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SPORE-FORMERS: MAJOR CONTAMINANTS OF "CHEESE MANAKISH"

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ABSTRACT

Food contamination is a major public health concern. "Manakish" are savory pastry popular in the Mediterranean region, as one of the traditional breakfasts. Freshly baked cheese Manakish from 16 popular bakeries were tested for the presence of bacterial contaminants, by using standard methods. While only three of the samples did not grow any bacterial contaminants, 10 samples from 10 different bakeries grew the endospores forming *Bacillus subtilis*, while 2 samples grew *Staphylococcus epidermidis*. Measures should be imposed to ensure proper handling of the Manakish and their ingredients, to avoid contamination that may be hazardous to the health of the consumers.

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INTRODUCTION

Food contamination is a universal public health concern. Although Food contamination can be unintentional during production, processing or food handling, sometimes contaminants are introduced as a way of prolonging a product's lifespan. The contamination can be microbial, physical or chemical. Microorganisms such as bacteria, viruses, and fungi are the most common food contaminants. Pathogenic bacteria cause food poisoning by either infecting the tissue of the individual or by producing a toxin that will later harm the individual (Doyle, 2018). Symptoms of food contamination depend on the type of pathogen; however, abdominal pain and cramps, diarrhea, nausea, and vomiting are the most common. After ingesting biologically contaminated food, there is usually a delay before symptoms develop (Laberge, 2013). In many cases, the food poisoning is mild but the young, the elderly, pregnant women, and immunocompromised patients (such as diabetes, cancer, HIV/AIDS, and transplant patients) are at a higher risk of developing severe food poisoning that may require hospitalization. Dairy products constitute a very favorable environment for the growth of different microorganisms due to their nutritional value (rich in lipids and proteins). Generally, several Gram-positive and Gram-negative bacterial species can contaminate these products. In particular, it was reported that *Pseudomonas* strains, ubiquitous in the environment, are the predominant species affecting dairy products (Raposo *et al*., 2017; Andreani and Fasolato, 2017). However, the type of dairy product dictates the type of microbial contamination. For instance, processed cheese was associated more with spore-forming bacteria. That dairy products may be carriers of pathogenic bacteria such as *Bacillus cereus, Brucella* spp., *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Mycobacterium paratuberculosis, Salmonella* spp., *Yersinia enterocolitica*, or *Staphylococcus aureus* has been established (Lu & Wang, 2017; Suilaiman & Hsieh, 2017). Suilaiman & Hsieh (2017) reported that cheese is very susceptible to *Salmonella sp.* as well as Shiga-like toxins producing *E. coli*. The presence of *E. coli* and coliform bacteria is an indication of poor hygienic conditions. The pathogenicity of enterotoxin-producing *Staphylococcus aureus* is linked to toxin-mediated virulence, invasive capacity, and antibiotic resistance (Carfora *et al*., 2015). The species *Bacillus cereus* that produce different toxins, is a spore former that challenges the dairy industry, adding the dairy products on the *Bacillus cereus*-contaminated food list (Tirloni *et al*., 2017; Grutsch *et al*., 2018). Bakery products are considered to be an important element in a balanced diet as a source of carbohydrates. As all food types, bakery products are also subject to contamination. Mostly, freshly baked products emerge with a sterile surface; it's the post-baking inappropriate handling of these products that lead to its contamination,

because of exposure to airborne contaminants and to contact with workers, equipment or surfaces. Manakish (singular: Mankousheh) is a savory pastry popular in the Eastern Mediterranean region, specifically in Lebanon, as one of the traditional Lebanese breakfasts. It consists of a dough topped with cheese, thyme, or ground meat. The dough is cooked in an oven with overly high temperature. It usually is folded in the middle, but could be sliced like a pizza, too. Manakish are also popular in other countries across the Levant as well as in Australia and the USA. Recently, Arabs and specifically Lebanese started opening Manakish bakeries in European countries, such as in France and England. The purpose of this study is to detect any bacterial contamination in samples of cheese Manakish bought from well-known bakeries in the Ras-Beirut area, a crowded region in the Lebanese capital Beirut.

MATERIALS AND METHODS

Samples tested: Sixteen freshly baked cheese Manakish samples were purchased from 16 different reputable and popular bakeries in the Ras Beirut area, a crowded region in Lebanese capital Beirut, during the months of February, March, and April, 2019. The Manakish were purchased by regular customers, left wrapped as purchased and immediately taken to the microbiology laboratory where they were aseptically processed within 10 to 15 minutes after purchase.

Processing of samples: Upon arrival to the microbiology laboratory, each sample was emptied into a sterile stomacher bag, mixed with 400 ml of sterile saline (0.85% NaCl) and homogenized. All the samples were processed in the same way. The extracts were immediately cultured. Sterile swabs were used to inoculate a Trypticase soy agar (TSA), MacConkey agar (MA), Salmonella-Shigella (SS) agar and Trypticase soy broth (TSB) and all were incubated for 18-24hr at 35.0°C. After incubation, any growth on the plates was isolated on TSA for identification. The TSB tube was used to inoculate a similar set of plates that were processed in the same way.

Identification of the isolates: The isolated colonies were identified by standard methods (Cowan and Steel, 1974). For the Gram positivecocci isolated, and in addition to the regular catalase and coagulase tests done, their growth on Mannitol Salt Agar (MSA) plates was used to reconfirm their identity.

RESULTS AND DISCUSSION

The results of this study showed that, unexpectedly, only 5 (31 %) of the freshly baked samples did not show bacterial growth, while 11 (69%) were heavily contaminated (Table 1). Of the 12 organisms isolated, 10 (91.5 %) were identified as the Gram-positive bacillus: *Bacillus subtilis* (91%), while the 2 remaining isolates were identified as the Gram-positive cocci: *Staphylococcus epidermidis* (9%). It was notable that the 10 *B. subtilis* isolates were from 10 different bakeries and that one sample grew both organisms (Table 1). It was noted that none of the samples grew Gram-negative organism. Foodborne diseases are major public health problem. These diseases result from microbial, chemical and/or physical contaminants. Microorganisms are a major source of such contamination (Doyle, 2018). Dairy products make a very favorable environment for bacterial growth due to its high nutrient value (Raposo *et al*., 2017; Andreani and Fasolato, 2017).

Table 1. The identity of the isolates from the different samples included in this study. NG: No growth

Sample / Bakery	Identified isolate
1	*Bacillus subtilis*
2	*Bacillus subtilis*
3	*Staphylococcus epidermidis.*
4	NG
5	NG
6	*Bacillus subtilis*
7	NG
8	NG
9	*Bacillus subtilis*
10	NG
11	*Bacillus subtilis*
12	*Staphylococcus epidermidis.* *Bacillus subtilis*
13	*Bacillus subtilis*
14	*Bacillus subtilis*
15	*Bacillus subtilis*
16	*Bacillus subtilis*

Bakery products usually emerge with a sterile surface upon heating in an oven, yet the post-baking inappropriate handling of the products is what usually leads to contamination (Saranraj and Geetha, 2012). The dough of Manakishis cooked in an oven with overly high temperature. Bacterial contaminants are expected to die at such a high temperature, except if they were in a spore form. Spores are known to endure extreme conditions such as exposure to wet and dry heat, desiccation, radiations, bases, acids and other chemicals. Resistance factors include their spore coat, protection of DNA by small-acid soluble proteins, accumulation of divalent cations such as Ca^{2+}, and dehydration (low water content) of the spore core. (Setlow, 2014; Hilbert, 2004; Kort *et al*., 2005; Setlow and E. A., 2001). Spores of some species cause food spoilage, food poisoning, and human diseases. The gram-positive Bacilli spore-formers could belong to either *Bacillus* or *Clostridium* species. Of these two genera, bakery products were specifically found to be contaminated by *Bacillus* species. The two genera were also isolated from milk products, even when milk is sterilized, pasteurized, dehydrated or fermented with heat treatment or storage temperature (André, Vallaeys, & Planchon, 2017). Moreover, the spores of members of the *Bacillus* genus, specifically *Bacillus subtilis* and *Bacillus cereus* can be present in raw ingredients like flour and yeast (Saranraj and Geetha, 2012). This indicates that spores of the *Bacillus* species could be found in cheese and/or dough even after heating.

Ten of the isolates in this study, isolated from the Mnakish from 10 different bakeries, were identified to be *B. subtilis*. Although *B. subtilis* is considered to be of low pathogenicity, yet some strains are responsible for causing ropiness, a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides in spoiled bread dough (Lefevre *et al*., 2017). Despite older reports that identified *B. subtilis* as a cause of serious infections (Bais, 1927), the organism has received little clinical attention, as it was later associated only with opportunistic infections of immunocompromised patients (Ihde and Armstrong, 1973; Reller, 1973). A recent study, however, proved that in principle *B. subtilis* is capable of being a very pathogenic organism as its genome harbored all the genes responsible for the pathogenicity of other *Bacillus* species (Gu *et al*., 2019). The results of this study also showed that 2 samples obtained from 2 different bakeries were contaminated with *Staphylococcus epidermidis*. *S. epidermidis*

is a non-motile, non-spore forming, facultatively anaerobic Gram-positive coccus that is found in the normal flora of humans as it colonizes the nasal mucosa and skin (Namvar *et al*., 2015). *S. epidermidis* is known to cause nosocomial infections with the increase use of medical implant devices, as it can switch from a harmless colonizing to an invasive life-style. Being an opportunistic pathogen is greatly linked to the ability of this bacterium to form a biofilm (Büttner, Mack, & Rohde, 2015). This potential pathogen, although implicated to cause manyhospital acquire infections, is still considered relatively safe in food (in certain quantities), but may be a clue to improper handling of food. A recent study in April 2019, by Chajęcka-Wierzchowska and colleagues, manifested that *Staphylococcus epidermidis* is widely present in Artisanal cheese made of raw milk in Poland. Their results demonstrated that 76.1% of *S. epidermidis* strains isolated from the cheeses were multidrug resistant to penicillins, tetracyclines, and erythromycin. In addition to antimicrobial resistance, those strains contained more virulence factors and carried mobile genetic elements which represented a possible source of resistance transmission to bacteria in humans (Chajęcka-Wierzchowska *et al*., 2019). Thus, although *S. epidermidis* can be abundant in the skin microflora, yet, their biofilm formation ability and antimicrobial resistance increase the possibility of them being pathogenic if found in food.

The health threat posed by these contaminants should not be underestimated. The presence of these contaminants, however, suggests that other more pathogenic food borne pathogens may also be able to contaminate the cheese Manakish. To prevent this contamination, regulation and control measures must be taken at all stages of food preparation; from milk processing, to cheese production, to flour production, transportation and storage before use, dough making and handling, ending in dough and cheese spreading for baking. In addition, strict measures must also be imposed for the post-baking handling of the food.

Conclusion

This study confirms that “cheese Manakish” can be contaminated with food borne pathogens causing a public health risk. Control measures must be imposed to minimize bacterial contamination and ensure safe consumption of the “cheese Manakish” by the public.

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