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## **Molecular typing of coliform species isolated from raw milk and some dairy products**

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## **SUMMARY**

Many enteropathogenic microorganisms have been found in milk and dairy products, which is usually produced under a septic conditions and stored under inadequate temperatures and consumed without any prior thermal treatment. They are frequently associated with outbreaks of foodborne diseases. A total of 125 samples of raw milk, ice cream Kariesh cheese, yoghurt and Laban rayeb (25 each) were collected randomly from different shops and supermarkets in Sharkia Governorate for enumeration and identification of coliform organisms

### **Enumeration and identification of coliforms:**

Results revealed that the logarithmic (log) mean values of total coliform count were  $11.20 \pm 1.020$ ,  $8.00 \pm 2.10$ ,  $5.80 \pm 1.90$ ,  $5.40 \pm 1.70$  and  $4.30 \pm 1.10$   $\log_{10}$  cfu/ml or gm in raw milk, ice cream, Kariesh cheese, yoghurt and Laban rayeb, respectively.

### **Identification of coliforms using biochemical tests:**

The results show that the isolation rates of *E. coli* from raw milk, ice cream, Kariesh cheese, yoghurt and Laban rayeb were 26, 20, 22, 28 and 18%, respectively.

*C. freundii*, *C. diversus*, *Ent. Aerogenes*, *Ent. Agglomerans*, *Ent. Cloacae*, *K. oxytoca* and *K. pneumoniae* were isolated from raw milk, ice cream, Kariesh cheese, yoghurt and Laban rayeb with different percentages.

### **Serodiagnosis of *E. coli* isolated from examined samples:**

Results achieved indicated that the incidences of serologically identified *E. coli* in raw milk samples were EPEC (*E. coli* O<sub>127</sub>:H<sub>6</sub>, O<sub>119</sub>:H<sub>6</sub> with a percentages of 15.38%, each), EHEC (*E. coli* O<sub>26</sub>:H<sub>11</sub>, O<sub>157</sub>:H<sub>7</sub> with a percentage of 7.69%, each), EIEC (*E. coli* O<sub>124</sub>:H<sub>30</sub> with a

percentage of 7.69%), EAEC (*E. coli* O<sub>125</sub>:H<sub>21</sub> with a percentage of 23.08%) and 23.08% of the strains was unidentified.

While in case of ice cream samples, the incidences of EPEC (*E. coli* O<sub>127</sub>:H<sub>6</sub>, O<sub>111</sub>:H<sub>2</sub>, O<sub>119</sub>:H<sub>6</sub>) were with percentages of 20.00, 20.00 and 30.00%, respectively), EHEC (*E. coli* O<sub>26</sub>:H<sub>11</sub>) were with a percentage of 10.00%), EAEC (*E. coli* O<sub>125</sub>:H<sub>21</sub>) were with a percentage of 10.00% and 10.00% of the strains was unidentified.

The incidences of serologically identified *E. coli* in Kariesh cheese samples were EPEC (*E. coli* O<sub>127</sub>:H<sub>6</sub>, O<sub>111</sub>:H<sub>2</sub>, O<sub>119</sub>:H<sub>6</sub> with percentages of 9.09, 18.18 and 18.18%, respectively), EHEC (*E. coli* O<sub>26</sub>:H<sub>11</sub>, O<sub>157</sub>:H<sub>7</sub> with a percentage of 9.09%, each), EIEC (*E. coli* O<sub>124</sub>:H<sub>30</sub> with a percentage of 9.09%), EAEC (*E. coli* O<sub>125</sub>:H<sub>21</sub> with a percentage of 18.18%) and 9.09% of the strains was unidentified.

In case of yoghurt samples, the incidences of EPEC were (*E. coli* O<sub>127</sub>:H<sub>6</sub>, O<sub>111</sub>:H<sub>2</sub>, O<sub>119</sub>:H<sub>6</sub> with percentages of 21.43, 14.29 and 21.43%, respectively), EHEC (*E. coli* O<sub>157</sub>:H<sub>7</sub> with a percentage of 7.14%), EIEC (*E. coli* O<sub>124</sub>:H<sub>30</sub> with a percentage of 7.14%), EAEC (*E. coli* O<sub>125</sub>:H<sub>21</sub> with a percentage of 14.29%) and 14.29% of the strains was unidentified.

While in case of Laban rayeb samples, the incidences of serologically identified *E. coli* were EPEC (*E. coli* O<sub>127</sub>:H<sub>6</sub>, O<sub>111</sub>:H<sub>2</sub>, O<sub>119</sub>:H<sub>6</sub> with percentages of 22.22, 33.33 and 11.11%, respectively), EHEC (*E. coli* O<sub>26</sub>:H<sub>11</sub> with a percentage of 11.11%), EAEC (*E. coli* O<sub>125</sub>:H<sub>21</sub> with a percentage of 11.11%) and 11.11% of the strains was unidentified.

#### **Identification of coliforms using MALDI-TOF MS:**

The results revealed that the incidence of *E. coli* in raw milk samples according to MALDI-TOF MS identification of coliforms was

50.00%, while *C. freundii*, *Ent. cloacae*, *K. variicola* and *Pantoea agglomerans* could be isolated with percentages of 16.67, 8.33, 16.67 and 8.33%, respectively.

The incidence of *E. coli* detected from ice cream samples was 14.29, while *Ent. cloacae*, *K. pneumoniae*, *Pluralibacter gergoviae* and *Serratia marcescens* could be isolated with percentages of 28.57, 14.29, 28.57 and 14.29%, respectively.

In addition, the incidence of *E. coli* isolated from Kariesh cheese samples was 33.33%, while *C. freundii*, *Ent. cloacae* and *K. pneumoniae* could be isolated with percentages of 16.67, 33.33 and 16.67%, respectively.

The incidence of *E. coli* isolated from yoghurt samples was 80%, while *K. variicola* and *Serratia marcescens* could be isolated with percentages of 10.00%, each.

The incidence of *E. coli* isolated from Laban rayeb samples was 20%, while *K. pneumoniae* and *Pluralibacter gergoviae* could be isolated with percentages of 60.00% and 20.00%, respectively.

Comparison between MALDI-TOF MS and biochemical methods for identification of coliforms revealed 81.06% similarity of both methods.

#### **Molecular typing of coliform:**

The results of *gyrB* gene sequencing for typing of isolated coliforms revealed detection of *K. pneumoniae*, *Serratia marcescens*, *K. variicola* and 2 strains of *Pluralibacter gergoviae* with a homology value of 100.00%.