Original article

**Genetic characterization of pathogenic** ***Escherichia coli* isolated from smallholding dairy buffaloes, their products and some environmental sources**

Mahmoud Elafify1, Basma Badawy2, Mohamed Sayed-Ahmed3, 4, Radwa Shata1, Marwa EL-Toukhy1, Mayada Gwida2\*

**1**Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

2Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

3Pharmacy Practice Unit, Department of Clinical Pharmacy, College of Pharmacy, Jazan University, Jizan, Saudi Arabia

4Department of Internal Medicine, Infectious and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

\*Corresponding author: Prof. Dr. Mayada Gwida

Email: [mayada.gwida@gmail.com](mailto:mayada.gwida@gmail.com), [mayada\_gwida@mans.edu.eg](mailto:mayada_gwida@mans.edu.eg)

Tel.:01003783173

Fax: 002(050)2200696

ORCID: https://orcid.org/0000-0001-6312-4703

**ABSTRACT**

So far, there has been scarce information regarding the circulation of *E. coli* in smallholding dairy buffaloes, and their products in Dakahlia governorate, Egypt. Hence, we aimed to characterize the pathogenic strains of *E. coli* that colonize smallholding dairy buffaloes, their product (including Kareish cheese) and to investigate the existence of the bacterium in some relevant environmental sources. The study included 150 samples including an equal sample sizes (*n* = 25) of raw milk, kareish cheese, water, feed, udder and rectal swabs. Phenotypic and genotypic procedures were used to characterize the bacterial population. Twenty-eight strains from the examined samples were confirmed as *E. coli* (18.7 %). The recovered isolates were categorized as enterohemorrhagic *E. coli* (*n* = 11), enteropathogenic *E. coli* (*n* = 9), enterotoxigenic *E. coli* (*n* = 5) and enteroinvasive *E. coli* (*n* = 3). All isolates showed resistance to erythromycin followed by oxacillin (89.28%), and nalidixic acid (67.9 %), while 96% of the recovered isolates displayed high sensitivity to imipenem. A great proportion of the recovered *E. coli* (67.9%) exhibited multidrug resistance (MDR). The majority of isolates (92.9%) harbored *stx1*either alone or in association with *stx2* which present in 60.7% (17/28); while *eae*A genes were detected in 50% among the recovered isolates in association with *stx1* or combined with *stx2* (*n* =11). Twelve isolates (42.86%) expressed all the examined beta lactamase encoding genes; while only 25% of the isolates harbored beta lactamase genes in common with *stx1, stx2* and *eae*A genes. Since pathogenic *E. coli* tested positive in the examined samples, it could pose a relevant threat and negative impact on animal as well as consumers health. The study could also emphasize the necessity of developing long-term strategies to assure dairy food safety.

**Keywords**: Pathogenic *E. coli*, Smallholding buffaloes, Environment, Dairy products.

1. **INTRODUCTION**

Food industry has developed several new modalities for hygienic production of milk and dairy products. However, consumption of dairy products that are contaminated by pathogenic microorganisms can cause serious public health concern **(Tabaran et al., 2016)**. Of these microbes, *Escherichia coli* (*E. coli*) is considered a diverse group of Gram-negative bacteria that has multitudinous characteristics and commonly found in the intestinal tract of human and warm-blooded animals **(Gwida et al., 2020)**.

The bacterium is classified according to their virulence factors into enterotoxigenic *E*. *coli*(ETEC), enteroaggregative *E*. *coli*(EAEC), enteropathogenic *E*. *coli*(EPEC), enteroinvasive *E*. *coli*(EIEC), diffusely adherent *E*. *coli* (DAEC), and verocytotoxigenic *E*. *coli* (VTEC) **(Ribeiro et al., 2019)**.

Animals` secretions and excretions, food materials, water and environment are considered the main sources for most pathogenic *E. coli* particularly shiga toxin-producing *E. coli* (STEC) which constitute a risk for food safety and have a relevant public health significance due to their life threatening diseases (**Elafify et al., 2020**). Although STEC have a lower occurrence than other zoonotic pathogens, they can provoke fatal health hazards such as hemorrhagic colitis, hemolytic uremic syndrome (HUS), renal failure and brain damage particularly in young children and immune-compromised patients (**EL Mahmoudy et al., 2021)**.

The pathogenicity of STEC is attributed to the presence of shiga-toxin type 1 and 2, Pathogenicity Island, LEE (Locus of Enterocyte Effacement), besides the presence of adherence factor called intimine encoded by *eae* gene. This gene can help the bacteria to form attaching and effacing lesions to intestinal epithelial cells and has a key role in the development of severe diseases **(Tabaran et al., 2016)**.

The improper and un-controlled usage of antibiotics, particularly third and fourth generations of cephalosporin for the medical management of various animal ailments or being used as growth promoters in food producing animals, can likely lead to the emergence of resistance among microbes makeing the antimicrobial therapies ineffective and posing a serious public health threat **(Gwida et al., 2020).**

Although there have been various studies in Egypt characterizing *E. coli* from different samples (**Elafify et al., 2020; Gwida et al., 2020; EL Mahmoudy et al., 2021),** there is still paucity information regarding the circulation of pathogenic *E. coli* particularly in smallholding dairy buffaloes, their products as well as some environmental sources. Therefore, the study was planned to characterize pathogenic *E. coli* that colonize smallholding dairy buffaloes, their product (including Kareish cheese) and to investigate the existence of the bacterium in some environmental sources owing to elucidate whether these different sources could be a potential vehicle for the transmission of pathogenic *E. coli* to humans in the selected communities.

1. **MATERIALS AND METHODS**
   1. **Animals and samples collection**

The study comprised twenty five apparently healthy household dairy buffaloes raised at different localities in Dakahlia governorate, Egypt during December 2020 to July 2021. Three different types of samples were collected from each animal. The first type of samples comprised composite milk, udder swabs and rectal swabs (25 samples each); the second set of samples included animal`s dairy products (Kareish cheese; *n* = 25); while the third set of samples involved environmental samples (i.e. water, and feed samples; 25 samples each). The study follows the principles and specific ethical guidelines presented by Mansoura University. An informed consent was also obtained from all owners prior to samples collection.

In brief, the teat ends were washed and swabbed with 70% ethyl alcohol then ten ml of mid streams milk were collected into sterile falcon tube. Teat swabs were then assembled from the four quarters by rotating sterilized cotton swabs on the teat barrel in a downward motion, from the base of the udder till the teat end as described previously **(Piccinini et al., 2009)**. Rectal swabs were collected by inserting sterilized swab moistened with tryptone soya broth (TSB; Oxoid, Hampshire, UK) in the anal opening and rotating the swab gently then all the collected swabs stored in the original swab collection container with 10 mL TSB.

For kareish cheese, 50 g were collected from each relevant animal’s milk and transferred in cooler to the laboratory. For the environmental samples, twenty five water samples were collected from the watering troughs in front of animals by using 50 ml capacity sterile syringe as well as 25 samples of dry feed concentrate (50gm) were gathered from animal fodders by using a sterile plastic bags. All collected samples were transferred under aseptic to the lab for further processing.

* 1. **Samples preparation**

Twenty five ml or grams of raw milk, cheese samples and feed concentrate were homogenized in 225 ml of TSB broth for one minute using a sterile stomacher. The collected udder and rectal swabs were inserted into 10 mL TSB; while for water samples ten mL was mixed well with 90 mL of TSB. All the inoculated broths were incubated at 37o C / 18-24 hours.

* 1. **Isolation and identification of *Escherichia coli* from the collected samples**

A loopful from the enriched broth was spread onto Eosin methylene blue (EMB, Oxoid, Hampshire, England) agar plate as a selective media and incubated at 37o C/ 18- 24 hours. Suspected isolates of *E*. coli which appeared as green metallic sheen were picked up and purified on EMB plate and incubated at 37°C for 18 h and subjected to further analysis which was carried out according to the methods described by **MacFaddin (2000)**.

* 1. **Serotyping of *E. coli* strains**

The identified strains were serotyped by slide agglutination test using rapid diagnostic *E. coli* antisera sets (Denka SeikenCo., Tokyo, Japan)**.** The procedures were carried out based on the manufacturer’s instructions **(Kok et al., 1996).**

* 1. **Antimicrobial susceptibility testing**

Susceptibility of  *E. coli* strains was evaluated by disk diffusion method using various antibiotics that represented eleven groups including [aminoglycoside](https://en.wikipedia.org/wiki/Aminoglycoside) (amikacin AK, 30 µg; gentamicin G,10 µg ; kanamycin K, 30 µg), penicillins (ampicillin AM,10 µg; oxacillin OX,1 µg ); lincosamide (clindamycin CL,10 µg ); 1st generation of cephalosporins (cefazolin CZ,30 µg); 3rd generation cephalosporins (cefotaxime CF,30 µg); fluoroquinolones (ciprofloxacin CP,5 µg); macrolides (erythromycin E, 15 µg); quinolones (nalidixic acid NA, 30 µg); carbapenems (imipnem IPM, 10 µ); tetracyclines (tetracycline T, 30 µg); sulfonamides (trimethoprim sulfamethoxaz-ole SXT, 25 µg). The interpretation follows the guidelines of Clinical and Laboratory Standards Institute guidelines **(CLSI, 2017).** The Multi Antibiotic Resistance (MAR) index for each strain was calculated according to **Singh et al. (2010)**.

* 1. **Genetic characterization of virulence and beta-lactamase encoding genes in *E. coli* strains**

GeneJET Genomic DNA Purification Kit was used to extract genomic DNA from the purified strains

(Thermo Fisher Scientific; US) according to manufacture instruction**.** Multiplex PCR was performed in a total volume of 25μl to determine shiga toxins (stx1&stx2) and intimin (eaeA) genes. The used primer sequences and their amplicone sizes were illustrated in **Table 1**. PCR reaction was conducted as described previously by **Paton and Paton (1998)**. The amplified products were exposed to ethidium bromide stained 2 % agarose gel electrophoresis and visualized by Gel Imaging System.

Beta-lactamase encoding genes were determined using multiplex PCR. Primers for *blaOXA*, *blaCTX-M1, and blaTEM* genes of E. coli were selected according to published references in Table 1. The reaction was performed in a final volume of 50 μl mixture containing 25 μl 2 × Taq master mix, 1 µl of both forward and reverse primer (10 pmol), 5 μl of purified DNA and 18 µl PCR water with the following cycling condition: initial denaturation at 94 °C for 10 min; 30 cycles of 30 sec at 94 °C for, 35 sec at 61 °C for 1 min at 72 °C; and ﬁnal extension at 72 °C for 9 min. The amplified products were separated in a 2.5 % stained agarose gel and visualized under an ultraviolet trans- illuminator.

1. **RESULTS**

The number of positive *E. coli* samples across the examined udder swabs, rectal swabs, composite milk and Kareish cheese were 7 (28%), 7 (28%), 3 (12%) and 6 (24%), respectively **(Table 2)**. Both the tested feed and drinking water samples were negative for *E. coli*. Out of the recovered positive samples (n =23), 28 *E. coli* isolates were obtained which were serotyped into four different categories including EHEC (11/28; 39.28 %), EPEC (9/28; 32.14 %), ETEC (5/28; 17.86 %), and EIEC (3/28; 10.71%) **(Table 3)**. The most prevalent serovar observed was O26:H11 (7/28; 25%) which was recovered mostly from Kareish cheese.

The great majority of isolates (92.9%; 26/28) harbored *stx1* as indicated by the amplification of 180 bp either alone or in association with *stx2* which present in 60.7% (17/28). The *eae*A genes were detected in 50% (14/28) among the recovered isolates in association with *stx1* or combined withstx2 (n =11). Only 11 isolates (39.3%) harbored the three virulence genes **(Table 4, Figure 1)**.The majority of *E. coli* isolates (78.57%, 22/28) harbored gene encoding *blaTEM,* while 57.14% (16/28) carried gene encoding *blaCTX-M1* and 50%(14/28) carried *blaOXA*. Twelve isolates (42.86%) expressed all the examined beta lactamase encoding genes which were commonly observed in Kareish cheese; while only 25% (7/28) of the isolates harbored all tested beta lactamase genes in common with *stx1, stx2* and *eae*A genes **(Figure 2)**.

A great proportion of the recovered *E. coli* (67.9%, 19/28) exhibited multidrug resistance. In the context, all isolates were resistant to erythromycin followed by oxacillin (89.28%), nalidixic acid (67.85%), clindamycin (53.57%), ampicillin (53.57%), kanamycin (50%), cefotaxime (42.86%) and ciprofloxacin (42.86%); while 96% of the recovered strains displayed high sensitivity toimipenem followed by gentamicin (92.9%) and cefazolin (85.7%) **(Figure 3)**.

**4. DISCUSSION**

The present study was performed to full fill the gap and provide updated information regarding the distribution of pathogenic and MDR *E. coli* in smallholding dairy buffaloes and their products as well as in some environmental sources collected from the animal surroundings. It is known that smallholding livestock are a critical source of red meat, milk, and milk products in the majority of developing countries including Egypt. Hence, diseases of dairy animals that are caused by bacterial pathogens including *E. coli* can not only affect animals and or milk production, but also pose a potential health hazard **(Gwida et al., 2020)**.

In the present study, the recovery rate of *E. coli* from the udder and rectal swabs was high. Our findings regarding the former set were higher than those previously described in several studies (**Palaha et al., 2012,** 17.03%; **Nalband et al., 2019,** 16.09%); however being lower than others (**Vanitha et al., 2018,** 16.67%**; Byomi et al., 2019,** 16.67%**; Fahim et al., 2019,** 14%**)**. The presence of E. coli on the skin of 28% of buffaloes’ teats poses a serious threat to the animals and the consumer health. It has also been reported that teat skin could act as the main reservoir for different pathogens which capable of infecting milk during milking (**Fahim et al., 2019**). In the same trend, variable detection rates from fecal samples were reported recently (**Ribeiro et al., 2019,** 80%**; Nalband et al*.,* 2019,** 59.09%**; EL Mahmoudy et al., 2021**, 20%). It has been well established that cattle are the main natural reservoirs of *E. coli* which inhabit their hindgut with the subsequent shedding in the feces **(Shridhar et al., 2017).**

Our findings showed that the recovery of *E. coli* from composite milk and Kariesh cheese was 12% and 24%, respectively. A low detection rate of *E. coli* was reported in raw milk (**Vanitha et al., 2018,** 8.8%; **Fahim et al., 2019,** 7.75**),** respectively; while high detection rates were also reported in raw milk samples (**El Nahas et al., 2015,** 55%**; Byomi et al., 2019,** 21.74%**;Ribeiro et al., 2019,** 66.67%). On the other side, several researchers have reported high rates of *E. coli* recovery from Kareish cheese (**Virpari et al., 2013, 28%; El Nahas et al., 2015, 50%; Amin et al., 2017, 73%; Maria et al., 2018, 40%)**. Comparing our results with other previous studies is difficult due to the diversity of sampling and methods used for screening. The previously discussed attribution of incidence in both udder and rectal swabs along with the lack of sanitary and hygienic measures that aid in the post-fecal contamination of bedding, teats and buckets used for manual milking of these household cattle result in the reasonable fade of the existence of *E. coli* in the freshly drawn raw milk. While in Kareish cheese several additional technical factors might be involved; such as the utensils used for milk storage till processing **(Ombarak and Elbagory, 2015)**, the floor, the brine, the cheese cloth, the packaging material, the curd cutting knives, the air in the production room, the cheese vats and last but not least the cheese maker or handler **(Sharaf et al., 2014**). Here, *E. coli* was not recovered from the examined water samples which complied with recent report **(Beauvais et al., 2018).** Nonetheless, variable detection rates of *E. coli* in other studies were recorded by several researchers in water samples **(Joris et al., 2013,** 8.7%**; Vanitha et al., 2018,** 11.11%**; Byomi et al., 2019,** 55.56%**; Fahim et al., 2019,** 20%**)**.

In the present study, eleven strains (39.3%) were confirmed as EHEC. Similar other reports have presumed that cattle predominantly harbored EHEC strains especially O26:H11 and O111:H2 **(Jenkins et al., 2003; Oliver et al., 2005)**. However, a low incidence of EHEC (4.7%) was recorded in fecal samples (**Atnafie et al., 2017)**, while **Beauvais et al. (2018)** detected EHEC in 95% of the tested fecal samples. **Kagambega et al. (2012)** also identified *E. coli* in the tested 304 fecal samples as 112 STEC (37%), 25 EPEC (8%) 12 ETEC (4%) with zero incidence of EIEC; while **Ribeiro et al. (2019)** reported a high percentage (6.25%) of EPEC that were derived from 10 rectal fecal samples and (16.25%) of STEC which were recovered from 26 rectal fecal samples.

On the level of dairy samples, **Osman et al. (2012)** recovered seven isolates of *E. coli* from mastitic milk, of which 77.4% were belonged to four different O serogroups (O26, O111, O86 and O127). On the other hand, **El Nahas et al. (2015)** recorded the occurrence of EHEC, EIEC, and EPEC in raw milk samples and Kareish samples at the following percentages (36.36%, 27.27%, and 13.64%, respectively) for raw milk samples, and 10%, 15% & 25%, respectively for Kareish samples. In the Mexican fresh cheese, **Maria et al. (2018)** detected 6% of (EPEC) and 5% of (ETEC). It was pretty obvious that EHEC could survive for long periods in contaminated farm environment, in terms of dissemination in between animals and the existence outside their cattle reservoirs; as these EHEC were adapted to survive in feed bunks, soil, feces and particularly dust which posed the risk of successive transmission and re-infection of other cattle in the herd **(Joris et al., 2013).**

Here, the great majority of isolates (92.9%) harbored *stx1* either alone or in association with *stx2* which present in 60.7% of the isolates; while 39.28% of the isolates harbored *Stx* genes and intimin. On the other side, 42.86% of *E. coli* isolates expressed all the examined beta lactamase encoding genes and only 25% of the isolates harbored all tested beta lactamase genes in common with *stx1, stx2* and *eae*A genes. These findings could indicate a potential impact of buffaloes or their products on the human health especially in rural areas where there are limited medical care. **Tabaranet al. (2016)** detected (18.62%) virulent genes particularly the *Stx genes* in raw milk and unpasteurized traditional cheese in Romania. Low incidences for shiga toxins were obtained by **Nalband et al. (2019)** (2.64%) and **Ribeiro et al. (2019)** (0.98%) from mastitic and raw milk samples, respectively. On the other hand, fecal samples showed positive results for virulent genes that were identified by **Shridhar et al. (2017)**; as *Stx1* only was found in 93 isolates (48.4%) while *Stx2* gene only was detected in 43 samples (22.4%), but the incidence of both of them together was (29.2%). Furthermore, **Ribeiro et al. (2019)** recovered 13 /28 isolates, of which 3 (2.94%) were positive for *Stx1,* 6 (5.88%) for *Stx2* and 4 (3.92%) for *eae* gene.

It was previously reported that hemolytic uremic syndrome cases in human was attributed to the presence STEC strains harbouring*stx2* gene as it is the most important virulence factor (**Ribeiro et al., 2019).**Intiman is another virulence factor it is thought to enhance the virulence of STEC**.** Not only the hazards of STEC has become a worrying issue but its increased resistance against antibiotics that highlights on a world-wide crises which is the human antimicrobial resistance resulted from the mal use of antimicrobial agents in food producing animals **(Da Costa et al., 2008)**; as the resistance genes are transferred leading to MDR posing a threat in the continuity of spreading this phenomenon specially along with the inappropriate use of antibiotics as growth promoters in developing countries.

Our findings illustrated that a great proportion of the recovered *E. coli* strains (67.9%) exhibited multidrug resistance, where the highest (100%) resistance was given to erythromycin and the lowest one was to impipenem. These results could be attributed to the fact that using these antibiotics has been considered the first choice of treatment for diseased cattle especially by the owners with limited income. Our pattern of resistance was supported by other studies which investigated the MDR of *E. coli* isolates; **Bonyadian et al. (2014)** detected lower resistance to nalidixic acid 56% and ampicillin 23.4% but they recorded higher resistance to both gentamycin 30% and sulphamethoxazol 28%. While **Tabaran et al. (2016)** recorded a nearly similar percentage 7.4% of resistance to gentamicin.

1. **CONCLUSION**

The results obtained from this work highlighted the potential occurrence of pathogenic and multidrug resistance *E. coli* strains in udder and rectal swabs and the milk of smallholding dairy buffaloes as well as in Kareish cheese which pose a relevant threat and negative impact on animal as well as consumer health. The study could emphasize the necessity of developing long-term strategies to assure dairy food safety. Strict hygienic preventive measures in smallholding buffaloes are needed to inhibit the bacterial growth and to improve the health of the animals as well as the wholesomeness of the milk.

**Conflicts of interest: No conflicts of interest**

**Author’s contributions**: MEL, BB and MG designed the research activity. RS and MELT helped in samples collection. MEL, BB, MS, RS and MELT conducted experiments. MEL and MG wrote the manuscript. All authors read and approved the final version of the manuscript for publication.

**Ethics approval:** The samples were collected based on the consent of their owners to participate in the current study. The study was complied with the standard guidelines and the ethical approval of Mansoura University and its committee (Code R/70).

**Consent to participate**

All authors participated in the research.

**Consent for publication**

All authors read and approved the final manuscript.

**REFERENCES**

Amin, W.F., Ahmed, E.H., Embarak, M.S., Abo-Shama, U.H., Thabit, A.G., Ismail, S.Y., 2017. Molecular Detection of Enterotoxigenic E. coli in raw Milk and Milk Products. Int J Curr Microbiol, 6 (11): 856–864. https://doi.org/10.20546/ijcmas.2017.611.100

[Atnafie](https://pubmed.ncbi.nlm.nih.gov/?term=Atnafie+B&cauthor_id=28122502), B.,  [Paulos](https://pubmed.ncbi.nlm.nih.gov/?term=Paulos+D&cauthor_id=28122502), D., [Abera](https://pubmed.ncbi.nlm.nih.gov/?term=Abera+M&cauthor_id=28122502), M.,  [Tefera](https://pubmed.ncbi.nlm.nih.gov/?term=Tefera+G&cauthor_id=28122502), G.,  [Hailu](https://pubmed.ncbi.nlm.nih.gov/?term=Hailu+D&cauthor_id=28122502), D.,  [Kasaye](https://pubmed.ncbi.nlm.nih.gov/?term=Kasaye+S&cauthor_id=28122502), S., [Amenu](https://pubmed.ncbi.nlm.nih.gov/?term=Amenu+K&cauthor_id=28122502), K.,  2017. Occurrence of Escherichia coli O157:H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. BMC Microbiol, 17(1):24. DOI 10.1186/s12866-017-0938-1

Beauvais, W., Gart, E., Bea, M., Blanco, A., Wilsey, J., Whinney, K., Bryan, L., Krath, M., Yang, C., Alvarez, D., Paudyal, S., Bryan, K., Stewart, S., Cook, P., Lahodny, G., Baumgarten, K., Gautam, R., Nightingale, K., Lawhon, S., Pinedo, P., Ivane, R., 2018.The prevalence of *Escherichia coli* O157:H7 fecal shedding in feedlot pens is affected by the water-to-cattle ratio: A randomized controlled trial. PLoS ONE, 13(2): e0192149.<https://doi.org/10.1371/journal.pone.0192149>

Bonyadian, M., Moshtaghi, H., Taheri, M., 2014. Molecular characterization and antibiotic resistance of enterotoxigenic and enteroaggregative Escherichia coli from raw milk and unpasteurized cheeses. Vet Res Forum, 5 (1): 29 – 34.

Byomi, A., Zidan, S., Hadad, G., Sakr, M., Sakr, E.S., 2020.Epidemiology of Mastitis in Dairy Cattle with Special Reference to Some Associated Risk Factors. J Vet Res, 2(1):35– 46.

Clinical and Laboratory Standards Institute (CLSI). 2015. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 12th ed. Wayne, PA: Clinical and Laboratory Standards Institute.

Da Costa, P.M., Bica, A., Vos-Pires, P., Bernardo, F., 2008. Effects of antimicrobial treatment on the selection of resistant Escherichia coli in broiler fecal flora. Microb Drug Resist, 14: 299–306.<https://doi.org/10.1089/mdr.2008.0859>

Elafify, M., Khalifa, H.O., Al-Ashmawy, M., Elsherbini, M., Abd El Latif, A., Okanda, T., Matsumoto, T., Koseki, S., Abdelkhalek, A., 2020. Prevalence and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* in milk and dairy products in Egypt.  J Environ Sci Health part B, 55 (3):265– 272.<https://doi.org/10.1080/03601234.2019.1686312>

EL Mahmoudy, S., Elgohary, A., Mohamed, A., Hassan, H., Gwida, M., 2021. Characterization of Shiga toxin-producing Escherichia coli isolated from cattle and their contacts. Mansoura Vet Med J, 22: 13–19.Doi10.21608/MVMJ.2021.57994.1026.

El Nahas, A., Mohamed, H., El Barbary, H., 2015. Incidence of *E. coli* in raw milk and its products. BVMJ, 29(1):112– 117.

Fahim, K., Ismael, E., Khalefab, H., Faragcand, H., Hamzad, D., 2019. Isolation and characterization of E. coli strains causing intramammary infections from dairy animals and wild birds. Int J Vet Sci Med, 7:61–70 <https://doi.org/10.1080/23144599.2019.1691378>.

Gwida, M., Awad, A., El-Ashker, M., Hotzel, H., Monecke, S., Ehricht, R., Müller, E., Reißig, A., Barth, S.A., Berens, C., Braun, S.D., 2020. Microarray-based detection of resistance and virulence factors in commensal Escherichia coli from livestock and farmers in Egypt. Vet Microbiol, 240: 108539.<https://doi.org/10.1016/j.vetmic.2019.108539>

Jenkins, C., Pearce, M.C., Smith, A.W., Knight, H.I., Shaw, D.J., Cheasty, T., Foster, G., Gunn, G.J., Dougan, G., Smith, H.R., Frankel, G., 2003.Detection of Escherichia coli serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques. Lett Appl Microbiol, 37:207–212. Doi:10.1046/j.1472-765X.2003.01379.x

Joris, M., Verstraete, K., Reuand, D., Zutter, L., 2013. Longitudinal follow up of the persistence and dissemination of EHEC on cattle farms in Belgium. Food borne Pathog Dis, 4. <https://doi.org/10.1089/fpd.2012.1277>.

Kagambega, A., Martikainen, O., Siitonen, A., Traore, A.S., Barro, N., Haukka, K., 2021. Prevalence of diarrheagenic Escherichia coli virulence genesin the feces of slaughtered cattle, chickens, and pigs in Burkina Faso. Microbiol, 1(3): 276–284. <https://doi.org/10.1002/mbo3.30>

Kok, T., Worswich, D., Gowans, E., 1996. Some Serological Techniques for Microbial and Viral Infections. In Practical Medical Microbiology; Collee J, Fraser A, Marmion B, Simmons A. eds., 14th ed.; Edinburgh, UK: Churchill Livingstone, 179–204.

MacFaddin, J.F., 2000. Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

Maria, C., Cadena-Ramirez, A., Tellez-Jurado, A., Gomez-Aldapa, C.A., Rangel-Vargas, E., Chavez-Urbiola, E., Castro-Rosas, J., 2018. Presence of Multidrug Resistance Shiga Toxin – Producing Escherichia coli, Enteropathogenic Escherichia coli and Enterotoxigenic Escherichia coli in Fresh Cheeses from Local Retail Markets in Mexico. J Food Prot, 81:1748 – 1754. <https://doi.org/10.1089/fpd.2015.2065>

Nalband ,S.M., Kolhe, R.P., Deshpande, P.D., Jadhav, S.N., Gandhale, D.G., Muglikar, D.M., Kolhe, S.R., Bhave ,S.S., Jagtap, U.V., Dhandore, C.V., 2020. Characterization of Escherichia coli Isolated from Bovine Subclinical Mastitis for Virulence Genes, Phylogenetic Groups and ESBL Production. Indian J Anim Res, 1:54 (10).DOI: 10.18805/ijar.B-3883

Ogutu, J., Zhang, Q., Huang, Y., Yan, H., Su , L., Gao, B., Zhang, W., Zhao, J., Cai, W., Li ,W., Zhao, H., Chen, Y., Song, W., Chen, X., Fu, Y., Zhang, F., 2015. Development of a multiplex PCR system and its application in detection of *blaSHV, blaTEM*, blaCTX*-M1*, *blaCTX-M-9* and *blaOXA-1* group genes in clinical *Klebsiellapneumoniae* and *Escherichia coli* strains. J Antibiot, 68: 725– 733. Doi:10.1038/ja.2015.68.

Oliver, S.P., Jayarao, B.M., Almeida, R.A., 2005. Food borne pathogens in milk and the dairy farm environment: Food safety and public health implications. Food borne Pathog Dis, 2: 115–129. <https://doi.org/10.1089/fpd.2005.2.115>

Ombarak, R., Elbagory, A.M., 2015. Bacteriological Quality and Safety of Raw Cow’s and Buffalo’s Milk Sold in Menoufia Governorate, Egypt. Minufiya Vet J, 9: 101–113.

[Osman](https://pubmed.ncbi.nlm.nih.gov/?term=Osman+KM&cauthor_id=22229945), K.M.,  [Mustafa](https://pubmed.ncbi.nlm.nih.gov/?term=Mustafa+AM&cauthor_id=22229945), A.M., [Aly](https://pubmed.ncbi.nlm.nih.gov/?term=Aly+MA&cauthor_id=22229945), M.A., [Abd Elhamed](https://pubmed.ncbi.nlm.nih.gov/?term=AbdElhamed+GS&cauthor_id=22229945), G.S., 2012. Serotypes, virulence genes, and intimin types of shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli isolated from mastitic milk relevant to human health in Egypt. Vector Borne Zoonotic Dis, 12: 297–305. DOI: 10.1089/vbz.2010.0257.

Palaha, R., Chaudhary, N., Kumar, H., 2012. Detection of Escherichia coli from the udder of the dairy farm buffaloes in Phagwara region, Punjab, India. Vet World, 5: 522–525. DOI:10.5455/vetworld.2012.522-525

Paton, A.W., Paton, J.C., 1998. Detection and characterization of Shiga toxigenic *E. coli* by using multiplex PCR Assays for *stx1, stx2, eaeA.Enterohemorrhagic* *E. coli* hylA, rfbo111, and rfbO157. J Clin Microbiol, 36:598–602.

Piccinini, R., Cesaris, L., Dapra, V., 2009. The role of teat skin contamination in the epidemiology of Staphylococcus aureus intramammary infections. Res J Dairy Sci, 76: 36–41.DOI: <https://doi.org/10.1017/S0022029908003671>

Perez, F., Jones, H., Hanson, N., Geyer, C., 2007. Global challenge of multidrug-resistant *Acinetobacterbaumannii.* Antimicrob Agents Chemother, 51:3471–3484.  <https://doi.org/10.1128/AAC.01464-06>

Ribeiro, L.F., Mayhara, M.C., Barbosa, O., Pinto, F.R., Lavezzo, L.F., Rossi, G.M., Almeida, H.M., Amaral, L.A., 2019. Diarrheagenic *Escherichia Coli* in Raw Milk, Water, and Cattle Feces in Non-Technified Dairy Farms. Med Vet, 20.Doi: 10.1590/1089-6891v20e-47449.

Sharaf, O.M., Ibrahim, G., Abd El-Khalik, D., Ibrahim, A., Tawfek, N.F., Effat, B., 2014. Prevalence of some pathogenic microorganisms in factories Domiati, Feta cheeses and UHT milk in relation to public health sold under market conditions in Cairo. Int. J. Chem Tech Res, 6(5): 2807–2814.

Shridhar, P., Slepker, C., Noll, L., Shi, X., Nagaraja, T.G., Bai, J., 2017. Shiga Toxin Subtypes of Non-O157 Escherichia coli Serogroups isolated from cattle feces. Front Cell Infect Microbiol, 7.  <https://doi.org/10.3389/fcimb.2017.00121>

Singh, A., Yadav, S., Singh, S., Bharti, P., 2010. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Int Food Res J, 43: 2027–2030. <https://doi.org/10.1016/j.foodres.2010.06.001>

Tabaran, A., Mihaiu, M., Tabaran, F., Colobatiu, L., Reget, O., Borzan, M., Dan, S., 2016. Characterization of virulence and antibiotic resistance genes in veterotoxigeneic and enterotoxigenic E. coli isolated from raw milk and unpasteurized traditional cheeses in Romania. Folia Microbiol, 62 (2):145–150.

### Vanitha, H.D., Sethulekshmi, C., Latha, C., 2018. An epidemiological investigation on occurrence of enterohemorrhagic *Escherichia coli* in raw milk. Vet World, [11 (8). 1164–1170](http://www.veterinaryworld.org/Vol.11/August-2018/22.html). Doi: [10.14202/vetworld.2018.1164-1170](https://dx.doi.org/10.14202%2Fvetworld.2018.1164-1170)

Virpari, P., Nayak, J., Brahmbhatt, M., Thaker, H., 2013. Study on isolation, molecular detection of virulence gene and antibiotic sensitivity pattern of *Escherichia coli* isolated from milk and milk products. Vet World, 6: 541–545. Doi:10.5455/vetworld.2013.541-545.

**Table (1): Primer sequences used for *E. coli* detection genes.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer** | **Oligonucleotide sequence (5′ → 3′)** | **Product size (bp)** | **References** |
| *stx1* (F) | 5′ ATAAATCGCCATTCGTTGACTAC ′3 | 180 | **Paton and Paton(1998)** |
| *Stx1* (R) | 5′ AGAACGCCCACTGAGATCATC ′3 |
| *Stx2* (F) | 5′ GGCACTGTCTGAAACTGCTCC ′3 | 255 |
| *Stx2* (R) | 5′ TCGCCAGTTATCTGACATTCTG ′3 |
| eaeA (F) | 5′ GACCCGGCACAAGCATAAGC ′3 | 384 |
| eaeA (R) | 5′ CCACCTGCAGCAACAAGAGG ′3 |
| *blaOXA*(F) | 5′ GGCACCAGATTCAACTTTCAAG ′3 | 654 | **Perez et al. (2007)** |
| *blaOXA*(R) | 5′ GACCCCAAGTTTCCTGTAAGTG ′3 |
| *blaCTX-M1* (F) | 5′ TTAGGAAGTGTGCCGCTGTA ′3 | 655 | **Ogutu et al. (2015)** |
| *blaCTX-M1* (R) | 5′CGGTTTTATCCCCCACAAC ′3 |
| *blaTEM*(F) | 5′ CATTTCCGTGTCGCCCTTATTC ′3 | 800 | **Perez et al. (2007)** |
| *blaTEM*(R) | 5′ CGTTCATCCATAGTTGCCTGAC ′3 |

**Table (2): Frequent distribution of *E. coli* in examined dairy and environmental samples.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source of Samples** | **No. of examined sample** | **No. of positive samples** | **No. of recovered isolates** | **% of positive samples** |
| Raw Milk | 25 | 3 | 6 | 12 |
| Kareish Cheese | 25 | 6 | 8 | 24 |
| Water | 25 | 0 | 0 | 0 |
| Feed | 25 | 0 | 0 | 0 |
| Udder Swab | 25 | 7 | 7 | 28 |
| Rectal Swab | 25 | 7 | 7 | 28 |
| Total | 150 | 23 | 28 | 15.3 |

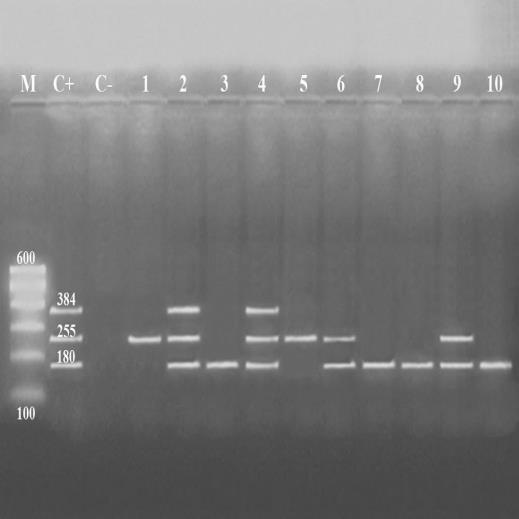
**Table (3**): **Sources and serotypes of *E. coli* isolated from different sources.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Pathotypes** | **Serotypes** | **Number of *E. coli* positive strains** | | | | | | |
| **Raw milk** | **Kareish cheese** | **Water** | **Feed** | **Udder swab** | **Rectal swab** | **Total number** |
| EPEC | O114:H4 |  |  |  |  | 1 |  | 1 |
|  | O119:H6 | 1 | 1 |  |  |  |  | 2 |
|  | O121:H7 |  |  |  |  | 1 |  | 1 |
|  | O55:H7 | 1 |  |  |  |  | 1 | 2 |
|  | O17:H18 | 1 |  |  |  |  |  | 1 |
|  | O146:H21 | 1 | 1 |  |  |  |  | 2 |
| ETEC | O128:H2 | 1 | 1 |  |  | 2 | 1 | 5 |
| EIEC | O159 |  |  |  |  | 1 | 2 | 3 |
| EHEC | O26:H11 | 1 | 4 |  |  | 1 | 1 | 7 |
|  | O111:H2 |  | 1 |  |  | 1 | 2 | 4 |
| Total |  | 6 | 8 | 0 | 0 | 7 | 7 | 28 |

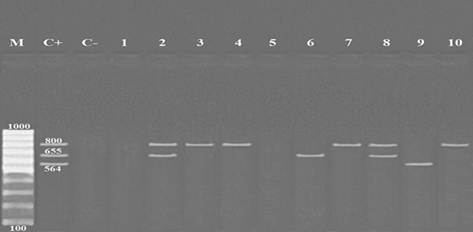
**Table (4): Virulence and antimicrobial characterization of *E. coli* isolates (n=28 isolates).**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Type of sample** | ***E. coli* strains** | **Antimicrobial resistance profile** | **MAR index** | **Virulence genes** | | | **Beta-lactamase genes** | | |
| ***Stx1*** | ***Stx2*** | ***eae*A** | ***blaOXA*** | ***blaCTXM1*** | ***blaTEM*** |
| 1 | Raw milk | O26 : H11 | NA, CL, K, CP, T, SXT, CF, CZ, G, AK,OX, IPM, E, AM | 1 | + | + | + | + | + | + |
| 2 | Kariesh cheese | O26 : H11 | CZ,K, E, NA, AM, CF, T, AK, CL, SXT, G, OX, CP | 0.928 | + | + | + | + | + | + |
| 3 | Kariesh cheese | O26 : H11 | SXT, AM,E, CL, K, CF, NA, T, AK, CZ, OX, CP | 0.857 | + | + | + | + | + | + |
| 4 | Kariesh cheese | O26 : H11 | CP, SXT, OX, NA, AM, K, CF, T, E, CL, AK | 0.786 | + | + | + | + | + | + |
| 5 | Kariesh cheese | O26 : H11 | CP ,E, NA, K, CL, CF, OX, AM | 0.571 | + | + | + | + | + | + |
| 6 | Udder swab | O26 : H11 | E, AM, K, NA, OX, CL | 0.428 | + | + | + | + | + | + |
| 7 | Rectal swab | O26 : H11 | OX, E | 0.143 | + | + | + | + | + | + |
| 8 | Raw Milk | O128 : H2 | CZ, CL, AM, CF, E, CP, T, AK, K, SXT, OX, NA | 0.857 | + |  | + | + | + | + |
| 9 | Kariesh cheese | O128 : H2 | CP, NA, AK , CL, AM, OX, K, E, CF, T | 0.714 | + |  | + | + | + | + |
| 10 | Udder swab | O128 : H2 | AM, E, NA, CL, CF, K, T, AK,OX,CP | 0.714 | + |  | + | + | + | + |
| 11 | Udder swab | O128 : H2 | CP, CL, E, AM, OX, NA, K, CF | 0.571 | + |  |  | + | + | + |
| 12 | Rectal swab | O128 : H2 | E, NA ,OX | 0.214 | + |  |  | + | + | + |
| 13 | Raw milk | O55 : H7 | E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT | 0.786 | + |  |  |  |  | + |
| 14 | Rectal swab | O55 : H7 | E, OX, NA, CL, AM | 0.357 | + |  |  |  |  | + |
| 15 | Kariesh cheese | O111 : H2 | E, OX, NA, CL, AM, K, CF, CP, T | 0.643 | + | + | + |  |  | + |
| 16 | Udder swab | O111 : H2 | CF, E, CL, K, OX, , AM, CP, T,NA | 0.643 | + | + | + |  |  | + |
| 17 | Rectal swab | O111 : H2 | E, OX | 0.143 | + | + | + |  |  | + |
| 18 | Rectal swab | O111 : H2 | E, OX | 0.143 | + | + | + |  |  | + |
| 19 | Raw milk | O119 : H6 | OX, E, NA | 0.214 | + | + |  |  | + |  |
| 20 | Kariesh cheese | O119 : H6 | E | 0.071 | + | + |  |  | + |  |
| 21 | Udder swab | O121 : H7 | AM, E, CL, K, NA, OX | 0.428 | + |  |  |  |  | + |
| 22 | Udder swab | O114 : H4 | OX, E | 0.143 |  | + |  |  |  |  |
| 23 | Udder swab | O159 | OX, E, NA | 0.214 | + |  |  |  |  | + |
| 24 | Rectal swab | O159 | OX, E, NA | 0.214 | + |  |  |  |  | + |
| 25 | Rectal swab | O159 | OX, E | 0.143 | + |  |  |  |  | + |
| 26 | Raw milk | O146: H21 | OX, E | 0.143 | + | + |  | + | + |  |
| 27 | Kareish cheese | O146: H21 | E | 0.071 | + | + |  | + | + |  |
| 28 | Raw milk | O17 : H18 | E | 0.071 |  | + |  |  |  |  |

T: Tetracycline, IMP: Ipipenem, NA: Nalidixic acid, CL: Clindamycin, SXT: Sulphamethoxazol, CZ: Cefazolin, E: Erythromycin, K: Kanamycin, G: Gentamicin, CF: Cefotaxime, AK: Amikacin, CP: Ciprofloxacin, OX: Oxacillin AM: Ampicillin.

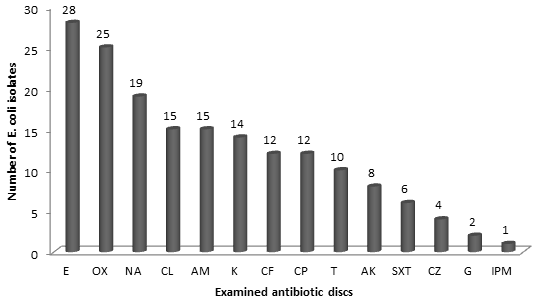


**Figure (1):** Agarose gel electrophoresis of multiplex PCR of *stx1* (180 bp), *stx2* (255 bp) and *eaeA* (384 bp) virulence genes for characterization of Enteropathogenic *E. coli.* Lane M: 100 bp ladder as molecular size DNA marker; Lane C+: Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes; Lane C-: Control negative ; Lanes 3 (O55), 7 (O121), 8 (O128) & 10 (O159): Positive strains for *stx1* gene; Lanes 1 (O17) & 5 (O114): Positive strains for *stx2* gene; Lanes 6 (O119) & 9 (O146): Positive strains for *stx1* and *stx2* genes; Lanes 2 (O26) & 4 (O111): Positive strains for *stx1*, *stx2* and *eaeA* genes.



**Figure (2):** Agarose gel electrophoresis of multiplex PCR of *blaOXA* (564 bp), *blaCTX-M1* (655 bp) and *blaTEM* (800 bp) as antibiotic resistance genes of Enteropathogenic *E. coli.*

Lane M: 100 bp ladder as molecular size DNA marker; Lane C+: Control positive for *blaOXA, blaCTX-M1* and *blaTEM* genes; Lane C-: Control negative; Lanes 3 (O55), 4 (O111), 7 (O121) & 10 (O159): Positive for *blaTEM* gene; Lane 6 (O119): Positive strain for *blaCTX-M1* gene; Lane 9 (O146): Positive strain for *blaCTX-M1* and *blaOXA* gene; Lanes 2 (O26) & 5 (O128): Positive for *blaOXA, blaCTX-M1* and *blaTEM* genes; Lanes 1 (O17) & 8 (O114): Negative for *blaOXA, blaCTX-M1* and *blaTEM* genes.



**Figure (3):** Resistance of *E. coli* isolates to antibiotics. CL: Clindamycin; K: Kanamycin; NA: Nalidixic acid; CF: Cefotaxime; SXT: Sulphamethoxazol; CZ: Cefazolin; T: Tetracycline; E: Erythromycin; AM: Ampicillin; G: Gentamicin; IMP: Ipipenem; AK: Amikacin; CP: Ciprofloxacin; OX: Oxacillin.