)	60 (3/5)	0 (0/3	100 (1/1)	0 (0/1)	0 (0/1)	40 (4/10)	100 (2/2)	0 (0/2)	0 (0/3)	20 (1/5)	0 (0/1)	0 (0/1)
TPZ		66.66 (6/9)	50 (2/2)	100 (2/2)	83.33 (5/6)	100 (4/4)	100 (1/1)	100 (5/5	66.66 (2/3	100 (1/1)	100 (1/1)	100 (1/1)	80 (8/10)	100 (2/2)	100 (2/2)	66.66 (2/3)	80 (4/5)	0 (0/1)	100 (1/1)

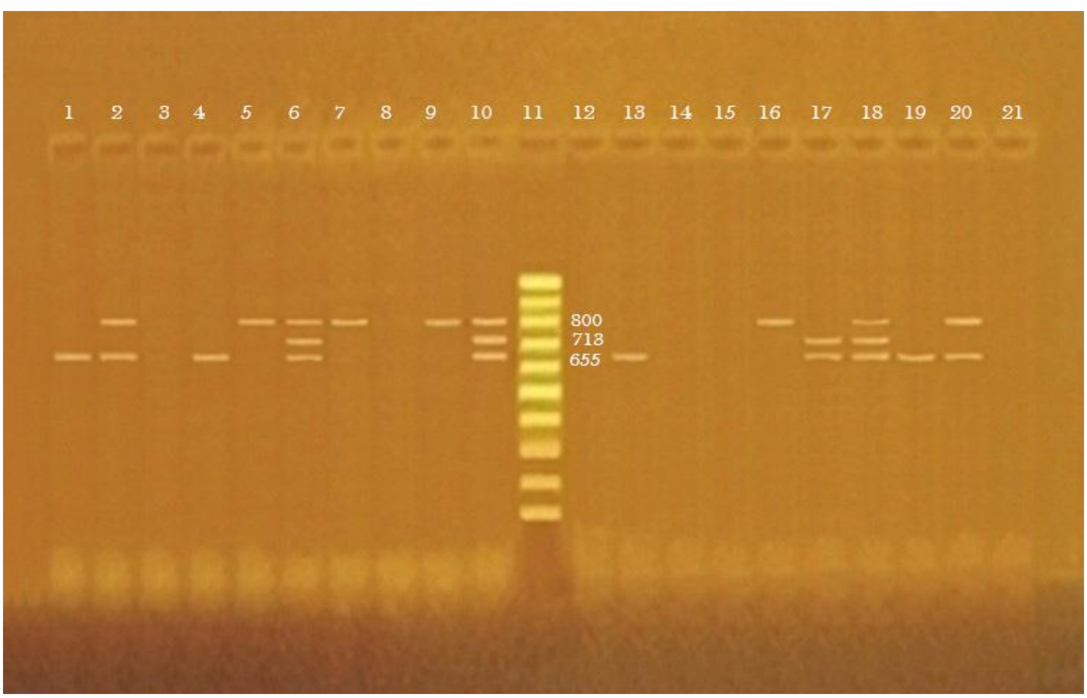

Figure 1: Agarose gel electrophoresis of multiplex PCR amplification of *blaCTX-M1*, *blaSHV* and *blaTEM* in *E. coli* with expected amplicon size ~ 655, 713, 800 bp respectively. Lane 11 is DNA ladder; lane 10 is positive control while lane12 is negative control

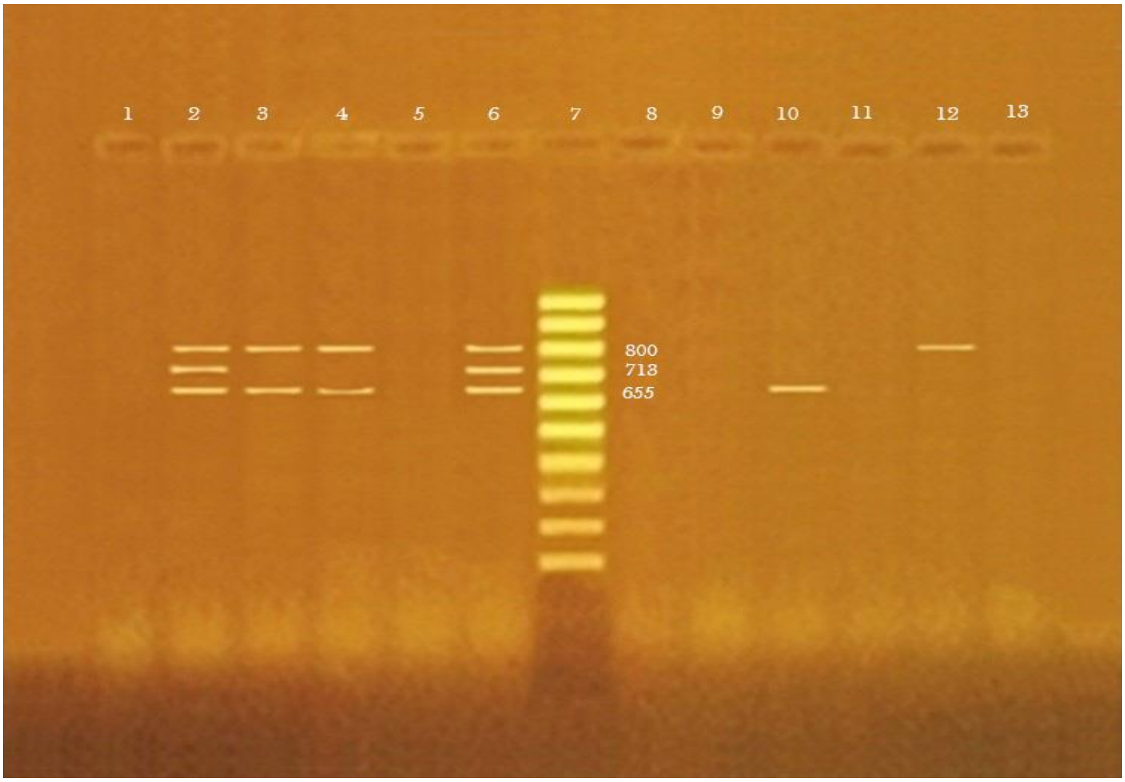

Figure 2: Agarose gel electrophoresis of multiplex PCR amplification of *blaCTX-M1*, *blaSHV* and *blaTEM* in *Klebseilla spp.* with expected amplicon size ~ 655, 713, 800 bp respectively. Lane 7 is DNA ladder; lane 6 is positive control while lane 8 is negative control

Discussion

In Egypt, there is a lack of data regarding ESBLs producing *Enterobacteriaceae* from milk and dairy product with a limited report about the surveillance of ESBLs producing *E. coli* in dairy cattle (3). Therefore, the present study was set to characterize ESBLs producing *Enterobacteriaceae* in some dairy products sold in Damietta city. In this study, the overall detection rate of ESBL producer *E. coli* in the examined dairy samples was 17%. A nearly similar detection rate was previously reported from bulk tank milk samples from German dairy farms (1); while a low frequency of ESBLs (0.7%) was reported in raw milk from the Czech Republic (14). In contrast, a higher detection rate 42.8% (114/266) of ESBL-producing *E.coli* was previously reported in Egypt from dairy cattle (3). It is really difficult to compare studies from Egypt and other countries due to lack of reports and different methods being used for determining the presence of ESBL producing *Enterobacteriaceae*. In one study, the authors failed to determine ESBL-producing isolates in bulk tank milk from 100 different Swiss dairy farms (15). In another study from Turkey, the authors identified ESBL-producing *Enterobacteriaceae* in 100 raw milk with the percentage of 43.6% (16). In a recent study from Sudan, the authors detected ESBL production in 17 out of the 22 isolated *E. coli* (29.3%) from 70 raw cow milk samples collected from different villages in Aljazira state (17). A prevalence of 42.22% ESBL-positive *E. coli* isolates out of 45 *E. coli* strains was identified from 24 typical Slovak cheeses made from raw milk (18).

In the present study, 13.5% of the identified isolates and 6% of the examined samples were classified as ESBL producing *Klebsiella pneumonia*. Different detection rate was reported from recent studies in India (19) and Sudan (17). In those studies, detection rate of 1.5% and 44.8% of *K. pneumonia* were identified as ESBL producing *Klebsiella pneumonia* from raw milk. ESBLs producing *Enterobacteriaceae* has been described for the first time from hospitalized humans but recently, several researchers from different countries reported its dissemination in the community and also in healthy food producing animals worldwide (1, 3. 5, 15).

Resistance to antimicrobial agents represents a real challenge in Egypt, with high burden on the Egyptian health care system (20). In that study, the authors found that 151 out of 355 (42.5%) of the *Klebsiella* spp. and 47 of 87 (54%) of *Escherichia coli* from the patient in intensive care units were identified as ESBL producers. Several studies have discussed the resistance spectra of different ESBL producers regardless their origin (i.e. human, animal or food origins) (5,15-16). Globally, ESBL-producing microorganisms are one of the most severe problematic multi-resistant and are being identified with increased frequency (4). Our results showed that resistance to two or more antibiotics was frequently encountered among the recovered isolates. As the production of most ESBLs is plasmids encoding, co-resistance to other groups of antibiotics is a common sequel (15). Remarkably, the range of resistance in our study was sufficiently worrisome. For instance, if an infection caused by any identified strains (as that observed in this study) it could represent a great concern due to limited therapeutic options. According to latest report of Infectious Diseases Society of America, ESBL-producing *E coli* and *Klebsiella* spp. were recognized as one of the six drug-resistant microbes that urgently required new remedies (21). Our genotypic characterization of the positive dairy samples by PCR assays revealed that 48% of the positive dairy samples were possessed *bla*CTX-M1encoding CTX-M broad-spectrum β-lactamases, while other genes like *blaTEM* and *blaSHV* were detected in 44% and 14.8%, respectively. This finding was in agreement with that previously reported (22, 24, 15, 23, 16). Unlike to our finding, several authors reported the absence of the *bla*CTX-M gene but identified only *bla*TEM and *bla*SHV genes in 15 and 4 isolates (18). The *bla*SHV-110, *bla*SHV-111, and *bla*CMY-41 genes have been identified from Domiati cheese from small producers in El Behera and Alexandria governments, Egypt (5). The production of broad-spectrum lactamases could provoke a significant clinical consequences with a resultant threaten the successful treatment of infectious diseases resulting in exacerbate public-health concerns (18).

Conclusion

In conclusion, there is clear evidence of circulation of antibiotic-resistant food borne ESBL producing Enterobacteriaceae in the examined dairy samples. As the majority of Egyptian population still consume raw milk and its product like soft cheese e.g. Domiati and Kareish cheese which contained ESBL producing Enterobacteriaceae , the concern about increasing the risk of a spread of such multi-drug resistant pathogens raises with a potential asymptomatic colonization and complication of systemic infection in human subjects and this could be achieved with safe use of antibiotics, reinforced sanitary measures, and a continues investigation of the phenomenon of antibiotic resistance in food producing animals.

Conflict of interest

The authors declare that they have no conflict of interest.

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