

Incidence of Pathogenic Bacteria in Cakes and Tarts Displayed for Sale in Tripoli, Libya

Sabrin M. Al-Jafaeri, Nuri S. Madi, and Mohamed H. Nahaisi

Abstract—This study was conducted to investigate the incidence of pathogenic bacteria: *Salmonella*, *Shigella*, *Escherichia coli* O157 and *Staphylococcus aureus* in cakes and tarts collected from thirty-five confectionery producing and selling premises located within Tripoli city, Libya. The results revealed an incidence of *S. aureus* with 94.4 and 48.0 %, *E. coli* O157 with 14.7 and 4.0 % and *Salmonella* sp. with 5.9 and 8.0 % in cakes and tarts samples respectively, while *Shigella* was not detected in all samples. In order to determine the source of these pathogenic bacteria, cotton swabs were taken from the hands of workers on the production line, the surfaces of preparation tables and cream whipping instruments. The results showed that the cotton swabs obtained from the hands of workers contained *S. aureus* and *Salmonella* sp. with an incidence of 42.9 and 2.9 %, the cotton swabs obtained from the surfaces of preparation tables 22.9 and 2.9 % and the cotton swabs obtained from the cream whipping instruments 14.3 and 0.0 % respectively; while *E. coli* O157 and *Shigella* sp. were not detected in all swabs. Additionally, other bacteria were isolated from the hands of workers and the Surfaces of producing equipments included: *Aeromonas* sp., *Pseudomonas* sp., *E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Serratia* sp. and *Acinetobacter* sp. These results indicate that some of the cakes and tarts might pose threat to consumer's health. Meanwhile, occurrences of pathogenic bacteria on the hands of those who are working in production line and the surfaces of equipments reflect poor hygienic practices at most confectionery premises examined in this study. Thus, firm and continuous surveillance of these premises is needed to insure the consumer's health and safety.

Keywords—Pathogenic bacteria, Cakes, Tarts, Confectionery producing and selling premises.

I. INTRODUCTION

FOOD diversity is essential in people life, and among the important types in the list of food are the confectionery which varies in forms, colors, decorations and ingredients. Cakes and tarts are famous confectionery in Libya. In addition to their high consumption rate during happy occasions, cakes and tarts became part of the daily requirements to the local consumer, where they are usually served with cold and hot drinks.

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The production of cakes and tarts are time consuming and characterized by very precise steps which include: preparation of raw materials and cake mixture, baking, cooling, filling, coating and ornamenting. The main ingredients in cakes and tarts include: sugar, flour, eggs, milk, fats along with other materials such as colors, flavors, emulsifiers, as well as the materials of ornamentation, which include fruits, nuts, natural and artificial ornamental materials, i.e. flowers, toys and others.

Because of their high content of carbohydrates, fats, proteins, minerals and vitamins, cakes and tarts considered of food products with high nutritional value, relatively expensive and sensitive to damage and spoilage [1], especially as they are usually sold fresh to the consumer. Thus, to protect public health, these products should be produced in accordance with good manufacturing and hygienic practices and displayed for sale under good hygienic practices. Unless these practices are followed, cakes and tarts exposed to microbial contamination.

The main sources which may contribute to the contamination of confectionery are the hands of workers on the production line, especially when they are suffering from infectious diseases and wounds or inflammations in their hands, in addition to the neglect of personal hygiene [2]. The hands of workers and the food contact surfaces may also contribute to microbial contamination during filling, coating and ornamenting, thus leading to an additional contamination in the final products [3].

The objective of this study was to investigate the incidence of pathogenic bacteria: *Salmonella*, *Shigella*, *Escherichia coli* O157 and *Staphylococcus aureus* in cakes and tarts collected from thirty five confectionery producing and selling premises located within Tripoli city in Libya.

II. MATERIALS AND METHODS

A. Collection of Samples

The samples were collected from thirty-five confectionery producing and selling premises located within Tripoli city in Libya. These samples included final products of cakes and tarts displayed for sale, and cotton swabs taken from the hands of workers on the production line and the surfaces of producing equipments.

Five samples of cakes and one sample of tart which were displayed for sale and handled by the personnel at the selling desk were taken from each confectionery premises.

The cotton swab samples were taken from the hands of workers on the production line, by taking swabs from the

hands, included under the nails and rings. Cotton swab samples were also taken from the surfaces of preparation tables and cream whipping instruments, including cracks and pits. Collected swab samples were kept separately on Amies Transport Medium, CM425.

All samples (cakes, tarts and cotton swabs) were transported to the microbiology laboratory at the biotechnology research center.

B. Preparation of samples for bacteriological analysis

Five samples of cakes and one sample of tart were mashed and mixed well separately in sterile conditions, then 10 g of each sample were mixed with 90 ml of Peptone Water, CM9 (Oxoid Ltd.), where homogenized for 1 to 2 min. For the isolation of *Staphylococcus aureus* and *Escherichia coli* O157 [4]. Meanwhile, 25 g of each sample were mixed with 225 ml of Buffered peptone water, CM509 (Oxoid Ltd.), where homogenized for 1 to 2 min. For the isolation of *Salmonella* and *Shigella* [5].

The cotton swab samples in Amies Transport Medium, CM425 (Oxoid Ltd.), were used for the isolation of pathogenic bacteria: *E. coli* O157, *S. aureus*, *Salmonella* and *Shigella*. and some other genera and species of bacteria.

C. Isolation and Identification of Pathogenic Bacteria from Cakes and Tarts

1. E. coli O157

The isolation of *E. coli* O157 was inoculated from homogenates into MacConkey broth, CM5 and Tryptone water, CM87 (Oxoid Ltd.), incubated at 37 °C ±0.5 for 24 to 48 h [6], also inoculated into Brilliant green lactose bile broth, CM31 and Tryptone water, CM87 (Oxoid Ltd.), incubated at 44 °C ±0.25 for 24 h [6].

The cultivating onto MacConkey Agar, CM7b and Eosin Methylene blue lactose Agar, CM69 (Oxoid Ltd.) from tubes whose the production of gas and positive indol test, incubated at 37 °C ±0.5 for 24 h, and identified typical colonies using the Indol, Methyl-red, Voges-Proskauer, Citrate utilization tests (IMViC tests) and Analytical Profile Index system (API 20E) to identify Enterobacteria (biomerieux Ltd.) [6], [7].

E. coli streaked onto Sorbitol MacConkey Agar, CM813 (Oxoid Ltd.), incubated at 37 °C ±0.5 for 18 to 24 h, then discriminated typical colonies (non-fermented sorbitol), and identified by Rapid Latex agglutination test for the detection of serological group by *E. coli* O157 test kit (WinLab Ltd.) [6].

2. S. aureus

The isolation of *S. aureus* was transferred 0.1 ml of the homogenates onto Mannitol salt Agar, CM85 (Oxoid Ltd.), by Surface spread Method, and incubated at 37 °C ±0.5 for 18 to 24 h, then discriminated typical colonies (fermented Mannitol), and gram staining, it was tested by biochemical tests (Catalase, Coagulase and DNase) [7], [8].

3. Salmonella and Shigella

The isolation of *Salmonella* and *Shigella* was prepared from 25 g of each sample into 225 ml of Buffered peptone water,

CM509 (Pre-enrichment medium), and incubated at 37 °C ±0.5 for 18 to 24 h, following incubation, 0.1 ml of each BPW incubated was transferred into 10 ml of Tetrathionate broth, CM29 and 10 ml of Selenite broth, CM395 (Oxoid Ltd.) (Selective-enrichment media), and incubated at 37 °C ±0.5 for 18 to 24 h. Then streaked onto Salmonella Shigella Agar, CM99 and Xylose Lysine Deoxycholate Agar, CM395 (Oxoid Ltd.) (selective media), and incubated at 37 °C ±0.5 for 18 to 24 h, then discriminated typical colonies, and identified by motile test and Biochemical tests (Hydrogen sulfide and Urease tests) and the API 20E to identify Enterobacteria (biomerieux Ltd.) [5], [7].

D. Isolation and Identification of Bacteria from the Hands of Workers and the Surfaces of Producing Equipments

The cotton swabs transferred from Amies Transport Medium, CM425, where cultivating onto different media such as Blood Agar, CM55, Mannitol Salt Agar, CM85, MacConkey Agar, CM7b, Eosin Methylene blue lactose Agar, CM69 (Oxoid Ltd.), these were incubated at 37 °C ±0.5 for 18 to 24 h.

For the isolation of *Salmonella* and *Shigella* was put cotton swabs into Buffered Peptone Water, CM509, and incubated at 37 °C ±0.5 for 18 to 24 h, following incubation, 0.1 ml of each BPW incubated was transferred into 10 ml of Tetrathionate broth, CM29 and 10 ml of Selenite broth, CM395 and incubated at 37 °C ±0.5 for 18 to 24 h. Then streaked onto Salmonella Shigella Agar, CM99 and Xylose Lysine Deoxycholate Agar, CM395, and incubated at 37 °C ±0.5 for 18 to 24 h.

All media were discriminated typical colonies, and identified using the biochemical tests [7], [9].

III. RESULTS AND DISCUSSION

A. Pathogenic Bacteria in Cakes and Tarts

Fig. 1 shows the incidence percentages of *S. aureus*, *E. coli* O157 and *Salmonella* sp. isolated from samples of cakes and tarts displayed for sale in confectionery premises.

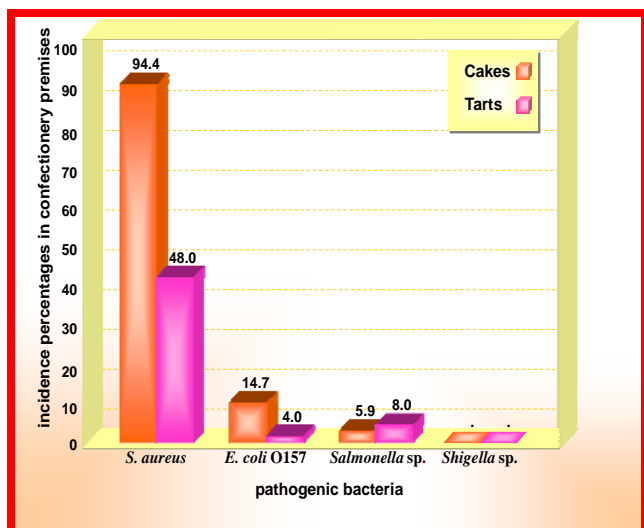


Fig. 1 Genera and species of pathogenic bacteria isolated from the cakes and tarts displayed for sale in confectionery premise

1. *S. aureus*

Incidence percentages of *S. aureus* in cakes and tarts were 94.4 and 48.0% respectively (Fig. 1). These results give an indication of poor of hygiene practices during the steps of producing, display and good cooling.

The recorded percentages of *S. aureus* in the current study were higher than those recorded by Viti & Marchi (1990) in Italy [10], for filled pastry with fresh cream, Pla et al (1996) in Spain [11], for cream cakes, chocolate cakes, coffee cakes and fruit cakes and Estefo & Savedra (1996) in Chile [12], for cakes filled with yoghurt, Chantilly cream and ricotta, i.e., 13.3, 3.2 and 2.6% respectively.

2. *E. coli* O157

Incidence percentages of *E. coli* O157 in cakes and tarts were 14.7 and 4.0% respectively (figure 1). There are several possibilities for the cause of the presence of this strain in cakes and tarts displayed for sale in confectionery premises which include: use fresh fruit for decoration, which is very likely to be contaminated with organic fertilizers and untreated sewage [13], especially if they are not washed properly, use of contaminated equipments for preparation and poor personal hygiene. In any way, it is in accordance with the directives of the microbiological quality of ready-to-eat food at the point of sale (FSAI Information Unit, 2001) [14], the cakes and tarts must not contain *E. coli* O157.

3. *Salmonella*

Salmonella sp. was isolated from cakes and tarts from two premises only, where the incidence percentages were 5.9 and 8.0% respectively (Fig. 1). These bacteria were isolated from cakes and a tart as well as from cotton swabs taken from the hands of workers on the production line and the surface of the preparation table in premise number 28. The possible source for these bacteria in the premise may be the worker that is very likely that he was diseased or carrier of bacteria, which led to the contamination of his hands, the table and the products, where the hygienic requirements at this premise were

very poor, especially in the toilets which is directly open into the manufacture room, in addition to the lack of cleaning and disinfection program. The other sources of contamination with this bacteria is likely some of the ingredients, especially eggs, raw milk and milk products, and fresh fruits [15].

The presence of *Salmonella* sp. in the final products of two premises (Number 28 and 31) and cotton swabs for the premise Number 28 represent a threat to the health of consumers for the products of these premises, and this is incompatible with the directives of microbiological quality of food ready to eat at the point of sale (FSAI Information Unit, 2001) [14], which provides cakes and tarts must not contain *Salmonella*.

The results of a study [10] about the presence of *Salmonella* sp. in samples of fresh confectionery filled with cream from confectionery premises in Italy were found to be all free of these bacteria. Also that *Salmonella* were not detected in 63 samples of cream cake displayed for sale in confectionery premises and supermarkets in Turkey [16].

4. *Shigella*

Presence of *Shigella* was not recorded in all cakes and tarts samples from premises considered in this study (Fig. 1). These results are in good agreement with the results of Pla et al., (1996) [11], where they found through the detection of bacteria in 311 samples of different cake types (cream cakes, chocolate cakes, coffee cakes and fruit cakes) in Spain that they do not contain *Shigella*.

B. Bacteria on the Hands of Workers and Surfaces of Equipments

Fig. 2 shows the incidence percentages of pathogenic bacteria and the genera and species of other bacteria isolated from hands of the workers and surfaces of equipments. The results showed that the cotton swabs obtained from the hands of the workers contained *S. aureus* and *Salmonella* sp. by 42.9 and 2.9%, the cotton swabs obtained from the surfaces of preparation tables 22.9 and 2.9% and the cotton swabs obtained from the cream whipping instruments 14.3 and 0.0% respectively; while *E. coli* O157 and *Shigella* were not detected in all swabs. Additionally, other bacteria were isolated from cotton swabs included *E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp., *Proteus* sp., *Aeromona* sp., *Acinetobacter* sp. and *Serratia* sp.

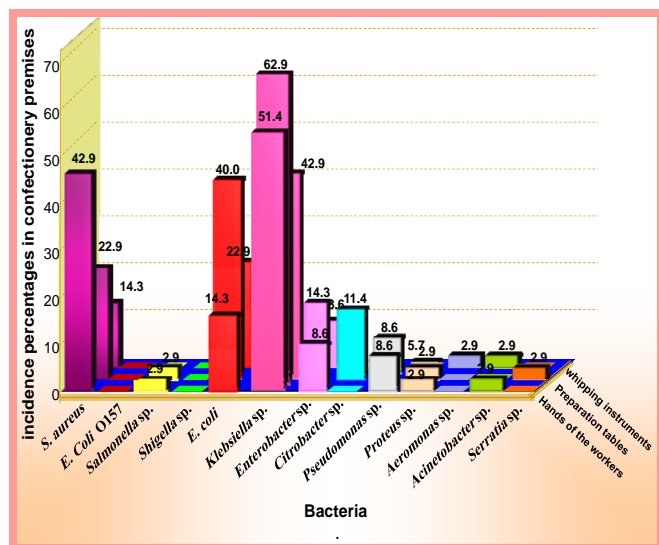


Fig. 2 The genera and species of bacteria were isolated from hands of the workers and surfaces of equipments in confectionery premises

The presence of pathogenic bacteria, genera and species of other different bacteria in cotton swabs which were taken from hands of the workers is an evidence to the poor level of cleanliness of workers' hands in confectionery premises considered in this study, which may conduce to the transfer of these bacteria from raw materials to final products during handling, especially as the production process of cakes and tarts usually need to be handled by the workers during the filling, coating and decorating process. Other accounts of contamination of the hands of the workers on the premises considered in this study are poor or inadequate facilities for personal hygiene in some premises, in addition to the lack of awareness of workers in most premises.

A study for the purpose of isolating *S. aureus* and *E. coli* from fingers of workers in 239 of food premises [17], did not isolate the *Salmonella* sp., a study has suggested that such cross-contamination becomes risk when there are accordant conditions for the generation of the bacteria in these products such premises, because of the contaminated hands of workers, where the surface preparation tables by cleaning when needed, were contaminated with *E. coli* by 24%, while those that cleaned after each use by 3%, noted in this study.

A relationship between contamination of hands, equipments and the cleaning system, where the conduce of contamination with *E. coli* contaminated cloth towels that were contaminated with the bacteria, which may have caused contamination of the hands and surfaces of equipment was reported by Tebbutt (1984) [17]. Similar observations were recorded on premises considered in this study in terms of use of cloth towels for cleaning, which are often dirty. Another study (Tebbutt and Southwell, 1989) [18] reported the isolation of *S. aureus* and *E. coli* by 27% and 16% respectively from samples taken from 136 rinse fingers of worker in 24 food premises and are less than that recorded in this study, i.e. 42.9% and 8.9% respectively. In another study conducted by Tebbutt (1991) [19] recorded the presence of *E. coli* in 5.1% of the hands of workers

and 9.8% of the surfaces of tables in 89 food premises, which is less than that recorded in this study, where the percentage of *E. coli* in the hands of workers and surfaces of tables by 14.3% and 40.0% respectively (Fig. 2).

Presence of *Klebsiella* sp. recorded the highest percentages among all bacteria isolated from swabs taken from hands of the workers and equipment surfaces in premises considered in this study, where the percentages of its presence on the surfaces of cream whipping instruments, preparation tables and hands of workers by 42.9, 51.4 and 62.9% respectively. Human stool, animal feces, soil and water represent the main sources of contamination with this bacteria, as well as fruits, milk and dairy products [20].

Less percentage of *Enterobacter* sp. were found in the hands of workers, cream whipping instruments and surfaces of preparation tables 8.6, 8.6 and 14.3%, respectively. The main sources of this bacterium are human stool, animal feces, water, soil, milk and dairy products. Presence of *Citrobacter* sp. on the surface of preparation tables by 11.4%. The main sources of this bacteria are human stool and animal feces, wastewater and others [20].

Pseudomonas sp. was found in the cream whipping instruments, surfaces of preparation tables and hands of workers by 5.7, 8.6 and 8.6%, respectively. These bacteria are spread widely in soil, water, fruits, milk and dairy products. It is possible that contamination of equipments with these bacteria from the spoilages in cool conditions, especially as a lot of types can hydrolyze protein and fat, and grow at low temperatures [20]. Other bacterial species, i.e., *Proteus* sp., *Aeromonas* sp., *Acinetobacter* sp. and *Serratia* sp. were isolated from cotton swabs, but with low percentages.

IV. CONCLUSION

The results obtained in this study showed the presence of some pathogenic bacteria in cakes and tarts displayed for sale in the confectionery premises located within Tripoli city, including: *S. aureus*, *E. coli* O157 and *Salmonella* sp. Pathogenic bacteria such as: *S. aureus* and *Salmonella* were not only isolated from the final products, cakes and tarts, but also from cotton swabs taken from the hands of some the workers on the production line and the surfaces of producing equipments.

The incidence of these pathogenic bacteria might pose threat to the health of the consumer. To minimize contamination, GHP, GMP and HACCP systems that are specific to the control of pathogenic bacteria at all steps of production, beginning from primary production, during the producing operations to display the final products.

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