Occurrence of multidrug resistant shiga-toxigenic E. coli in retailed cheese in Zagazig city, Egypt

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ABSTRACT

Cheese is regarded as an essential dairy product that cover part of the human needs with vitamins and minerals. However, cheese is also implicated in many food poisonings outbreaks worldwide. This study conducted to estimate coliforms and E. coli, and to investigate the occurrence of shigatoxigenic E. coli in four cheese types mostly consumed in Zagazig city, Egypt. In addition, detection of coding genes for shiga toxins (stx1 and stx2) was further screened using PCR. Besides, antimicrobial susceptibility of the isolated E. coli was further examined. The obtained results revealed that Kariesh cheese had the highest coliforms and E. coli counts, followed by Rumy, Domiati, and Feta cheeses, respectively. The isolation rates of E. coli were 52%, 24%, 20%, and 8% in Kariesh, Rumy, Domiati and Feta cheeses, respectively. The identified E. coli serotypes were E. coli O55:H7, E. coli O86:H11, E. coli O103:H4, E. coli O111:H4, and E. coli O26:H11 harbored both stx1 and stx2; however, E. coli O127:H6 did not express any of the tested genes. Antimicrobial sensitivity testing revealed multidrug resistance profiles, particularly among E. coli O86:H11, E. coli O78:H-, and E. coli O26:H11. Therefore, strict hygienic measures should be adopted during all manufacture steps of these kinds of cheese.

1. INTRODUCTION

Cheese is rich in the essential amino acids, trace elements and minerals such as calcium and magnesium, and vitamins (Gerosa and Skoet, 2013; Ma et al., 2020). In Egypt there are many cheese types that are made from either raw or pasteurized milk such as Kariesh, Feta, Domiati, and Rumy cheeses. Cheese consumption is associated with many food poisonings outbreaks worldwide. One of the major organisms responsible for such food poisoning outbreaks is Escherichia coli (E. coli) (Mc Sweeney, 2007). E. coli is a normal inhabitant in the intestinal flora of the farm animals. Detection of E. coli in foods of animal origin indicates fecal contamination. At the same time, E. coli is associated with several cases of human hospitalization (Darwish et al., 2015). E. coli strains are broadly classified into enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and shiga toxin-producing E. coli (STEC). This classification is dependent on their pathogenicity and virulence attributes (Ruttler et al., 2006; Xia et al., 2010). There are more than 100 E. coli serotypes that are classified as STEC. Of these, E. coli O157 causes 50 to 90% of E. coli-related human food poisoning cases, with most of the remaining cases caused by O45, O26, O111, O103, O121 and O145 (Scallan et al., 2011).

The uncontrolled usage of the antimicrobials in the animal production units had resulted in the development of antimicrobial resistance among microorganisms making treatment of infectious diseases as a challenging problem (Darwish et al., 2013). The present study was conducted to estimate the coliforms and E. coli counts and to examine the isolation rates of different E. coli serotypes in four cheese types (Kariesh, Feta, Domiati, and Rumy) mostly consumed in Zagazig city, Egypt. In addition, detection of shiga toxin-coding genes (stx1 and stx2) was done using PCR. Besides, antimicrobial sensitivity testing of the identified serotypes was further examined using the disk diffusion method.

2. MATERIAL AND METHODS

2.1. Sample collection:
One hundred cheese samples of Kariesh, Feta, Domiati, and Rumy, (n = 25/each cheese type) were collected from different
grocery stores and street vendors in Zagazig city, Egypt. The microbiological examination of cheese samples was done at Faculty of Veterinary Medicine, Zagazig University.

2.2. Sample preparation:
Twenty-five grams from each sample was blended aseptically in buffered peptone water 0.1% (225 ml) for 2 min at 2500 rpm to obtain a dilution of 10^4, followed by making decimal serial dilutions (APHA, 2001).

2.3. Estimation of MPN of coliforms:
The three tubes method recommended by APHA (2001) for the estimation of MPN of coliforms was followed.

2.4. Estimation of MPN of E. coli:
Loopfuls from the positive tubes (with acid and gas production) were aseptically inoculated into previously warmed tubes (44.5°C) containing 7 ml of E. coli (EC) broth (Himedia, Mumbai) and then incubated at 44.5°C for 24-48 hrs (APHA, 2001). The positive tubes were used to estimate MPN of E. coli according to the recommended tables.

2.5. Isolation of Escherichia coli:
From each positive tube (acid and gas of EC broth, a loopful was streaked onto Eosin Methylene blue (EMB) agar plates, followed by incubation at 37°C for 24 hrs. Typical colonies of E. coli appear greenish, metallic with dark purple center. E. coli identification was done according to the staining and biochemical tests (APHA, 2001).

2.6. Sero-diagnosis of E. coli:
The rapid diagnostic E. coli antisera sets purchased from Difco, Detroit, USA were used for the serological identification of the confirmed E. coli isolates (Kok et al., 1996).

2.7. DNA preparation:
DNA was extracted from the confirmed E. coli isolates using the method reported before (Darwish et al., 2015).

2.8. Detection of shiga toxin-coding genes in the identified isolates:
Further detection of the coding genes for shiga toxins (stx1 and stx2) in the identified E. coli isolates was carried out using multiplex PCR. The primer sequences and the amplified products sizes were presented in Table 1. The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Germany). PCR assays were carried out using the method reported before (Darwish et al., 2015).

2.9. Antimicrobial sensitivity testing of E. coli:
Antimicrobial sensitivity testing was conducted using the disk diffusion method (Wayne, 2013). The tested antimicrobials were ampicillin (AM) 10 µg, cephalothin (CN) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CP) 5 µg, enrofloxacin (En) 5 µg, erythromycin (E) 15 µg, gentamicin (G) 10 µg, kanamycin (K) 30 µg, nalidixic acid (NA) 30 µg, neomycin (N) 30 µg, oxacillin (Ox) 1 µg, oxytetracycline (T) 30 µg, penicillin (P) 10 IU and trimethoprim/sulfamethoxazole (SXT) 25 µg.

2.10. Statistical analysis
Coliforms and E. coli counts were expressed as means ± SE for log 10 cfu/g. Statistical analysis was done using Tukey–Kramer HSD test where, p <0.05 indicated statistical differences (Gomez and Gomez, 1984).

3. RESULTS
Figure 1A showed that Kariesh cheese had the highest coliforms counts (log 10 cfu/g) among the examined cheese samples with a value of 4.15 ± 0.15, followed by Rumy cheese (3.87 ± 0.17), Domiati cheese (3.45 ± 0.21), and Feta cheese (2.45 ± 0.11), respectively. E. coli counts (log 10 cfu/g) in the examined cheese samples were corresponding to the coliforms counts as Kariesh cheese had significantly the highest value (3.85 ± 0.18), followed by Rumy cheese (3.17 ± 0.12), Domiati cheese (2.75 ± 0.19), and Feta cheese (2.11 ± 0.22), respectively (Fig. 1B).

The prevalence rates of E. coli were 52%, 24%, 20%, and 8% in Kariesh, Rumy, Domiati and Feta cheeses, respectively (Fig. 2). The isolated E. coli were further identified into six serotypes namely E. coli O55:H7, E. coli O111:H4, E. coli O127:H6, E. coli O86:H11, E. coli O78:H, and E. coli O26:H11. Kariesh cheese had the highest contamination level with different E. coli serotypes, particularly with E. coli O78:H (20%), and E. coli O26:H11 (16%), while Feta cheese had the lowest contamination level (Fig. 3A, B).

Table 1. Oligonucleotide sequences of shiga toxin-coding genes

| Primer | Oligonucleotide sequence (5′ → 3′) | Product size (bp) | References |
|--------|----------------------------------|------------------|------------|
| stx1 (F) | 5′-ACAATGCAGTGACCTAGCTAGGTG-3′ | 614 | Dhanaashree and Mallya (2008) |
| stx1 (R) | 5′-CTGAATCCCCCTCCATTATG-3′ | 779 | |
| stx2 (F) | 5′-CCATGACAGCAGCACACGATT-3′ | | |
| stx2 (R) | 5′-CTATGACACAGTAGCGACACTTGG-3′ | | |

Figure 1. Coliforms and E. coli counts in the examined cheese samples. Values represent means ± SE (Log 10 cfu/g) of A) Coliforms B) E. coli counts in Kariesh, Feta, Domiati, and Rumy cheeses. Columns with different letter are significantly different at p <0.05.
We further detected the expression of shiga toxin-coding genes among the identified *E. coli* serotypes. *E. coli O135:H7* harbored only *stx1*, *E. coli O111:H4*, and *E. coli O86:H11* harbored only *stx2*, *E. coli O78:H1*-, and *E. coli O26:H11* harbored both *stx1*, and *stx2*; however, *E. coli O127:H6* did not express any of the tested genes (Fig. 4).

Antimicrobial sensitivity testing revealed drug resistance for more than one antimicrobial, particularly in *E. coli* O86:H11, *E. coli O78:H1*-, and *E. coli O26:H11* (Table 2).

Table 2: Antimicrobial sensitivity testing of *E. coli* serotypes identified in the examined cheese samples.

| Antimicrobial | AM | CN | CP | En | E | G | K | NA | OX | P | SXT | No. | %   |
|---------------|----|----|----|----|---|---|---|----|----|---|----|------|-----|-----|
|               | 1  | 1  | 1  | 1  | 1 | 1 | 1 | 1  | 1  | 1 | 1   | 100  | 40  |
| **Stx1**      | 1  | 1  | 1  | 1  | 1 | 1 | 1 | 1  | 1  | 1 | 1   | 100  | 40  |
| **Stx2**      | 1  | 1  | 1  | 1  | 1 | 1 | 1 | 1  | 1  | 1 | 1   | 100  | 40  |
| **Stx1+Stx2** | 1  | 1  | 1  | 1  | 1 | 1 | 1 | 1  | 1  | 1 | 1   | 100  | 40  |

4. DISCUSSION

In the present study, Kariesh cheese had the highest coliforms counts as well as *E. coli* counts among the examined cheese samples followed by Rumy, Domiati then Feta cheeses, respectively. Such high values of coliforms and *E. coli* counts reflect improper hygienic measures adopted during cheese preparation, storage, or distribution, particularly in the case of Kariesh cheese (Mossel et al., 1995). Similarly, unsatisfactory hygienic measures were reported for the retailed fresh cheese in Mexico (de la Rosa-Hernández et al., 2018), and in Kariesh cheese, Ras cheese, and Tallaga cheese retailed in Beni-Suef city, Egypt (Hassan et al., 2019).

*E. coli* is considered as a major foodborne pathogen responsible for many cases of hospitalization and deaths especially among children and elderly. For instance, a cluster of *E. coli O157:H7* hemorrhagic colitis was identified in Canada, between October 2002 and February 2003 (Honish et al., 2005). Besides, *E. coli O104:H4* was responsible for an outbreak occurred in Germany in May 2011 infecting more than 3000 peoples and left 50 deaths (Frank et al., 2011). In addition, another 19 persons were infected with the shiga toxin producing *E. coli O121* in six of United States (CDC 2014). In the present study, the highest prevalence rate of *E. coli* was in Kariesh cheese followed by Rumy, Domiati and Feta cheeses, respectively. In agreement with the obtained results in the present study, Hassan and Elmalt (2008) isolated toxigenic *E. coli* from retailed Kariesh cheese in Qena city at 47.8%. Besides, Ombark et al. (2016) isolated *E. coli* of enteropathogenic and enterohemorrhagic types at 74.5% from Kariesh cheese, and 21.7% from Ras cheese retailed in Egypt. Furthermore, Hussein et al. (2019) isolated *E. coli* at 16%, and 5.3% from Kariesh cheese and Ras cheese, respectively, sold in Menoufia Governorate.

*E. coli O55:H7, E. coli O111:H4, E. coli O127:H6, E. coli O86:H11, E. coli O78:H1*, and *E. coli O26:H11* were further identified as the prevalent serotypes in the current investigation. Kariesh cheese had the highest contamination level with different *E. coli* serotypes, particularly with *E. coli O78:H1* and *E. coli O26:H11*, while Feta cheese had the lowest contamination level. Similarly, de Campos et al. (2018) could identify *E. coli O127, O75:H12, and O64474:H8* from Minas cheese in Brazil. Furthermore, Hussein et al. (2019) identified eight *E. coli* serotypes from Kariesh and Ras cheeses, namely, *E. coli O26: H11, O91: H21, O111: H2, O103: H2, O125: H21, O171: H2, O86:H1*, and O119: H6.
The expression of shiga toxin-coding genes among the identified *E. coli* serotypes were detected. The obtained results revealed that *E. coli* O55:H7 harbored only stx1, *E. coli* O111:H4, and *E. coli* O86:H11 harbored only stx2, *E. coli* O78:H-, and *E. coli* O26:H1 harbored both stx1, and stx2; however, *E. coli* O127:H6 did not express any of the tested genes. *E. coli* of non-O157 serogroups such as O26, O103, O111 were reported to be the most substantial food poisoning pathogen groups, especially O26 that able to cause wide range of illness in human (Dambrosio et al., 2007). Similarly, *E. coli* expressing shiga toxin coding genes were isolated from a Spanish raw ewe’s milk cheese (Caro et al., 2007). In addition, Elhadidy and Mohammed (2013) isolated shiga toxin-producing *E. coli* including serotypes O22:H8, O26:H11, O86:H21, O103:H2, O113:H21 and O146:H21 from Kariesh and Domiaty cheese retailed in Egypt. Hussein et al. (2019) could also identify eight *E. coli* serotypes from Kariesh and Ras cheeses producing shiga toxins (stxl, and stx2). STEC is implicated in many cases of hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Karch et al., 2005).

The uncontrolled usage of antimicrobials in the livestock production is the major cause for the development of drug-resistance pathogens, which is regarded as a major health concern. In the current study, the multidrug resistance profiles particularly among *E. coli* O86:H11, *E. coli* O78:H-, and *E. coli* O26:H11 were prominent. For instance, 50% or more of the identified *E. coli* O26:H11 showed resistance to AM, P, NA, and OX. While 50% or more of *E. coli* O86:H11 showed resistance to AM, G, NA, P, OX, and SXT. Isolation of multidrug resistant *E. coli* from cheese was reported in studies conducted in Romania (Tabaran et al., 2017), Brazil (de Campos et al., 2018), and Egypt (Obrahak et al., 2018). Therefore, rational use of antimicrobials in the animal farms and livestock production is highly recommended.

In conclusion, the current study revealed isolation and identification of multidrug resistant and shiga toxin-producing *E. coli* from Kariesh, Domiaty, Rumy and Feta cheese retailed in Zagazig city, Egypt. Therefore, strict observation of hygienic measures should be adopted during all manufacture steps of these kinds of cheese. In addition, continuous monitoring studies for the prevalence of STEC in other dairy products are highly recommended.

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