Prevalence of Yersinia enterocolitica in Ice-Creams in Baghdad

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ABSTRACT
In order to investigate the prevalence of Yersinia enterocolitica in ice-creams, forty pooled samples of ice-creams with a vanilla, chocolate, strawberry and fruit flavor (five replicates from each type, n=200) were collected randomly from different markets in Baghdad during February till October (2015), in which they processed and analyzed by different food microbiological procedures. The results showed recovery of 16 (30%) isolates out of 40 pooled samples as 2 (5%) isolates from vanilla type, 5 (12.5%) isolates from chocolate type, 2 (5%) isolates from strawberry type and 7 (17.5%) isolates from fruit flavor type. The mean log count of Y. enterocolitica in vanilla was 0.477, in strawberry was 1.397, in fruit flavor was 1.968 and range from 0.845 to 1.826 log10 cfu g-1 in chocolate type. Escherichia coli and their pathogenic serotype O157 H7 was detected in some samples. Results profile provide useful information on biosafety and hazard analyses critical control points of hygienic measurements of ice-creams marketed in Baghdad.

INTRODUCTION
Yersinia enterocolitica was a small, psychrotrophic, Gram-negative facultative anaerobic coccobacillus, isolated from a variety of environmental sources, foods and clinical samples (1-4, 8, 15). It’s a causative organism in several outbreaks of gastroenteritis, in which foods were implicated (3-5, 8). The pathogenic bacterium Y. enterocolitica has become increasingly important as a food contaminant. Of special significance in food hygiene was the ability of Y. enterocolitica to grow in refrigerated foods especially ice creams. The psychrotrophic ability to grow at temperatures close to 0 °C was characterized by temperature-dependent adaptations. Yersinia has been frequently isolated from a variety of foods like untreated milk, chocolate milk, dairy cream and ice cream, vegetables like carrots, tomatoes, lettuce, celery and mushrooms, raw hare, beef and lamb. It has also been isolated from drinking water. Milk and dairy products were the most consumed foods with animal origins. Y. enterocolitica has a particular public health importance, because of its capability of growing in raw milk and viability at refrigeration temperatures for long time (6-16). Therefore, consumption of milk and dairy products has a higher chance of infection by Y. enterocolitica in humans. Consumption of raw milk and traditional dairy products was common Bessie in Iraq; but, the most important issue about Y. enterocolitica was its survivance in pasteurized milk and even commercial dairy products. Y. enterocolitica infection causes the disease yersiniosis, which was a zoonotic disease occurring in humans, as well as a wide array of animals such as cattle, deer, pigs and birds. Many of these animals recover from the disease and become asymptomatic carriers (16). It infects the host by sticking to their cells by trimeric autotransporter adhesins (TAAs) and secreted outer membrane proteins (Yops) (16).

The genus Yersinia includes 11 species: Y. pestis, Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. bercovieri, Y. mollaretii, Y. rohdei, Y. aldovae and Y. ruckeri. Among them, only Y. pestis, Y. pseudotuberculosis, and certain strains of Y. enterocolitica were of pathogenic importance for humans and certain warm-blooded animals, whereas the other species were of environmental origin and may act as opportunists. Y. enterocolitica was a heterogeneous group of strains, which were traditionally classified by biotyping into six biogroups on the basis of phenotypic characteristics, and by serotyping into more than 57 O serogroups, on the basis of their O (lipopolysaccharide or LPS) surface antigen (4, 8 & 16).

Ice-creams were a popular products consumed particularly in summer as well as throughout all year and continues to be a dominant interest of large segment of the population. The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, coloring materials, flavors, fruits, nuts, sweetening agents, eggs and eggs products, and stabilizers. Any of these may contribute microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria. Temperature-dependent heating during the ice cream making reduces largely the vegetative forms of the microorganisms. On the other hand, spore bearing microorganisms may well pose risks through consumption of this kind of milk products. Furthermore, the presence of pathogens in ice cream samples was mostly by means of tools and equipment, water, workers, environment, packaging materials and contaminations during the transportation and distribution of ice cream (4, 8, 15).

The objective of this study was to evaluate the contamination level and microbial load of Y. enterocolitica in ice-creams with a vanilla, chocolate, strawberry and fruit flavor (ice-sticks, ice-cups and machine types) commercially available in the Baghdad markets.

MATERIALS and METHODS
Collection and Processing of Samples: a total of forty pooled samples (five replicates from each type, totally; two hundred replicates) were randomly collected from different markets in Baghdad during February till October (2015), in which they processed and analyzed by different food microbiological procedures with some modifications (17-25). Samples were collected aseptically in sterile plastic bags and containers, in which they transported to zoonotic lab as soon as possible.

Each pooled and well thawed and mixed replicates were divided in to two separate units (direct and indirect processing): directly well mixed replicates units were enriched in freshly prepared tryptone soya yeast extract broth for 24-72 hours at 4, 10, 25 & 37 °C (50 g well mixed sample part was added to 250-500 ml tryptone soya yeast extract broth, then mixed well with vortex and streaked by sterile loops and swabs on selective chromogenic Yersinia CIN (cefsludin-irgasan-novobiocin) agar plates (Oxoid UK) according to the method of the FDA for each pooled unit), then incubated at 37 °C for 18-48 hours. In this pathway samples units were cultured by dilution formula: one pooled part of sample unit to five-ten parts of broth diluent (14-16). Indirectly pooled replicates were thawed and refrigerated at 4 °C for 3 days then either enriched with tryptone soya yeast extract broth or diluted with sterile phosphate buffered saline (one-unit part of sample to ten parts of diluent) at 10, 25 & 37 °C for 18-48 hours. In this pathway samples units were cultured by dilution formula: one pooled part of sample unit to five-ten parts of broth diluent (14-16). Pure isolated pink bull eyes colonies with clear watery borders were counted by droplet technique in accordance to Mcfarland’s opacity tubes, then pure seeds were prepared for further identification procedures. Electronic computerized biochemical RapIDSTM One panel test system for Enterobacteriaceae was used for confirmation procedure of isolates. All the isolates which were negative for utilization
of citrate, positive for urease activity and giving an alkaline slant/acid butt without gas or H2S on Triple sugar iron slant were submitted to further testing. In order to identification and bio grouping of isolates as Y. enterocolitica; activities of oxidase, lysozyme deacylase, ornithine decarboxylase, β-D-glucosidase, lipase and pyrazinamidase, utilization of rhamnose, sucrose, xylose and trehalose were evaluated. Further analyses were also conducted applying Indole and Voges Proskauer tests to isolates (17-25).

Testing for Pathogenicity Markers: Y. enterocolitica strains were tested for virulence by Temp-Dependent autoagglutination (25 °C-35 °C) in Methyl Red-Voges Proskauer broth, occur of small red colonies on Congo Red-Magnesium Oxalate agar and Congo red / crystal violet binding assays (calcium binding and biofilm formation) as well as, esculin hydrolysis, fermentation of salicine and formation of formazan red tree on modified 2,3,5-tritetrazoliūm chloride semisolid nutrient agar tubes (17-25).

MacConkey, Sorbitol-MacConkey and Eosin Methylene Blue agars were used with biochemical panel test and Remel serotyping kit for identification of Escherichia coli and their pathogenic serotype O157 H7. Data were statistically analyzed by Chi-square test in accordance with SPSS (26).

**RESULTS & DISCUSSION**

Y. enterocolitica has a particular public health importance, because of its capability of growing in raw milk and viability at refrigeration temperatures for long time. Therefore, consumption of milk and dairy products especially raw ones give a higher chance for infection by Y. enterocolitica in humans (4, 8 & 15). Results profile reflect contamination of ice-creams samples sold in Baghdad markets with Y. enterocolitica and Escherichia coli and their pathogenic serotype O157 H7 as shown in tables (1 & 2) and photographs (1). The results showed recovery of 16 (30%) isolates out of 40 pooled samples as 2 (5%) isolates from vanilla type, 5 (12.5%) isolates from chocolate type, 2 (5%) isolates from strawberry type and 7 (17.5%) isolates from fruit flavour type. The mean log count of Y. enterocolitica in vanilla was 0.477, in strawberry was 1.397, in fruit flavour was 1.968 and range from 0.845 to 1.826 log10 cfu g−1 in chocolate type.

### Table (1): Isolation percentages and mean log10 count of Y. enterocolitica from ice-creams in Baghdad.

<table>
<thead>
<tr>
<th>Type of Ice-Cream</th>
<th>Number</th>
<th>Isolation %</th>
<th>Mean log10 count cfu g−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla Flavour</td>
<td>10 (50r)</td>
<td>2 (5%)</td>
<td>0.477*</td>
</tr>
<tr>
<td>Chocolate Flavour</td>
<td>10 (50r)</td>
<td>5 (12.5%)</td>
<td>0.845# - 1.826#</td>
</tr>
<tr>
<td>Strawberry Flavour</td>
<td>10 (50r)</td>
<td>2 (5%)</td>
<td>1.397#</td>
</tr>
<tr>
<td>Fruit Flavour</td>
<td>10 (50r)</td>
<td>7 (17.5%)</td>
<td>1.968#</td>
</tr>
<tr>
<td>Total</td>
<td>40 (200r)</td>
<td>16 (30%)</td>
<td>1.222</td>
</tr>
</tbody>
</table>

a,b,c,d: Indicate significant differences among isolates for mean log10 count vertically at level (P<0.05).

### Table (2): Isolation percentages of Escherichia coli and their serotype O157 H7 in Baghdad.

<table>
<thead>
<tr>
<th>Type of Ice-Cream</th>
<th>Number</th>
<th>Escherichia coli %</th>
<th>Escherichia coli serotype O157 H7 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla Flavour</td>
<td>10 (50r)</td>
<td>1 (2.5%)</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Chocolate Flavour</td>
<td>10 (50r)</td>
<td>3 (7.5%)</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Strawberry Flavour</td>
<td>10 (50r)</td>
<td>None (0%)</td>
<td>None (0%)</td>
</tr>
<tr>
<td>Fruit Flavour</td>
<td>10 (50r)</td>
<td>None (0%)</td>
<td>None (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (200r)</td>
<td>4 (10%)</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>
was more active biochemically at 25 °C than at 35-37 °C, meaning that disparate results for a given test may be obtained depending on incubation temperature. This characteristic, coupled with the known temperature-sensitivity of the Y. enterocolitica virulence plasmid, makes strict adherence to temperature and time requirements a necessity (18 & 19).

Unrestricted hygienic monitoring systems and food policies like absence of biosafety and hazard analysis critical points during production and handling of healthy ice-creams, absence of risk assessments during importation of dairy products, all these and others result in contamination of mincemeat in Baghdad markets with different invaders. Using raw milk without pasteurization, milking with unsanitary methods, and using traditional dairy products produced in unsatisfactory conditions and probably from unpasteurized milk, were the main resources for growth, proliferation and survival of Y. enterocolitica. These factors cause several disorders for human. Therefore, improving the methods of milking, monthly checking of the milking halls to detect Y. enterocolitica especially in the animal feces, fumigating the milking halls frequently, inspecting the hygiene during milking, boiling the milk, using pasteurized and even sterilized milk for dairy products, keeping dairy products in cool and dry places away from the sunlight, and finally preventing from contamination of dairy products with extrinsic factors such as insects and dust, are the best ways to prevent Y. enterocolitica infections (4).

However, while this study has focused on the detection and/or enumeration of Y. enterocolitica at near consumer levels, effective action to reduce or eliminate the risks posed by this organism will involve diverse and coordinated actions at a number of stages of the food chain. These include the incorporation and consistent application of Good Agricultural practice (GAP), Good Manufacturing practice (GMP), and Hazard Analysis of Critical Control Points (HACCP) at every stage of the food supply chain, from the farm to the retailer, and those involved with the handling and processing of such raw milk products in the home environment. In addition, suitable intervention measures may be necessary to eliminate the pathogen in food reaching the consumer.

REFERENCES


