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Prevalence and Molecular Characterization of TEM and CTX-M Beta-Lactamase Genes of Escherichia *coli* Isolated From Cow Milk

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Abstract

Objective: The study objective was to investigate the presence and antibiotic resistance of toxigenic *E. coli* strains in the raw milk of dairy cattle and to determine the prevalence of bla_{TEM} and bla_{CTX-M} genes in the selected isolates.

Methods: Two hundred raw milk samples were collected from 20 dairy farms located in Kafr El Sheikh City.

Results: Among the samples, 60 were positive for *E. coli*. The sensitivity of these isolates was detected against different antibiotics. Using the disk diffusion test, all the isolates were resistant to at least two beta-lactam antibiotics. The resistance to beta-lactam antibiotics by the selected *E. coli* was varied to be highly significant. Five percent of the tested *E. coli* was highly resistant with a multi-antibiotic resistant (MAR) index, of 1.0, while 8% had the lowest MAR (0.14). Both bla_{TEM} and bla_{CTX-M} resistant genes were detected in isolate No. 52, while, bla_{CTX-M} was detected in isolate No. 34 and bla_{TEM} was detected in isolates No. 9, 21, 28, and 40. The PCR products of bla_{TEM} and bla_{CTX-M} genes were sequenced and deposited in the GenBank of the NCBI database with the accession numbers OR450046 and OR879117, respectively.

Conclusion: The macrolides group of antibiotics especially erythromycin should not be a suitable treatment of dairy herds for mastitis by *E. coli* in Egypt. The majority of *E. coli* was multiple-antibiotic resistant and co-carried many virulence genes, and it may pose a great potential risk to public health.

Keywords: *E. coli*, raw milk, antibiotic resistance, bla_{CTX-M} gene, bla_{TEM} gene

Introduction

E. coli was a typical resident in animal intestines (Tark et al., 2016; Liu et al., 2021)[1,2] and was discovered to typically infect the cows' mammary glands after parturition and the first few weeks of lactation, which may result in acute and localized mastitis.[3] The various pathogenic types of *E. coli* strains are increasingly acknowledged as posing a significant risk to public health. Bacterial mastitis in cows is primarily brought on by *E. coli*, often only lasts two to three days, and results in an infection. However, in a few instances, *E. coli* has been shown to produce persistent infections.[4] Due to its virulence, pathogenic *E. coli* can cause problems in both animals and humans.[5] Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC) are pathogenic types of *E. coli* and classified based on epidemiological, clinical, and pathogenic characteristics.[6] Several outbreaks of *E. coli* in milk and other foods have previously been documented.[7-9]

Public health professionals in both developed and developing nations continue to face a significant challenge from the rise in antibiotic resistance. However, the overuse of antibiotics and their expanding use in both human and animal healthcare contribute to the development of antimicrobial resistance. *E. coli* is associated not only with the probable emergence of antibiotic resistance bacteria but also with the rapid growth of antibiotic resistance bacteria.[2,5] Furthermore, *E. coli* can acquire resistance to other antibiotics through mutation, plasmids transportation, or transposons (Gonggrijp et al., 2016),[10] and it has been reported to be the most common cause of clinical mastitis in well-managed dairy herds with low milk somatic cell counts (SCC) in the United Kingdom.[11]

E. coli is one of the most prevalent infectious agents isolated from severe mastitis cases in modern dairy farms.[12] Lang and Smith (2007)[13] confirmed that *E. coli* O157:H7 is capable of moving through the soil profile with water after rainfall or irrigation and can even reach groundwater. Ruminants, particularly cattle, are reservoirs of *E. coli* O157:H7, which can live asymptotically in cow intestines and be excreted intermittently in feces.[14] Shiga toxins are the most important virulence factors in *E. coli*, and Stx1 and Stx2 have been linked to the severity of human infections.[15] Aidar-Ugrinovich et al. (2007)[16] discovered STEC in dairy and beef cattle excrement, water and animal feed, milk and dairy products, and ground beef. In reality, the pathophysiology of non-O157 shiga toxin *E. coli* (STEC) is not entirely characterized.[17] Many studies on *E. coli* strains have been conducted, particularly on the diverse virulence factors.[18]

bla_{TEM} and bla_{CTX-M} genes are the most significant extended-spectrum beta-lactamase (ESBL) genes.[19,20] It is thought that these genes were created by mutation from narrow-spectrum beta-lactamase genes.

E. coli isolates that induce mastitis and a variety of distinct virulence factors has been discovered individually or in combinations.[21,22] The majority of mastitis isolates lacked any of the virulence characteristics investigated in the risk profile when shiga toxin-producing *E. coli* was present in raw milk.[21,23,24] Multiplex PCR is an excellent approach for detecting specific STEC serotype virulence genes such as shiga toxins 1 and 2, intimin, and enterohaemolysin A.[25] One strategy for mastitis prevention would be the genetic alteration of dairy cows to express recombinant immunomodulation proteins in their milk.[26] Non-antimicrobial techniques for treating *E. coli* mastitis have been researched as alternatives to antimicrobial

agents; non-steroidal anti-inflammatory medicines, frequent milking, and fluid therapy have all been widely advocated for supportive treatment of coliform mastitis.[27] This study aimed to investigate the presence of *E. coli* strains in the raw milk of dairy cattle in Kafr El-Shaikh City, Egypt, to study the antibiotic susceptibility of these strains, and to determine the prevalence of bla_{TEM} and bla_{CTX-M} genes among beta-lactamase-producing *E. coli*.

Materials and Methods

Eosine methylene blue agar medium (EMB) was used as a differential selective medium for *E. coli* which has a characteristic green metallic sheen on EMB agar. MacConkey's agar medium (Oxoid, 1987)[28] was used as a selective and differential medium for isolation and differentiation between members of the family Enerobacteriaceae. Nutrient agar medium was used to enrich, purify, and study bacterial isolates' culture characteristics (Pigment production).[28] MacConkey's broth (Oxoid, 1987)[28] was used as a selective media for *E. coli*. Muller Hinton agar medium (Oxoid, 1987)[28] was used for the antibiotic sensitivity test. Semi-solid nutrient agar medium (Cruickshank et al., 1975)[29] was used for the detection of bacterial motility and preservation of bacterial isolates.

Sample Collection

A total number of two hundred milk samples were collected aseptically in clean, sterile 20 ml falcon tubes from twenty farms in Kafr El-Sheikh Governorate in February to April 2021. They were transferred instantly in an icebox and preserved at –20°C. Milk samples were diluted; one milliliter of the sample was diluted in 9 ml buffer peptone water (Oxoid) and incubated at 37°C for 18–24 hrs. Subsequently, a sterile loop was used to transfer the bacteria from the inoculated buffer peptone water and was inoculated on a MacConkey agar plate (Oxoid). Plates were incubated at 37°C for 24 hr. The suspected colonies were inoculated in eosin methylene blue (Oxoid). After the procedure, green metallic sheen colonies were selected for biochemical identification using the IMVIC reaction and triple sugar iron test.

Characterization and Identification of Bacteria From Milk Samples

According to Quinn et al. (2002),[30] the selected bacteria isolated from milk were identified based on morphological and some physiological methods. Gram stain, shape, motility, and production of some enzymes were detected in all isolates. Catalase, oxidase, and nitrate reduction production, methyl red/Voges-Proskauer (MR/VP), hydrolysis of esculin, citrate test, fermentation of glucose, etc., were used to identify the bacterial samples. Furthermore, growth on mannitol, growth at 25–45°C, pH range, NaCl range, gas production, and hemolytic activity were recorded to identify bacteria.[31]

Antibiotic Susceptibility Evaluation of Bacterial Strains

Antimicrobial susceptibility test for *E. coli* isolates was conducted by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI).[32,33] The sensitivity of each isolate was determined against 12 different antibiotics: Oxacillin (1 µg), trimethoprim (5 µg), tetracycline (30 µg), sulfamethoxazole-trimethoprim (25 µg), gentamicin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg), penicillin G (10 µg), nalidixic acid (30 µg), nitrofurantoin (30 µg), clindamycin (10 µg), and rifampin (30 µg) (Oxoid).

Multi-Antibiotic Resistant (MAR) Index

MAR index for the isolate identified as the number of antibiotics resisted by the isolate (a) / the total number of the tested antibiotics (b), according to the calculating formula MAR index = a/b.[34]

DNA Extraction and PCR Amplification

DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen GmbH, Germany) with changes to the manufacturer's recommendations. Virulence genes in *E. coli* strains were detected by Uniplex PCR primers for the following genes; bla_{TEM} and bla_{CTX-M} (Table 1) in a 25 µL reaction containing 12.5 µL of Emerald Amp Max PCR Master Mix (Japan), 1 µL of forward primer, 1 µL for reverse primer, 5.5 µL of water, and 5 µL of DNA template. The thermocycling settings for the two primers were as follows: a five-minute initial denaturation at 94°C, then 35 cycles of 30 sec denaturation at 94°C, 40 sec annealing at 54°C, and 45 sec extension at 72°C. The PCR reaction was then completed after a final extension of 10 min at 72°C. The reaction was conducted in an Applied Biosystem 2720 thermal cycler. The PCR products were run on a 1.5% agarose gel with 0.5 µg/ml ethidium bromide at 100 volts for 45 min. The DNA was then seen under a UV light source and photographed by a gel documentation system.

Phylogenetic Analysis

QIAquick PCR Product extraction kit (Qiagen Inc. Valencia CA) was used for the purification of the PCR product directly. A purified PCR product was sequenced in the forward and/or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA), with Cat. No. 4336817. A BLAST® analysis, Basic Local Alignment

Table 1. **Oligonucleotide primer sequences used for bla_{TEM} and bla_{CTX-M} gene detection**

Gene	Sequence	Amplified product	Reference
bla_{TEM}	F: ATCAGCAATAAACCAGC	516 bp	Colom et al. (2003)[35]
	R: CCCCGAAGAACGTTTTC		
bla_{CTX-M}	F: ATG TGC AGY ACC AGT AAR GTK ATG GC	593 bp	Archambault et al. (2006)[36]
	R: TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		

Search Tool (Altschul et al., 1990)[37] was initially performed to establish sequence identity to GenBank accessions. A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 12.1 of MegAlign module of Lasergene DNAStar software Pairwise (Madison, Wisconsin, USA) which was designed by Thompson et al. (1994).[38] Phylogenetic analyses were done using maximum likelihood, neighbor-joining, and maximum parsimony in MEGA 6.[39]

Results

Occurrence of E. coli in Cow Milk

Out of 200 milk samples, 60 samples were positive for the presence of *E. coli*. Sixty different bacterial isolates were obtained and identified as *E. coli* strains after examination using some biochemical reactions. Out of these isolates, only 14 isolates proved to be multidrug-resistant (MDR). The colonies of *E. coli* grow with a green metallic sheen, which is due to the metachromatic property of dyes (eosin and methylene blue in the ratio of 6:1) and the lactose fermenting property of *E. coli*, which changes the pH of the medium to acidic. Hence, making the medium more selective for *E. coli* makes the identification much easier as shown in Figure 1.

Antimicrobial Susceptibility Testing

The sixty isolates were examined by the disk diffusion method for susceptibility to 14 antibiotics (Table 2). All tested *E. coli* strains were resistant to erythromycin and azithromycin (Table 2). The sensitivity to other tested antibiotics was varied significantly according to the *E. coli* strain (Figure 2). The highest multi-antibiotic resistant (MAR) index (1.0) was obtained by strains Nos. 34, 52, and 56 followed by 0.86 for isolates No. 40, 47, and 58. On the other hand, the most sensitive strains were isolate No. 2, 3, 4, 5, 6, and 29. They formed various inhibition zones ranged from 13 mm to 22 mm, respecting to the antibiotic type. The highest inhibition zone was detected for these strains were against levofloxacin (22 mm), amikacin (22 mm), ofloxacin (21 mm), gentamycin (21mm), amikacin (19 mm), and ciprofloxacin (22 mm), respectively (Table 2).

Fig. 1 **The growth of *E. coli* on eosin methylene blue agar showed green metallic sheen colonies.**

Table 2. **Biological activity of different antibiotics against *E. coli* isolates**

Isolate no.	Inhibition zone (mm)														MAR index
	OFX	AX	LEV	E	AZM	GN	CIP	DO	CXM	RD	CFP	CTX	CRO	AK	
1	13	17	12	–	–	18	17	17	17	14	14	16	13	13	0.21
2	16	19	22	–	–	20	19	16	20	17	16	21	15	18	0.14
3	21	20	19	–	–	18	16	18	16	16	14	19	18	22	0.14
4	21	17	20	–	–	20	17	20	16	19	17	17	20	17	0.14
5	20	16	19	–	–	21	16	19	21	16	19	19	17	16	0.14
6	15	18	13	–	–	18	16	16	14	18	17	17	15	19	0.14
7	18	–	16	–	–	18	16	16	13	18	17	17	16	14	0.21
8	19	18	20	–	–	18	–	16	16	–	14	18	–	17	0.36
9	17	–	18	–	–	8	17	14	10	–	–	15	–	9	0.64
10	17	15	15	–	–	17	15	19	14	14	15	18	13	11	0.21
11	14	18	–	–	–	17	16	16	15	18	11	17	13	12	0.36
12	13	18	16	–	–	14	15	18	16	16	14	19	13	11	0.21
13	15	18	14	–	–	18	15	11	19	17	14	18	15	13	0.21
14	13	18	16	–	–	15	13	11	16	15	10	15	19	15	0.29
15	16	14	19	–	–	14	11	19	17	11	13	18	11	15	0.36
16	15	11	19	–	–	16	17	13	12	18	17	11	15	12	0.43
17	10	16	13	–	–	10	10	16	19	15	15	19	13	12	0.43

(Continued)

Table 2. **Biological activity of different antibiotics against *E. coli* isolates—Continued**

Isolate no.	Inhibition zone (mm)														MAR index
	OFX	AX	LEV	E	AZM	GN	CIP	DO	CXM	RD	CFP	CTX	CRO	AK	
18	17	15	11	–	–	19	17	17	19	10	14	12	17	15	0.36
19	13	16	13	–	–	15	13	13	18	19	10	12	15	11	0.36
20	16	14	19	–	–	18	10	14	19	13	11	10	17	15	0.36
21	21	11	–	–	–	19	12	6	–	7	–	13	–	7	0.79
22	17	14	10	–	–	18	10	10	15	11	10	15	18	11	0.57
23	11	17	13	–	–	19	10	16	10	14	10	11	17	14	0.50
24	17	14	19	–	–	21	18	15	21	10	15	19	13	19	0.21
25	21	20	22	–	–	18	21	17	20	16	19	17	14	11	0.21
26	16	14	19	–	–	17	10	21	16	19	14	19	15	14	0.21
27	21	17	16	–	–	17	21	18	16	22	20	10	17	13	0.21
28	13	15	–	–	–	–	17	11	–	9	–	16	–	7	0.71
29	20	17	15	–	–	19	22	21	19	16	21	19	16	20	0.14
30	15	12	19	–	–	17	15	19	14	14	15	18	13	10	0.29
31	11	18	14	–	–	17	16	16	15	14	11	17	13	12	0.36
32	19	14	11	–	–	17	11	18	16	16	14	19	19	11	0.36
33	17	19	10	–	–	21	10	9	15	11	18	15	18	16	0.43
34	11	6	9	–	–	6	9	7	–	8	–	–	6	–	1.00
35	15	18	11	–	–	14	15	18	13	11	16	13	11	11	0.43
36	16	16	14	–	–	18	11	17	13	12	13	10	17	13	0.36
37	15	17	14	–	–	16	14	19	13	11	17	11	19	21	0.29
38	16	15	10	–	–	21	20	18	15	12	10	15	17	12	0.43
39	15	12	14	–	–	18	15	10	19	17	14	21	15	17	0.29
40	16	–	9	–	–	8	8	–	8	–	9	18	11	–	0.86
41	17	15	15	–	–	17	15	19	14	14	15	18	13	11	0.21
42	14	16	5	–	–	17	16	16	15	18	11	17	13	12	0.36
43	13	18	16	–	–	14	15	18	16	16	14	19	13	11	0.21
44	15	18	14	–	–	18	15	11	15	17	14	12	15	13	0.29
45	13	18	16	–	–	15	13	11	16	15	10	15	19	15	0.29
46	16	14	19	–	–	14	11	19	17	11	13	18	11	15	0.36
47	6	19	7	–	–	–	6	–	–	8	9	14	9	8	0.86
48	16	14	19	–	–	14	11	19	17	11	13	18	11	15	0.36
49	15	11	19	–	–	16	17	13	12	18	17	11	15	12	0.43
50	10	16	13	–	–	10	10	16	19	15	15	19	13	12	0.43
51	17	15	11	–	–	19	17	17	19	10	14	12	17	15	0.36
52	9	–	8	–	–	8	9	–	–	10	–	–	–	–	1.00
53	14	11	19	–	–	13	18	11	15	13	16	14	19	12	0.36
54	16	17	13	–	–	17	11	15	12	11	15	11	19	17	0.43
55	10	10	16	–	–	15	19	13	12	17	10	16	13	21	0.43
56	10	–	–	–	–	9	9	–	–	7	9	11	8	10	1.00
57	15	18	11	–	–	14	15	18	13	11	16	13	11	13	0.36
58	–	19	–	–	–	10	–	9	–	–	9	17	9	6	0.86
59	15	12	10	–	–	17	21	16	19	21	16	19	12	15	0.36
60	17	15	19	–	–	10	16	18	21	19	20	16	21	19	0.21

CLSI zone diameter for *E. coli* (>16 mm susceptible; from 13 mm to 15 mm intermediate; < 12 mm resistant); multi-antibiotic resistant (MAR); ofloxacin (OFX); amoxicillin (AX); levofloxacin (LEV); erythromycin (E); azithromycin (AZM); gentamycin (GN); ciprofloxacin (CIP); doxycycillin (DO); cefuroxime (CXM); rifampicin (RD); cefoperazone (CFP); cefotaxime (CTX); cefotriaxone (CRO); amikacin (AK).

Fig. 2 **Antimicrobial activity of cefotriaxone (CRO); amoxicillin (AX); erythromycin (E); cefotaxime (CTX); gentamycin (GN); against isolate No. 27 of *E. coli*.**

Characterization of Beta-Lactamases (β-Lactamases) Resistance Genes Profiles

Molecular analysis of β-lactamase resistance genes in the most resistant *E. coli* isolates showed the presence of bla_{TEM} encoding genes in *E. coli* isolates No. 9, 21, 28, 40, 52, and 56 which represented 60% of the tested isolates. Table 3 summarizes the presence of blaTEM and bla_{CTX-M} in the selected E. coli isolates. Amplification of 516 bp product corresponding to bla_{TEM} gene was obtained in these isolates (Figure 3a). However, two bands of 593 bp product corresponding to bla_{CTXM} gene were observed in strains Nos. 34 and 52 (Figure 3b). These means that strain No. 52 has the two tested genes bla_{TEM} and $bla_{CTX-M.}$ These bands (516 and 593 bp) were sequenced and aligned with the partial sequences of the neighbor-joining sequences in GenBank and then deposited under accession numbers of OR450046 and OR879117, respectively.

The alignment of bla_{TEM} gene isolated from *E. coli* of raw milk in NCBI showed 70% similarity with those of *Proteus mirabilis* and *E. coli* H258, however, it has about 84 and 93% similarity with lentivirus shuttle vector and certain synthetic construct clone, respectively. Examination of phylogenetic tree of bla_{TEM} gene sequence demonstrates OR450046 gene variant from those of *E. coli* strain D72, *E. coli* strain MS 69-1, and *Mycoplasma mycoides* that are out-groups (Figure 4a). While, the bla_{CTX-M} gene sequence showed 100% similarity with those reported of *Actinobacter* sp. strain SH17, *Klebsiella pneumonia*, *Enterobacter* sp. strain SD1, *Bacillus* sp. strain SD10, *E. coli* strain EcCT, and *E. coli* strain Ec35 (Figure 4b).

Discussion

The existence of harmful microorganisms in milk has grown to be a significant issue for public health.[34,40] Pathogenic *E. coli* found in unpasteurized milk is one of these pathogens and has been connected to foodborne outbreaks and the emergence of antibiotic resistance.[41] In this study, 33.3% of raw milk samples tested positive for *E. coli*. These findings are much lower than those seen in some investigations. About 81.1% of Indian raw milk samples investigated by Bhoomika et al. (2016)[42] had *E. coli*, while, 75% of raw milk in Bangladesh (Islam et al., 2016),[43] 64.5% of raw milk in Malaysia (Jayarao and Henning, 2001),[44] and 45% of raw milk in Northern China (Lan et al., 2017)[45] were found. In Sharkia Governorate, Egypt, *E. coli* was present at a substantially lower prevalence (22.4%) in raw milk.[46] Furthermore, our findings are comparable to those of Ntuli et al. (2016),[5] who found a 36% prevalence rate in bulk milk in South Africa, and Sharma et al. (2015),[47] who reported

Table 3. **Detection of bla_{TEM} and bla_{CTX-M} in the selected *E. coli* isolates**

Bacterial isolates	bla_{TEM}	bla_{CTX-M}
8	–	–
9	+	–
21	+	–
28	+	–
34	–	+
40	+	–
47	–	–
52	+	+
56	+	–
58	–	–

–: Negative result; +: Positive results.

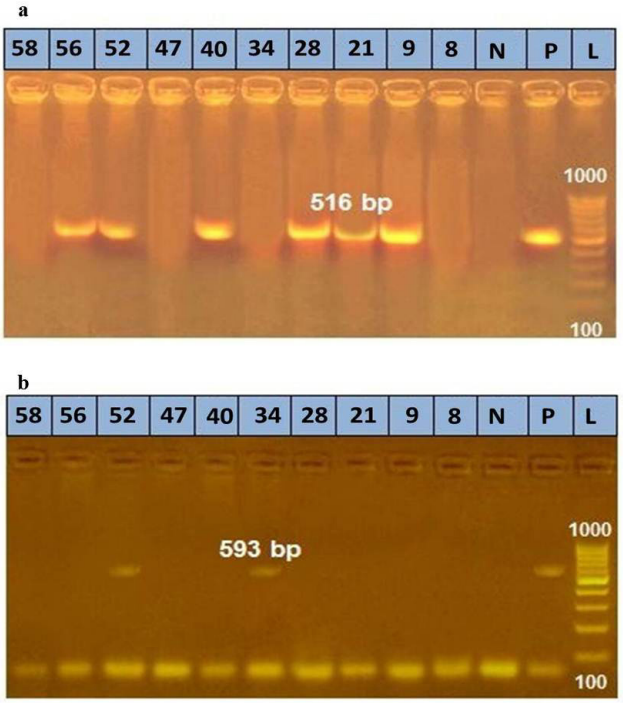

Fig. 3 **Gel image showing amplification of 516 (a) and 593 bp (b) product corresponding to bla_{TEM} and bla_{CTXM} gene, respectively of *E. coli* isolated from raw milk. (L): DNA ladder; (P): positive control; (N): negative control.**

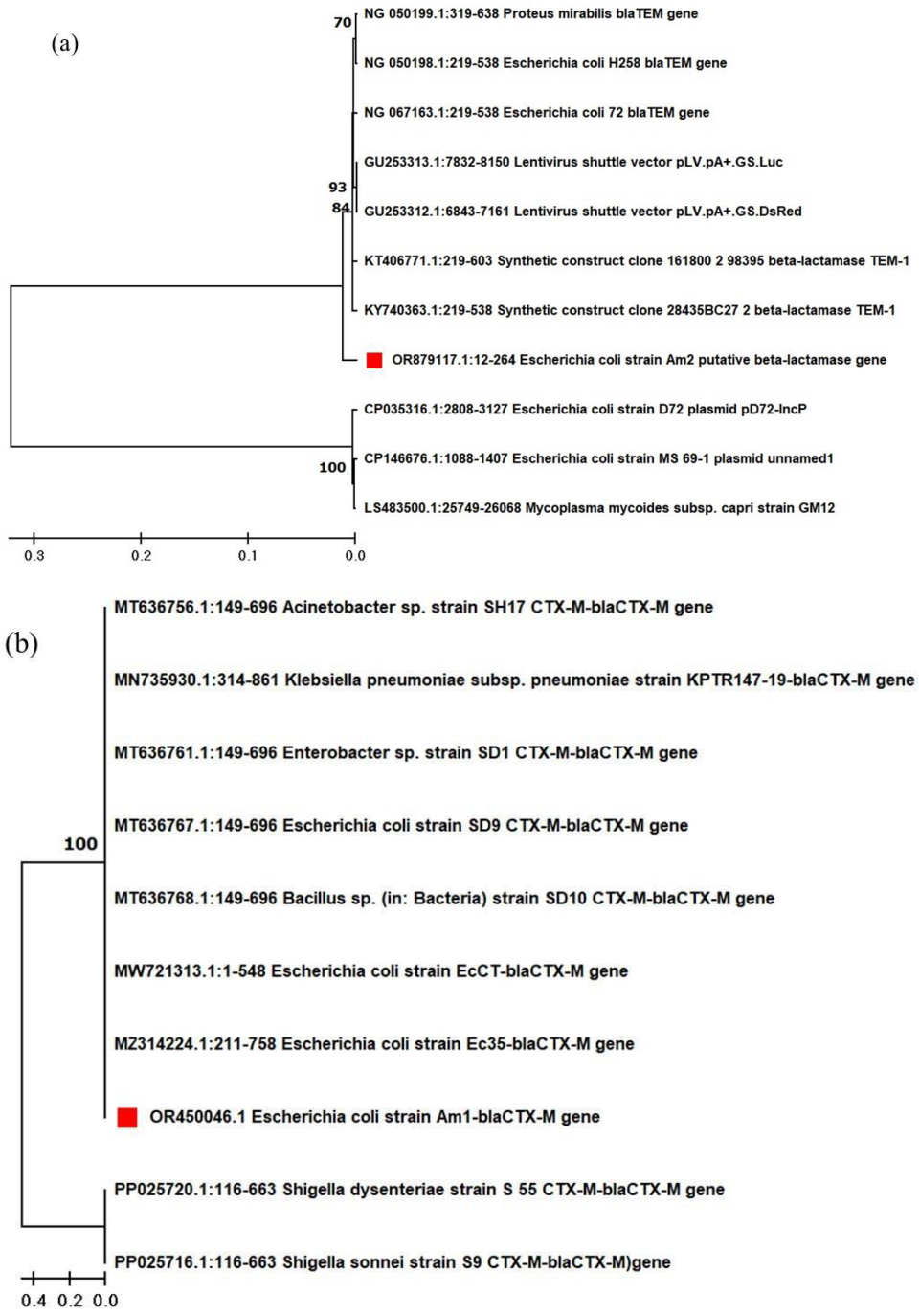

Fig. 4 **Phylogenetic analysis trees based on (a) *bla*$_{TEM}$ and (b) *bla*$_{CTX-M}$ gene sequences obtained from *E. coli*.**

35.6% occurrence rate of *E. coli* in raw milk in the Jaipur, capital of Rajasthan. Overall, many findings suggested that *E. coli* is a prevalent bacterium in raw milk obtained from dairy ranches in Northern China.[2,48] The high prevalence of *E. coli* in raw milk and dairy products is cause for concern because it is linked to fecal contamination and the danger of enteric pathogenic bacteria in food.[9] Virulence genes are important factors of *E. coli* resistance to certain antibiotics. Some *E. coli* strains carried specific virulence genes could be potentially harmful to public consumers.[3] In this study, the presence of two β-lactam resistance genes (*bla*$_{TEM}$ and *bla*$_{CTX-M}$) was examined in the most resistant *E. coli* by PCR test. Among ten β-lactam resistance *E. coli*, six isolates were positive to *blaTEM* gene, while, the *bla*$_{CTX-M}$ gene was detected in two isolates only. It was found that the same isolate had more than one beta-lactamases. Isolate No. 52 had the two tested genes (*bla*$_{TEM}$ and *bla*$_{CTX-M}$). This result confirmed that the *bla*$_{TEM}$ has become more prevalent than *bla*$_{CTX-M}$ in β-lactam resistance *E. coli*. This is in disagreement with several studies (Hagel et al., 2019)[49] who showed the *bla*$_{CTX-M}$ was more prevalent than *blaSHV* and *bla*$_{TEM}$ in β-lactam resistance in some Enterobacteriaceae species. Many works showed that the *bla*$_{CTX-M}$ gene was predominant in *E. coli*.[50] Several results suggested that the *bla*$_{CTX-M}$ gene spread is a worldwide pandemic. It was associated with the geographic area.[51] In Germany, the Netherlands, France, Japan, and the United Kingdom, *bla*$_{CTX-M}$ was the most important ESBL-related gene.[52] It has been found that the *bla*$_{TEM}$ and *bla*$_{CMY}$ genes were more common in *E. coli* than the *bla*$_{CTX-M}$, *bla*$_{CMY}$, *bla*$_{TEM}$,

and bla_{SHV} genes, according to several earlier investigations.[3,53] On the other hand, the resistance of the other *E. coli* isolates, which have not bla_{TEM} and bla_{CTX-M} genes may be due to another gene or/and non-ribosomal factors. It is necessary to conduct more studies to fully understand the genetic factors affecting antibiotic resistance using a whole-genome approach, which may help to explain the differences between many strains' phenotypes and genotypes.

Conclusion

In conclusion, the antibiotic resistance of *E. coli* isolated from raw milk in Egypt, kafr El-Sheikh Governorate was assessed for many samples. Our data indicated that *E. coli* isolates were widely present in raw milk samples in Egypt. More than 23.3% of the tested *E. coli* possessed one or more virulence genes, which showed potential pathogenic strains of *E. coli,* had different levels of antimicrobial resistance to different antibiotics except for gentamicin. Macrolides group of antibiotics especially erythromycin should not be a suitable treatment of dairy herds for mastitis by *E. coli* in Egypt. The majority of *E. coli* was multiple-antibiotic resistant and co-carried many virulence genes, and it may pose a great potential risk to public health. The possibility of transferring and transmitting resistance genes, between non-pathogenic and pathogenic *E. coli* isolates may be documented in further studies.

Conflict of Interest

The authors declare no conflict of interest.

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