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Research Paper

Prevalence and Molecular Characterization of Antimicrobial Resistance in *Escherichia coli* Isolated from Raw Milk and Raw Milk Cheese in Egypt

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ABSTRACT

The goal of this study was to examine antimicrobial resistance and characterize the implicated genes in 222 isolates of *Escherichia coli* from 187 samples of raw milk and the two most popular cheeses in Egypt. *E. coli* isolates were tested for susceptibility to 12 antimicrobials by a disk diffusion method. Among the 222 *E. coli* isolates, 66 (29.7%) were resistant to one or more antimicrobials, and half of these resistant isolates showed a multidrug resistance phenotype (resistance to at least three different drug classes). The resistance traits were observed to tetracycline (27.5%), ampicillin (18.9%), streptomycin (18.5%), sulfamethoxazole-trimethoprim (11.3%), cefotaxime (4.5%), kanamycin (4.1%), ceftazidime (3.6%), chloramphenicol (2.3%), nalidixic acid (1.8%), and ciprofloxacin (1.4%). No resistance to fosfomycin and imipenem was observed. Tetracycline resistance genes *tetA, tetB,* and *tetD* were detected in 53 isolates, 9 isolates, and 1 isolate, respectively, but *tetC* was not detected. Aminoglycoside resistance genes *strA, strB, aadA,* and *aphA1* were detected in 41, 41, 11, and 9 isolates, respectively. Sulfonamide resistance genes *sul1, sul2,* and *sul3* were detected in 7, 25, and 3 isolates, respectively. Of 42 ampicillin-resistant isolates, bla_{TEM}, $bla_{\text{CTX-M}}$, and bla_{SHV} were detected in 40, 9, and 3 isolates, respectively, and 10 (23.8%) ampicillin-resistant isolates were found to produce extended-spectrum β-lactamase. Each *bla* gene of extended-spectrum β-lactamase–producing *E. coli* was further subtyped to be $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-104}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{SHV-12}}$. The class 1 integron was also detected in 28 resistant isolates, and three different patterns were obtained by PCR-restriction fragment length polymorphism. Sequencing analysis of the variable region revealed that four isolates had *dfrA12*/*orfF*/*aadA2,* two had *aadA22,* and one had *dfrA1*/*aadA1*. These data suggest that antimicrobial-resistant *E. coli* are widely distributed in the milk production and processing environment in Egypt and may play a role in dissemination of antimicrobial resistance to other pathogenic and commensal bacteria.

Key words: Antimicrobial resistance; Dairy products; Egypt; *Escherichia coli*

Worldwide antimicrobial resistance (AMR) is one of the most serious problems for both public and animal health, with inappropriate use of antimicrobial agents in humans and animals being one of the main causes of this problem *(7, 14)*. Food may act as a vector for the transfer of antimicrobial-resistant bacteria to humans *(53)*. Contamination of foods with such bacteria could be a major public health threat because there is a possibility that genes encoding AMR determinants could be transferred to other bacteria of clinical significance *(48)*. The rapid dissemination of the AMR genes among bacteria could be facilitated if these genes are encoded on mobile genetic elements such as plasmids, transposons, and integrons *(31, 45)*. Because *Escherichia coli* acquires resistance easily and is a common inhabitant of the intestinal tract of humans and animals, *E. coli* has been selected as a sentinel organism in AMR surveillance studies *(55)*.

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The study of AMR in clinical and environmental isolates is crucial to prevent the spread of multidrug-resistant bacteria *(50, 51)*. Information concerning the problem of AMR in the whole African continent is limited *(56)*. Similar to many other developing and African countries, food hygiene and safety conditions in Egypt, specifically in the dairy sector, require more attention. In addition, AMR is exacerbated by the lack of stringent controls on antimicrobial use in human health as well as animal production systems *(2),* which increases the risk of emergence of foodborne microbes possessing an array of resistance genes. Egypt, from ancient times, has been considered to be one of the leading countries in the manufacture of dairy products. Dairy products, especially cheese, play an important role in the Egyptian diet *(8, 18, 23)*. Many people eat a certain amount of cheese at least once every day *(47)*. However, very little is known about the prevalence of AMR and its molecular basis in bacteria isolated from Egyptian food.

Therefore, the aim of the present study was to check for the presence of antimicrobial-resistant *E. coli* in Egyptian

TABLE 1. *Primers for detection of antimicrobial-resistant genes and for identification of gene cassettes in the class 1 integron*

Target	Primer	Sequence 5′ to 3′	Size (bp)	Reference
tetA	tetA-F	GCTACATCCTGCTTGCCTTC	210	*28*
	tetA-R	CATAGATCGCCGTGAAGAGG		
tetB	tetB-F	TTGGTTAGGGGCAAGTTTTG	659	*28*
	tetB-R	GTAATGGGCCAATAACACCG		
tetC	tetC-F	CTTGAGAGCCTTCAACCCAG	418	*28*
	tetC-R	ATGGTCGTCATCTACCTGCC		
tetD	tetD-F	AAACCATTACGGCATTCTGC	787	*28*
	tetD-R	GACCGGATACACCATCCATC		
bla_{SHV}	bla-SHV.SE	ATGCGTTATATTCGCCTGTG	747	*34*
	bla-SHV.AS	TGCTTTGTTATTCGGGCCAA		
bla_{TEM}	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	*34*
	TEM-165.AS	ACGCTCACCGGCTCCAGATTTAT		
bla_{CTX-M}	CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	593	*34*
	CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG		
aphA1	aphA1-F	ATGGGCTCGCGATAATGTC	600	*48*
	aphA1-R	CTCACCGAGGCAGTTCCAT		
aadA	4F	GTGGATGGCGGCCTGAAGCC	525	*27*
	4Ra	AATGCCCAGTCGGCAGCG		
strA	2Fa	CCTGGTGATAACGGCAATTC	546	*27*
	2Ra	CCAATCGCAGATAGAAGGC		
strB	3Fa	ATCGTCAAGGGATTGAAACC	509	*27*
	3Ra	GGATCGTAGAACATATTGGC		
sul1	Sul1-Lb	GTGACGGTGTTCGGCATTCT	779	*27*
	Sul1-Rb	TCCGAGAAGGTGATTGCGCT		
sul2	Sul2-Lb	CGGCATCGTCAACATAACCT	721	*27*
	Sul2-Rb	TGTGCGGATGAAGTCAGCTC		
sul3	Sul3-Fc	GAGCAAGATTTTTGGAATCG	880	*27*
	Sul3-Rc	CATCTGCAGCTAACCTAGGGCTTTGGA		
intI1	INT-1U	GTTCGGTCAAGGTTCTG	923	*28*
	INT-1D	GCCAACTTTCAGCACATG		
qacEΔ1	Qac-F	GGCTGGCTTTTTCTTGTTATCG	287	*40*
	Qac-R	TGAGCCCCATACCTACAAAGC		
IntI1 variable region	5′-CS	TCGGGCATCCAAGCAGCAAGCGC	Variable	*28*
	3′-CS	TAAAAGCAGACTTGACCTGATAG		

dairy products, including raw milk and the two most popular cheeses, Karish and Ras, and to determine the antimicrobial susceptibility and genetic mechanisms of AMR.

MATERIALS AND METHODS

***E. coli* isolates.** Two hundred twenty-two *E. coli* isolated and identified in our previous study *(35)* were used in this study. One hundred eleven isolates were from raw milk, and 89 and 22 isolates were isolated from Karish and Ras cheeses, respectively.

Antimicrobial susceptibility test. Antimicrobial susceptibility testing was carried out for each of the 222 *E. coli* isolates. Twelve different antimicrobials were tested by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines *(12)*. *E. coli* strain ATCC 25922 was used as a quality control. The following antimicrobials (BD, Franklin Lakes, NJ) were used: ampicillin (AMP; 10 μg), cefotaxime (CTX; 30 μg), ceftazidime (CAZ; 30 μg), cefotaxime–clavulanic acid (CTX-CLA; 30 μg/10 μg), ceftazidime–clavulanic acid (CAZ-CLA; 30 μg/10 μg), imipenem (IPM; 30 μg), kanamycin (KAN; 30 μg), streptomycin (STR; 10 μg), tetracycline (TET; 30 μg), ciprofloxacin (CIP; 5 μg), nalidixic acid (NAL; 30 μg), sulfamethoxazole-trimethoprim (SXT; 1.25 μg/23.75 μg), chloramphenicol (CHL; 30 μg), and fosfomycin (FOS; 50 μg). The isolates were classified as susceptible, intermediate, and resistant according to the zone diameter interpretative standards recommendations by the Clinical and Laboratory Standards Institute *(13)*.

ESBL detection. Ampicillin-resistant *E. coli* were examined for extended-spectrum β-lactamase (ESBL) production by the combination disk diffusion test using CTX or CAZ with or without CLA according to the Clinical and Laboratory Standards Institute criteria *(12)*.

Detection of AMR genes. Sixty-six *E. coli* isolates that exhibited phenotype of resistance to one or more of the antimicrobial agents tested, were examined for AMR genes respective to each phenotype. The presence of genes associated with tetracycline resistance (*tetA, tetB, tetC,* and *tetD*), β-lactam resistance (bla_{CTX-M}, bla_{SHV}, and bla_{TEM}), aminoglycoside resistance (*strA, strB, aadA* [encoding streptomycin resistance] and *aphA1* [encoding kanamycin resistance]), and sulfonamide resistance (*sul1, sul2,* and *sul3*) were detected by PCR as described previously *(10, 28, 34, 48)*. Primer sequences and predicted sizes of PCR products of different AMR genes are listed in Table 1. The PCR products were analyzed by electrophoresis in 1.5% agarose gels (Seakem LE Agarose, Lonza, Basel, Switzerland), stained with ethidium bromide, and visualized under UV light by using a gel documentation system (Bio-Rad Laboratories, Hercules, CA).

TABLE 2. *Antimicrobial resistance of* E. coli *isolated from dairy products*

Antimicrobial agent[a]	No. of isolates ($n = 222$)		
	Resistant	Intermediate	Susceptible
TET	61	0	161
AMP	42	9	171
STR	41	30	151
SXT	25	2	195
CTX	10	4	208
KAN	9	34	179
CAZ	8	5	209
CHL	5	0	217
NAL	4	10	208
CIP	3	8	211
FOS	0	0	222
IPM	0	0	222

[a] TET, tetracycline (30 μg); AMP, ampicillin (10 μg); STR, streptomycin (10 μg); SXT, sulfamethoxazole-trimethoprim (1.25/23.75 μg); CTX, cefotaxime (30 μg); KAN, kanamycin (30 μg); CAZ, ceftazidime (30 μg); CHL, chloramphenicol (30 μg); NAL, nalidixic acid (30 μg); CIP, ciprofloxacin (5 μg); FOS, fosfomycin (50 μg); IPM, imipenem (10 μg).

DNA sequencing of *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{TEM}}$ genes. *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{TEM}}$ genes were PCR amplified from ampicillin-resistant isolates, and their products were sequenced to determine their subtypes as described previously *(32, 34)*. In brief, the amplified products were purified using a Wizard SV Gel and PCR Clean-Up system (Promega, Fitchburg, WI) and sequenced on both DNA strands by using primers as described previously *(32, 34)* and the BigDye Terminator v1.1 Cycle Sequencing kit (Thermo Fisher Scientific, Waltham, MA). The products were then purified using a CleanSEQ kit (Beckman Coulter, Beverly, MA) and subjected to sequencing in an ABI PRISM 3130-Avant Genetic analyzer (Thermo Fisher Scientific). The DNA sequence data were compared with those in the GenBank database (Nucleotide collection database [nr/nt]) by using the BLAST algorithm available at the National Center for Biotechnology Information Web site (www.ncbi.nlm.nih.gov). Multiple DNA alignments were performed by using ClustalW of the MegAlign program (DNASTAR software package, Lasergene, Madison, WI).

Detection and sequencing of class 1 integron. Detection of the class 1 integron gene *intI1* was done as described previously *(28)*. In brief, the presence of the *intI1* gene (encoding class 1 integrase) was examined by PCR using a primer set of INT-1U and INT-1D (Table 1). The variable region of the class 1 integron was amplified using 5′-CS and 3′-CS primers (Table 1), and PCR products were purified and sequenced as described below. The presence of *qacEΔ1-sul1* genes in the 3′ conserved region of the class 1 integron was also examined in all *intI1*-positive isolates as described by Sáenz et al. *(40)*.

Typing and sequencing of the variable region of class 1 integron genes. The PCR product for the variable region of the class 1 integron was gel-cut purified with a Wizard SV Gel and PCR Clean-Up system (Promega). The purified PCR products were subjected to restriction fragment length polymorphism (RFLP) by digesting with *Hin*fI enzyme (TaKaRa Bio Inc., Shiga, Japan) as described previously *(36)*. The digest showing the same restriction pattern was considered to be identical to each other, and one representative product of them was further sequenced.

TABLE 3. *Antimicrobial resistance patterns of* E. coli *isolates*

Resistance pattern[a]	No. of isolates
TET	16
AMP	2
STR	1
AMP-TET	7
TET-STR	4
CHL-STR	1
AMP-TET-STR	3
TET-KAN-STR	2
AMP-CTX-STR	1
AMP-SXT-TET-STR	14
AMP-CHL-TET-STR	1
AMP-TET-KAN-STR	1
AMP-CHL-TET-KAN-STR	1
AMP-CTX-CAZ-SXT-TET-STR	6
AMP-SXT-TET-NAL-KAN-STR	1
AMP-TET-NAL-CIP-KAN-STR	1
AMP-CTX-SXT-TET-NAL-CIP-STR	1
AMP-SXT-TET-NAL-CIP-KAN-STR	1
AMP-CTX-CAZ-CHL-SXT-TET-KAN-STR	2
Total	66

[a] TET, tetracycline (30 μg); AMP, ampicillin (10 μg); STR, streptomycin (10 μg); CHL, chloramphenicol (30 μg); KAN, kanamycin (30 μg); CTX, cefotaxime (30 μg); SXT, sulfamethoxazole–trimethoprim (1.25 μg/23.75 μg); CAZ, ceftazidime (30 μg); NAL, nalidixic acid (30 μg); CIP, ciprofloxacin (5 μg).

RESULTS

Antimicrobial resistance phenotypes of *E. coli* isolates. Two hundred twenty-two *E. coli* isolates from raw milk, Karish cheese, and Ras cheese were tested for antimicrobial susceptibility to 12 antimicrobial agents. As shown in Table 2, 61, 42, 41, 25, 10, 9, 8, 5, 4, and 3 isolates of *E. coli* were resistant to TET (27.5%), AMP (18.9%), STR (18.5%), SXT (11.3%), CTX (4.5%), KAN (4.1%), CAZ (3.6%), CHL (2.3%), NAL (1.8%), and CIP (1.4%), respectively. None of them were resistant to FOS and IPM. Production of ESBLs was detected in 10 (23.8%) of 42 AMP-resistant *E. coli*. The phenotypic resistance patterns of the *E. coli* are shown in Table 3. Among the 222 *E. coli* isolates, 66 (29.7%) were resistant to one or more antimicrobials; 33 (50%) of these 66 isolates showed multidrug resistance, i.e., they showed resistance to at least three different classes of antimicrobial agents.

Antimicrobial resistance genotype of *E. coli* isolates. The distribution and type of genes encoding resistance for tetracycline, β-lactams, aminoglycosides (streptomycin and kanamycin), and sulfonamides in *E. coli* isolates are described in Table 4. Among 61 tetracycline-resistant *E. coli, tetA, tetB,* and *tetD* genes were detected in 53 isolates, 9 isolates, and 1 isolate, respectively. Both *tetA* and *tetB* or *tetA* and *tetD* genes were detected in one isolate each (Table

TABLE 4. *Distribution of antimicrobial resistance genes in resistant* E. coli

Antimicrobial agent (no. of resistant isolates)	Resistance gene	Positive isolate	
		No.	%
Tetracycline (61)	*tetA*	53	86.9
	tetB	9	14.8
	tetD	1	1.63
	tetC	0	0
β-Lactam (42)	bla_{TEM}	40	95.2
	bla_{CTX-M}	9	21.4
	bla_{SHV}	3	7.14
Streptomycin (41)	*strA*	41	100
	strB	41	100
	aadA	11	26.8
Kanamycin (9)	*aphA1*	9	100
Sulfonamides (25)	*sul2*	25	100
	sul1	7	28
	sul3	3	12

5). In contrast, *tetC* was not detected in any of the isolates tested.

Among β-lactam resistance genes in 42 ampicillin-resistant *E. coli,* bla_{TEM}, bla_{CTX-M}, and bla_{SHV} were detected in 40, 9, and 3 isolates, respectively (Table 4). Among 42 ampicillin-resistant *E. coli,* 10 were ESBL producers, and bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes were detected in 9, 8, and 1 ESBL-producing *E. coli,* respectively (Supplemental Table S). Among them, five ESBL-producing *E. coli* carried both bla_{CTX-M} and bla_{TEM} genes and two carried bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes (Supplemental Table S). Sequence analysis of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes in ESBL-producing *E. coli* ($n = 10$) revealed that bla_{CTX-M} was typed to be $bla_{CTX-M-15}$ (eight isolates) and $bla_{CTX-M-104}$ (one isolate) and that the bla_{TEM} type was bla_{TEM-1}. In contrast, bla_{SHV} was typed to be bla_{SHV-12}. $bla_{CTX-M-15}$ alone was present in one isolate, and the same for $bla_{CTX-M-104}$, whereas $bla_{CTX-M-15}$ together with bla_{TEM-1} was present in five isolates and $bla_{CTX-M-15}$ together with bla_{TEM-1} and bla_{SHV-12} was present in two isolates.

The *strA, strB,* and *aadA* genes encoding streptomycin resistance were detected in 41, 41, and 11 of 41 streptomycin-resistant isolates, respectively (Table 4). The *aphA1* gene encoding kanamycin resistance was detected in all kanamycin-resistant *E. coli* (Table 4). Different combinations of sulfonamide resistance genes such as *sul1, sul2,* and *sul3* were detected in 25 sulfamethoxazole-trimethoprim–resistant *E. coli* (Table 5). All isolates harbored the *sul2* gene; seven (28%) harbored the *sul1* gene, in addition to *sul2*; and three (12%) harbored *sul3* with the *sul2* gene (Tables 4 and 5).

Prevalence and sequencing of integron genes. The class 1 integron was detected in 28 (42.4%) of the 66 resistant isolates. Amplification of the variable region of the class 1 integron resulted in three size fragments—1, 1.5, and 2 kb—by PCR in 7 of the 28 *intI1*-positive isolates in which the *qacEΔ1-sul1* genes were also amplified (data not shown). RFLP showed that four of the 2-kb fragments and two of the 1-kb fragments gave the same RFLP patterns, indicating that each fragment in four and two strains was identical, respectively. Furthermore, sequencing analysis revealed that 2-, 1.5-, and 1-kb fragments contained *dfrA12*/*orfF*/*aadA2, dfrA1*/*aadA1,* and *aadA22,* respectively.

TABLE 5. *Antimicrobial resistance genotypes of* E. coli *isolates (*n *= 66)*

Genotype	No. of isolates
tetA	12
tetB	3
bla_{TEM}	2
tetA-bla_{TEM}	7
strA-strB	2
tetA-tetB	1
tetA-strA-strB	3
strA-strB-$bla_{CTX-M-15}$	1
tetA-strA-strB-bla_{TEM}	3
tetA-strA-strB-aphA1	2
tetA-strA-strB-sul2	1
tetA-strA-strB-bla_{TEM}-*sul2*	8
tetB-strA-strB-bla_{TEM}-*sul2*	2
tetA-strA-strB-aphA1-bla_{TEM}	1
tetA-strA-strB-$bla_{CTX-M-15}$-bla_{TEM-1}-*sul2*	5
tetB-strA-strB-aphA1-bla_{TEM}-*sul2*	1
tetA-tetD-strA-strB-bla_{TEM}-*sul2-sul3*	1
tetA-strA-strB-aadA-bla_{TEM}-*sul1-sul2*	4
tetA-strA-strB-aadA-$bla_{CTX-M-104}$-*sul1-sul2*	1
tetA-strA-strB-aadA-aphA1-bla_{TEM}-*sul2-sul3*	1
tetA-strA-strB-aadA-aphA1-bla_{TEM}-*sul1-sul2*	1
tetA-strA-strB-aadA-bla_{TEM}-*sul1-sul2-sul3*	1
tetA-strA-strB-aadA-aphA1-bla_{SHV}-bla_{TEM}-*sul2-sul3*	1
tetA-strA-strB-aadA-aphA1-$bla_{CTX-M-15}$-bla_{SHV-12}-bla_{TEM-1}-*sul2-sul3*	2
Total	66

DISCUSSION

There have been very few published studies on the occurrence of antibiotic resistant bacteria in food samples in Egypt *(2, 20, 21)*. In addition, the molecular characteristics of antibiotic-resistant bacteria from food samples in Egypt are almost unknown. To the best of our knowledge, this is the first report regarding the comprehensive analysis of AMR and AMR genes in *E. coli* isolated from raw milk and raw milk cheese in Egypt.

This study shows that AMR is widespread in *E. coli* in such products. The most prevalent resistance traits were tetracycline (27.5%) followed by ampicillin (18.9%), aminoglycosides (streptomycin, kanamycin) (18.5%), and sulfamethoxazole-trimethoprim (11.3%) (Table 2). These findings are in agreement with many studies from different countries that found that resistance to tetracycline, penicillins, aminoglycosides, and sulfonamides is generally the most prevalent among *E. coli* isolated from food products or healthy food-producing animals *(15, 24, 33, 38, 39, 41, 44, 48, 52)*. Different use patterns of antimicrobial agents are expected to have some impact on the distribution of AMR

phenotypes and possible resistance determinants *(1, 29)*. Our data on the distribution of resistance phenotypes support this hypothesis, as higher levels of resistance were observed to the common antimicrobials that have been widely used in Egypt. In most of the developing countries, including Egypt, the low cost and easy availability of these antibiotics are the main reasons behind their wide use to treat diseases, particularly diarrhea *(49)*. In contrast, in this study, 2.3% of *E. coli* showed resistance to chloramphenicol. Chloramphenicol has been banned and has not been approved for use in food-producing animals in many countries, but resistance to chloramphenicol still remains in Egypt.

Multidrug resistance bacteria can come into contact with humans through the food supply (e.g., meat, fish, dairy products, and eggs) and direct contact with animals or indirectly through environmental pathways *(4)*. In the present study, 50% of antimicrobial-resistant *E. coli* isolates showed resistance to more than three classes of antimicrobials (Table 3). Moreover, all phenotypically resistant isolates ($n = 66$) carried at least one AMR gene, and 74.2% of these isolates carried multiple (two or more) AMR genes (Table 5). Among tetracycline resistance genes, *tetA* was detected in the highest frequency (86.9%), a finding that is in agreement with several previous studies *(9, 19, 37, 51)*. The *tetB* and *tetD* genes were also detected in our study, but in a lower frequency than those reported previously *(38)*. In this study, *tetC* was not detected in any isolates, although Srinivasan et al. *(44)* reported a high frequency of *tetC* in *E. coli* isolates from dairy cows with mastitis. The difference could be owing to the different sources of *E. coli* between these two studies.

In agreement with the previous findings *(11, 19, 21, 24, 40, 48)* that TEM β-lactamase was the most prevalent in ampicillin-resistant *E. coli* isolates from food and food-producing animals, all ampicillin-resistant *E. coli* ($n = 42$) carried the bla_{TEM} gene, except for two isolates (Supplemental Table S). ESBL-producing *E. coli* have become a serious problem worldwide and $bla_{\text{CTX-M}}$ has been distributed worldwide *(42)*. However, little is known about ESBL-producing bacteria and the types of β-lactamases occurring in Egypt. In this study, 10 ESBL-producing *E. coli* were isolated from raw milk and Karish and Ras cheeses. Among them, nine $bla_{\text{CTX-M}}$, eight bla_{TEM}, and three bla_{SHV} genes were detected. Furthermore, each β-lactamase gene was subtyped to be $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-104}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{SHV-12}}$. $bla_{\text{CTXM-15}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{SHV-12}}$ genes have been previously reported in clinical *E. coli* isolates in Egypt *(3, 17)*. The $bla_{\text{CTX-M-104}}$ gene is a novel variant of $bla_{\text{CTX-M}}$; it differs from $bla_{\text{CTX-M-14}}$ by a point mutation that leads to a change of only one amino acid (N275S). This new $bla_{\text{CTX-M-14}}$–like gene was reported for the first time in an *E. coli* isolate from chicken in China *(57)*, and more recently in an *E. coli* isolate from swine, also in China *(30)*. Among 10 ESBL-producing *E. coli*, three isolates were positive for either $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-104}}$, or $bla_{\text{TEM-1}}$, and five isolates were positive for both $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$, whereas two were positive for $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{SHV-12}}$. *E. coli* isolates harboring β-lactamase genes such as $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{SHV-12}}$ have been previously reported from hospital waste water in India *(16)*; to our knowledge, this is the first report of *E. coli* isolates harboring this β-lactamase gene combination from food sources.

All the 41 streptomycin-resistant isolates carried both *strA* and *strB* genes. Eleven of them carried *strA*/*B* along with *aadA*. The *strA* and *strB* genes were always present together in our isolates. This finding is in agreement with the results of other reports *(29, 38, 44, 46)*. In this study, the *sul2* gene was detected in all sulfamethoxazole-trimethoprim–resistant *E. coli* followed by *sul1* (28%) and *sul3* (12%) (Table 4). This tendency is in agreement with the data previously reported in *E. coli* isolates from food or animals *(19, 22, 24, 40, 45)*.

Integrons play a major role in the storage and spread of AMR among gram-negative bacteria *(43)*. Class 1 integrons were detected in 42.4% of the resistant isolates ($n = 66$) investigated in this study. All of them were phenotypically resistant to four different classes of antimicrobials, with the exception of one isolate that showed resistance to two classes only, indicating that the multidrug resistance observed in these isolates may be associated with the class 1 integron. Furthermore, we observed that 24 of 28 *intI1*-positive isolates were sulfamethoxazole-trimethoprim resistant. However, when we analyzed the remaining four isolates for the presence of sulfonamide resistance genes, *sul2* was detected in these four isolates and three of them also carried *sul3*, indicating that the class 1 integron is often associated with *sul* genes, as was reported previously *(6)*.

The trimethoprim resistance genes (*dfrA1* and *dfrA12*) and aminoglycoside resistance genes (*aadA1*, *aadA2*, and *aadA22*) detected in this study were also previously reported in human *E. coli* isolates and food isolates *(19, 21, 24, 25, 40)*. Interestingly, the rare aminoglycoside resistance gene *aadA22*, which was reported for the first time in an *E. coli* isolated from Egyptian Domiati cheese *(21)*, was detected in two isolates from raw milk in this study. Twenty-one *intI1*-positive *E. coli* lacked the *qacEΔ1-sul1* region in the 3′-conserved region of the class 1 integron. The existence of these defective integrons has been reported *(5, 26, 54)*, and our laboratory is currently determining the complete structure and characterizing these integrons more precisely.

Some of the multidrug-resistant strains isolated in this study were also found to be harboring virulence genes (Supplemental Table S). The presence of *E. coli* strains carrying resistance and virulence genes is worrisome as this could enhance the emergence of pathogens that might be difficult to treat with antibiotics *(10)*.

In conclusion, results of this study highlight the importance of *E. coli* present in dairy products in Egypt as a reservoir for AMR *E. coli* and suggest that AMR genes carrying *E. coli* are widely distributed in the milk production and processing environment in Egypt. These AMR *E. coli* may play a role in dissemination of these genes to other pathogenic and commensal bacteria. The presence of multidrug-resistant strains is alarming, because such strains are considered a serious threat to public health. This potential threat emphasizes the need for effective hygienic and sanitation procedures in milk and cheese production and the wise use of antimicrobial agents in food-producing

animals to reduce the risks of contamination with antibiotic resistant bacteria.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-17-277.s1.

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