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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 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. 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.

KEYWORDS : Yersinia enterocolitica, ice-creams.

INTRODUCTION

Yersinia enterocolitica was a small, psychrotrophic, Gram-negative facultative anaerobic coccobacilli, 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 significant 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 surveillance 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 segments 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. Time-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 on 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 by vortex and streaked by sterile loops and swabs on selective chromogenic *Yersinia* CIN (cefsulodin-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 3,7,10 & 14 days, then sub cultured on CIN agars at same temperatures and incubation periods above (17-25).

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 RapID™ 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, lysine decarboxylase, 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 ℃-35 ℃) 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 & DISSCUTION

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 $\log_{10}$ 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.

Table (1): Isolation percentages and mean $\log_{10}$ count of *Y. enterocolitica* from ice-creams in Baghdad.

Type of Ice-Cream	Number	Isolation %	Mean $\log_{10}$ count cfu\g^{-1}
Vanilla Flavour	10 (50r)	2 (5%)	0.477[a]
Chocolate Flavour	10 (50r)	5 (12.5%)	0.845[b] - 1.826[d]
Strawberry Flavour	10 (50r)	2 (5%5	1.397[c]
Fruit Flavour	10 (50r)	7 (17.5%)	1.968[d]
Total	40 (200tr)	16 (30%)	1.222

a,b,c,d: Indicate significant differences among isolates for mean $\log_{10}$ count vertically at level (P≤0.05).

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

Type of Ice-Cream	Number	*Escherichia coli* %	*Escherichia coli* serotype *O157 H7* %
Vanilla Flavour	10 (50r)	1 (2.5%)	1 (2.5%)
Chocolate Flavour	10 (50r)	3 (7.5%)	1 (2.5%)
Strawberry Flavour	10 (50r)	None (0%)	None (0%)
Fruit Flavour	10 (50r)	None (0%)	None (0%)
Total	40 (200r)	4 (10%)	2 (5%)

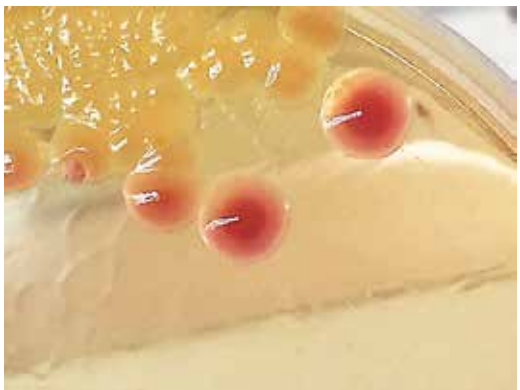
CIN (Cefsulodin, Irgasan, Novobiocin) agar was a highly selective medium designed to isolate *Yersinia enterocolitica*. The properties of this medium were based on selective chemical agents, antibiotics, dyes, and the basal medium. The characteristic deep red center (bulls-eye) with a transparent margin appearance of *Yersinia* colonies was important for identification, and was due to the fermentation of mannitol in the medium, producing an acid pH which gives the colonies their red color and the "bull's eye" appearance. Sodium deoxycholate, cefsulodin, irgasan, and novobiocin were added as selective agents. Colonies of *Y. enterocolitica* appeared small, medium and large mucoid with convex and shiny dew drop. Colonies of *Y. enterocolitica* have very offensive odour with characteristic pink pigmentation as clouds surrounding bull eyes colonies on CIN medium. Highly selective biochemical RapID™ One panel test kit (Oxoid, 2015) was used for identification of *Y. enterocolitica* and *E. coli*. Characteristic features of *Yersinia* colonies on MacConkey agar were tested as pale, large, mucoid, glistening tooth like structures. Biofilm producing strains were detected in most isolates by Christensen microtiter plate assay and Freeman Congo Red agar, then tested for antibiotic resistancy in another field (17-25).

A

A

Monographs (1): Y. enterocolitica colonies on CIN agar (A) and on MacConkey agar (B).

A



A

B

E. coli and their pathogenic serotypes *O157 H7* were recovered from some samples as a major contaminant pathogen even in cold stages of isolation, this may indicate strong relationship between these isolates in ice-creams.

The serotyping and biotyping of isolates within *Y. enterocolitica* species can be helpful in determining whether they are potential pathogens. In the absence of the antisera to serogroup *Y. enterocolitica* isolates in routine microbiology laboratories, significance remains a function of assessing an isolate for plasmid-encoded virulence factors. These tests were indirect but simple markers of pathogenicity that can be determined in most laboratories and include autoagglutination, production of V (immunogenic protein) and W antigens (nonprotective lipoprotein), serum resistance, calcium dependency for growth at 37 °C, Congo red and crystal violet binding tests, and even plasmid profiles. Other virulence assays include lethality for mice, production of conjunctivitis in guinea pigs (Sereny test), absence of pyrazinamidase activity, hydrolysis of esculin (25 °C), and fermentation of salicin (35 °C) (17-25).

Recovery of pathogenic *Y. enterocolitica* was contingent upon a number of factors including: the level of background flora on the product; the amount of background flora coming through enrichment and plating; the level of pathogenic *Y. enterocolitica* present on the sample; the numbers of non-pathogenic *Y. enterocolitica* and non-pathogenic *Yersinia spp*. present on the product; and loss of virulence factors during enrichment and plating. Furthermore, a recovery method which gives good recovery of one serotype of pathogenic *Y. enterocolitica* may not be suited to other serotypes. In order to recover any of the important pathogenic serotypes of *Y. enterocolitica* which might be present, multiple enrichment broths and plating media are usually recommended for the recovery of the organism from naturally-contaminated foods.

A great deal of effort must be expended in the recovery and characterization of presumptively-pathogenic *Y. enterocolitica*. Sequential levels of characterization tests include: identification of presumptive *Yersinia*, speciation to *Y. enterocolitica*, biogrouping the *Y. enterocolitica*, followed by testing for pathogenicity markers. *Y. enterocolitica* 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 control 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 unsanitary 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.

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