Prevalence and virulence determinants of *Escherichia coli* isolated from raw cow's milk

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Raw milk plays an important role in the survival and transport of pathogenic bacteria including *Escherichia coli* strains. This study was performed to determine the existence of *E. coli* in raw milk intended for human consumption and its associated virulence determinants. In this context, a total of 232 milk samples were obtained from different dairy shops located at Mansoura city and its surrounding villages. Milk samples were subjected for bacteriological and serological examination of *E. coli*. Furthermore, *E. coli* strains were tested for its haemolytic activity on blood agar plates. The recovered *E. coli* strains were also screened by Polymerase chain reaction (PCR) for the presence of enterotoxins including heat –labile (LT), heat- stable (ST) toxins and haemolysin (*hly*) genes. The recovery rate of *E. coli* was 14.65% (34/232). Among the recovered *E. coli* strains, 12 different *E. coli* serotypes were identified namely, O26:H11 (n=6), O111:H2 (n=5), O128:H2 (n=5), O91:H21 (n=4), O124 (n=3), O127:H6 (n=3), O103:H21 (n=2), O153 (n=1), O113:H4 (n=2), O6 (n=1), O121:H7 (n=1) and O146 (n=1). Regarding PCR results, 31(91.19%) *E. coli* strains harbored *STa* and seven strains carried *hly* gene (20.59%) while non *E. coli* isoates harbord *LT* gene. Conclusively, raw milk can be considered as serious source of pathogenic *E. coli*, therefore, proper management practices and effective control measures are recommended to improve milk hygiene and sanitation.

**Key words:** Raw milk, *Escherichia coli*, enterotoxin genes, haemolysin gene.

**INTRODUCTION**

Raw milk harbor variable microorganisms, considered as an important source of food borne pathogens because it is regarded as perfect media for microbial growth (Laba and Udosek, 2013). Consumption of raw milk may be associated with the occurrence of food-poisoning outbreaks (Christidis et al., 2016). The presence of different food borne pathogens in milk may be contributed to the fecal contamination during milking process (Rehman et al., 2014). *E. coli* is a normal inhabitant of the gastrointestinal tract of both man and animals. Most of *E. coli* strains are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra

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Isolation of E. coli from milk represent a serious public health hazard because some strains of E. coli may be belongs to enteropathogenic or toxigenic or both groups which causes sever gastrointestinal disturbance (Thomas et al., 2017). Enterotoxigenic E. coli (ETEC) is one of the most common bacteria responsible for diarrhea in different parts of the world (Bagheri et al., 2014). Like other gastrointestinal infectious diseases, they are caused by lack of sanitation and most often contamination transfers from contaminated food or water (Walker et al., 2007; Marchou, 2013). There are two enterotoxins, Heat-stable toxin (ST) and Heat-labile toxin (LT). These two toxins are considered as the main virulence factors which influence the pathogenesis of ETEC strains (Kolenda et al., 2015; Sjöling et al., 2015). Alpha-hemolysin (HlyA) of E. coli is one of cytolytic pore-forming toxins (PFTs) produced by Gram-negative bacteria. E. coli HlyA lyses erythrocytes shows strong cytotoxic and cytolytic action against diversity of nucleated cells (Söderström et al., 2017). HlyA does not only kill and lyse cells but also affects target cells at sublytic concentrations. Haemolysin (hlyA) is produced mainly by extraintestinal pathogenic E. coli (ExPEC) strains and occasionally by ETEC, STEC and EPEC (Burgos and Beutin, 2010). Therefore, the main purpose of this study was to examine E. coli isolated from raw milk for the presence of enterotoxins including heat labile (LT) and heat stable (ST) toxins and haemolysin.

### MATERIALS AND METHODS

#### Sampling

A total of 232 raw milk samples were collected randomly from different dairy shops, groceries and supermarkets in Mansoura city and its surrounding villages at Dakhalia Governorates, Egypt during the period from January to April, 2017. All samples were collected in sterile tubes and transported in an ice box to the laboratory as quick as possible for bacteriological examination with minimal of delay.

#### Isolation and identification of E. coli

All samples were immediately centrifuged and the sediment were streaked onto the surface of MacConkey’s agar plates and incubated aerobically at 37°C for 24 h (Quinn et al., 2002). Lactose fermenting (Pink colored) colonies was sub-cultured on Eosin Methylene Blue (Oxoid) agar medium. Colonies showing characteristic metallic green sheen on EMB agar were identified as E. coli. Presumptive E. coli colonies were subjected for gram staining and standard biochemical tests (Quinn et al., 2004). Additional identification of E. coli isolates was performed using commercial biochemical test kits (bioMerieux API, France).

### Serological identification of E. coli

E. coli strains were transferred to Food Analysis Center, Faculty of Veterinary Medicine, Benha University for serological identification using rapid diagnostic E. coli antisera sets (Kok et al., 1996).

#### Haemolytic activity

E. coli isolates were cultured on blood agar containing 5% sheep blood, for detection of its haemolytic activity. Haemolysis was recorded after an overnight incubation at 37°C. A clear halo was defined as haemolysin positive (Brauner et al., 1990).

#### PCR assay for detection of enterotoxin genes (Sta-Lt) and haemolysin gene (hly)

Bacterial genomic DNA was extracted from E. coli isolates according to Ramadan et al. (2016). E. coli isolates were screened by Polymerase chain reaction (PCR) for the presence of enterotoxins (LT, STA) and haemolysin (hly) genes. Oligonucleotide primers sequences and its amplicons sizes were described in Table1. Amplification reaction of PCR targeting haemolysin and enterotoxins was performed as previously described by Piva et al. (2003) and Lee et al. (2008), respectively (Table 1). Amplified DNA products for each gene were analyzed by 1.5% agarose gel electrophoresis in 1x TBE buffer stained with ethidium bromide visualized under UV transilluminator.

### RESULTS

In the present study, 34 (14.65%) E. coli strains have been recovered out of 232 examined milk samples. Among E. coli strains, twelve different E. coli serotypes were identified including, O26, O111, O128, O91, O124,
O127, O103, O113, O153, O6, O121 and O146 with a prevalence rate of 17.6, 14.7, 14.7, 11.7, 8.8, 8.8, 5.8, 5.8, 2.9, 2.9, 2.9 and 2.9% respectively.

E. coli isolates were tested for hemolytic activity on 5% sheep blood agar, 52.94% (18/34) of E. coli strains revealed different degrees of hemolysis. Based on the PCR results, 91.19% of E. coli isolates are potentially pathogenic, which carry one or more investigated virulence genes. From a total of 34 E. coli strains, 7(20.59%) strains carried hly gene (Figure 1), 31(91.17%) strains carried STa (Figure 2) while none E. coli isolates carried LT gene (Table 2).

DISCUSSION

Raw milk is a perfect medium that supports the growth and multiplication of E. coli. Consumption of such milk appeared as main threat to health concerns, especially for those people who still drink raw milk without heat treatment (Claeys et al., 2013). In the present study, E.coli was recovered with 14.65% prevalence rate. Similarly, E. coli has been isolated by several researchers from raw milk of cattle and buffaloes (Caine et al., 2014; Islam et al., 2008; Hossain et al., 2011). Comparing to present results, a higher percentage of E. coli in milk was reported by Bandyopadhyay et al. (2012), Farzan et al. (2012), Mohd et al. (2013), Ali and Abdelgadir (2011) and Gwida and EL-Gohary (2013), who could isolate E. coli from raw milk in a percentage of 26.43, 30.28, 33.96, 63 and 41.2% respectively. However, lower results were recorded by Kivaria et al. (2006) who detected E. coli in 6.3% of the examined raw milk samples.

In the present study, 12 different E.coli serotypes were identified; nearly the same serotypes were recovered
In this study, 52.94% of the isolates harbored hly gene, 20.59% of E. coli harbored hly gene. A lower percentage was recorded by Ombarak et al. (2016), who identify hly gene in 2 (2.25%) isolates from karish cheese and one isolate (0.90%) from raw milk while, a higher percentages (42.85%) were reported by Osman et al. (2012). The presence of E. coli in milk especially enteropathogenic and/or toxigenic strains has a public health hazards which lead to sever gastrointestinal disturbance. Among E. coli isolates, 7 (20.59%) strains carried hly gene, 31 (91.17%) strains carried STa while, LT gene was not identified in all E. coli strains. Comparing to these results, Eid (2014) revealed that, only one strain were tested positive for STa gene out of eight E. coli isolates.

In Brazil, Paneto et al. (2007) studied the frequency of toxigenic E. coli in raw milk and cheese whereby, 1 (2%) of E. coli isolates were ETEC. In Romania, Tabaran et al. (2017) analyzed 145 E. coli strains isolated from raw milk and traditional dairy cheeses, for the presence of STa and STb. In LT, none of the samples carries the estl gene, but 14 (9.7%) of the E. coli isolates were positive for both eltA and estl. Caine et al. (2014) examined 100 E. coli strain for the presence of enterotoxins which could identify enterotoxins in 4% of the total examined isolates.

Bonyadian et al. (2014) tested 120 isolates of E. coli, isolated from milk and unpasteurized cheeses which identified LT and STb in 2 (1.66%) and 12 (10.00%), respectively but could not identify STa gene. In this study, it was interesting that, all E. coli strains carry hly gene along with enterotoxin gene. These results suggest that food of animal origin represents a significant source of pathogenic E. coli strains.

**Conclusion**

The high contamination of milk with toxigenic E. coli represents a serious public health hazards which necessity high and strict preventive measures, to minimize the bacterial contamination within the food chain such as regular washing and sterilization of dairy equipment, utensils, milkers hands, animal udders as well as heat treatment of milk before distribution to consumers.

**Significance statement**

This study provided a data about the prevalence of E. coli in cow’s raw milk, especially enterotoxigenic (ETEC) E. coli. These data is required for the establishment of food control systems which required the prevention and control of foodborne illnesses.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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