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Examination of Pathogenic Bacteria in Some Cake Samples

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Abstract

Thirty cake samples of twelve different companies were collected from Damietta and Dakahlia governorates in September and October 2012. All samples were in production date except one (A4) was expired. Thehigh contamination of examined cakes with mesophilic bacteria were found in A4 sample (286.76×10^3cfu/g) while the lowest value was found with B22 sample (0.12×10^3cfu/g). Samples of C11 (21.30×10^3) and A5 (0.07×10^3) cfu/g were the highest and the lowest values of thermophilic bacteria, respectively. Staphylococci presented in all examined cake samples except seven samples. The highest value was 3.50×10^3cfu/g in C13 sample followed by 2.90×10^3cfu/g in A14 sample. *Aeromonas* spp. was presented in all examined cake except A4 sample. The highly contaminated samples with *Aeromonas* spp. in case of A5 being 2.73×10^3cfu/g. The pathogenic enteric bacilli were found in all examined cake samples except sixteen which gave negative results. Results in positive samples were between 0.02×10^3 to 0.23×10^3cfu/gin D28 and C13, respectively. Eleven samples gave a negative results and nineteen were positive for coliform bacteria. Seven of cake sample gave negative results of *Bacillus cereus* while other samples, gave positive results ranged between 0.02×10^3 and 1.54×10^3cfu/g in C21 and D9, respectively. Twenty examined samples have not *Vibrio* spp. Results proved that C12 was the highest count being 2.92×10^3cfu/g. All samples were not in conformity with the Egyptian Organization for Standardization and Quality of cake (ES: 4037/2005). Forty-two bacterial isolates were obtained and identified according to the microscopic and biochemical examination. Obtainedbacterial isolates were identified as *Bacillus cereus*, *Bacillus sublilis*, *Aeromonas* spp., *Pseudomonas* spp., *Echerichia* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Micrococcus* spp. and *Staphylococcus aureus*.

Keywords

Cake, Bacterial Examination, *Bacillus cereus*, *Aeromonas* spp., *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus*

1. Introduction

Microbial spoilage is the major problem causing deterioration in bakery products. It is caused mainly by moulds and yeasts and occasionally by bacteria. Losses due to microbial spoilage vary between 1% and 5% of products depending on season, type of product and method of processing as reported before [1]. The interference between low pH, high water activity (a_W) filling materials and low a_W cake may support growth and toxins production by microorganisms even when the individual ingredients do not support growth. Similarly, preservatives in an aqueous ingredient may migrate to a fatty phase when mixed with a high fat ingredient, which may subsequently allow growth of spoilage microbiota or pathogens [2]. As reported before, ambient temperatures, product pH levels between 5.4 and 7.5, and water activity in range of 0.75–0.98 promote spoilage of baked cereal foods with mold, yeast, and spore forming bacteria. Water activity (a_w) is a particularly important factor influencing spoilage of cereal products, and many bakery products such as breads and cakes have levels above 0.94. Although relatively harmless, their visible presence deters customers and can lead to substantial economic losses to whole sale bakeries [3]. Pastries include cakes and baked shells filled with custard, cream, or sauces.

They can be spoiled by microorganisms coming with the ingredients that are added after baking, such as icing, nuts, toppings, and cream. Most products, because of low a_w, allow only molds to grow. However, some materials used as fillings may have high a_w, which allows for bacterial growth [4]. As with bread, mold growth is the predominant spoilage problem for pastries. However, the pastry filling or topping may be more susceptible to microbial growth than the cereal product. Many fillings support the growth of spoilage bacteria, especially if they have high a_w, near to neutral pH, and contain high protein ingredients such as meat, egg or milk. Cooked fillings spoilage from spore formers that survive the cooking, other bacteria introduced after the cooking step, or those that survive inadequate cooking [5]. The purpose of this work was to examine the pathogenic bacteria contaminated some of cake samples in local markets. Moreover, the bacterial isolates were identified based on the microscopic examination and biochemical tests using different cultivation media.

2. Material and Methods

2.1. Cake Samples and Collection

Thirty samples of different production companies (12 companies) were collected from Damietta and Dakahlia governorates in September and October 2012. These samples were 9 chocolate cake (first group); 6 chocolate and other additions (second group); 6 cream cake (third group) and 9 cream cake and other additions (fourth group). These samples were divided into four groups. The first group was chocolate cake, and it was composed of nine samples.

2.2. Preparation of Cake Samples for Bacterial Examination

Ten gram of each examined sample mentioned above was suspended in 90.0 ml sterilized tap water then serial dilutions were prepared. Each sample was well mixed to be homogenized solution using a Vortex (VM-300 power: 220 VAC, 50Hz, 0.16A/Made in Taiwan-Associated with Cannic, Inc U.S.A.). One ml of each dilution was put into Petri dish, and then melted suitable cultivation medium was poured and mixed well then left to solidify. Petri dishes were then incubated at the suitable temperature for appropriate period of time (Table 1) and obtained developed single microbial colonies of different morphologies in all used cultivation media were picked-up. Colonies were transferred onto suitable media plates for sub culturing to obtain pure bacterial isolates [6].

2.3. Bacterial Groups Count of Cake Samples

Pouring plat method was used for counting misophilic bacteria (MB); thermophilic bacteria (TB); Staphylococci (SC); *Aeromonas* (AC); Enteric bacilli (EB); *Bacillus cereus* (BC) and *Vibrio* count (VC) (Table 1). Staphylococcal count monitoredon *Staphylococcus* agar No. 110 medium that produce colonies with yellow-orange pigment. *Aeromonas* selective agar medium was used for counting *Aeromonas* colonies that showing a visible yellow colour. Pathogenic enteric bacilli count was done on *Salmonella Shigella* agar medium where *Salmonella* spp. was black-center colonies, *Shigella* spp. colorless colonies and *E. coli* pink to red colonies. Blue color colonies surrounded by a good precipitate of the same color is considered *Bacillus cereus* on *Bacillus cereus* selective agar medium. A yellow, shiny colony on alkaline peptone agar medium was *Vibrio* spp. [7]. The presence of coliform bacteria was tested by adding one ml of each dilution to test tube containing Durham tubes in three replicates; containing 7.0ml of MacConkey broth medium and then placed in incubator at 37°C for 24 h. the tubes which had yellow color and gas production is considered positive results [8].

Table 1. *Cultivation media and incubation conditions used for the pathogenic bacterial examination of cake samples.*

Examined bacteria	Cultivation media	Incubation conditions	
		Temperature (°C)	Time (h.)
Misophilic bacterial count (MB)	Nutrient agar	30	72
Thermophilic bacterial count (TB)	Tryptone glucose beef extract agar (TGBE)	45	72
Staphylococci count (SC)	*Staphylococcus* agar No. 110	37	72
Aeromonas count (AC)	*Aeromonas* selective agar	30	72
Enteric bacilli count (EB)	*Salmonella Shigella* agar	37	48
Bacillus cereus count (BC)	*Bacillus cereus* selective agar	37	72
Vibrio count (VC)	Alkaline peptone agar	37	72
Coliform bacteria (TC)	MacConkey broth	37	24

2.4. Isolation and Identification of Obtained Bacterial Isolates

Single bacterial colonies of different morphologies developed on different media were picked-up. Colonies were transferred onto suitable cultivation medium for sub-culturing to obtain pure isolates. Isolation was done on suitable media as mentioned above (Table 1). Identification the obtained bacterial isolates was done according to [4, 9, 10 and 11]. Morphological tests such as shape, arrangement of the bacterial cells as well as the Gram reaction was microscopically examined in stained preparations of 24-48 hrs old bacterial cultures. Motility test was observed in fresh cultures, by hanging drop preparation, for 18hrs old broth cultures. Presence and shape of endospores were recognized in stained smears using Schaeffer and Fulton's method after 4 days old cultures. Also, Biochemical tests such as Catalase test, Kovacs oxidase test, Indole production, Methyl red test (M.R.), Voges Proskauer test (V.P.) and Citrate utilization test.

2.5. Maintenance of Bacterial Isolates

Bacterial isolates were maintained on specific cultivation medium slants at 5°C till use. Before use, the bacterial isolates were sub-cultured on new slants of specific cultivation medium and incubated at the suitable temperature for appropriate period of time (Table 1).

3. Results and Discussion

3.1. Bacterial Examination of Cake Samples

The pathogenic bacterial examination of cake samples were presented in Table 2. The components of cake samples differed in size, shape, quality, taste and fillings. The fillings of examined cake samples were chocolate cake filled with chocolate; cake with chocolate and other additions; cake with cream and other additions and cream cake. These samples were divided into four groups. The first group was chocolate cake, and it was composed of nine samples.

3.1.1. Mesophilic Bacteria

Results presented in Table 2 showing that, the highest value of mesophilic bacterial count in the first group (chocolate cake) was 286.76×10^3cfu/g in A4 sample. while the lowest value was 0.51×10^3cfu/g in A25 sample. The second group was chocolate cake and other additives. The highest value of the second group was 13.31×10^3cfu/g in B10 sample, while the lowest value was 0.12×10^3cfu/g in B22 sample. The highest contaminated sample was D9 having 5.42×10^3cfu/g in the cream cake samples (third group), followed by D16 and D28 sampleswhich giving 3.09×10^3 and 2.45×10^3cfu/g, respectively. The highest value of total count of mesophiles of cream cake with other additives (fourth group) was C12 being 93.28×10^3cfu/g, but the lowest value was C21 being 0.28×10^3cfu/g.

Generally, high contamination of examined cakes with mesophilic bacteria was found in A4 sample (286.76×10^3cfu/g) while the lowest value was B22 sample (0.12×10^3cfu/g).

These results are in a good line with those obtained by [12]. They found that cakes of all types rarely undergo bacterial spoilage due to their unusual high concentrations of sugars, which restrict the availability of water. The most common form of spoilage displayed by these products is moldiness. The obtained results are in accordance with those obtained before [13], who reported that, total plate counts varied between 6.80×10^3 to 13.63×10^3cfu/g cream cake samples. In a German survey of cakes, 50% of examined samples had aerobic microbial counts of 1–5 × 10^6cfu/g, 16% were>10^6cfu/g, 6.5% were >10^7cfu/g, with the highest count was more than 10^9cfu/g [5]. All samples were not in conformity with the standard specifications of the Egyptian cake ES: 4037/2005 [14].

Table 2. *Total Bacterial count and collection of cake samples from different companies.*

Sample No.	Examined cake samples	production company Code	Total count of examined microbial groups by (cfu/g)×10^3			
			Mesophiles	Thermophiles	Staphylococci	*Aeromonas* spp.
A1	Chocolate cake (Frist group)	CO.1	4.82	0.10	ND	0.06
A2		CO.1	19.42	0.26	ND	0.03
A3*		CO.11	4.89	0.14	ND	0.04
A4**		CO.5	286.76	3.25	ND	ND
A5*		CO.11	1.99	0.07	ND	2.73
A8		CO.6	33.11	16.05	0.36	0.10
A14		CO.7	0.92	0.24	2.90	0.13
A15		CO.2	0.91	0.35	0.94	0.35
A25		CO.8	0.51	0.09	0.27	0.22
B6*	Chocolate cake and other additives (Second group)	CO.11	4.70	1.88	ND	1.38
B10		CO.4	13.31	2.96	0.21	0.89
B18		CO.1	2.28	0.10	0.43	0.43
B19		CO.2	0.19	0.11	0.16	0.85
B22		CO.9	0.12	0.21	0.95	0.30
B27		CO.2	1.19	0.96	0.04	0.13
D9	Cream cake (Third group)	CO.3	5.42	2.33	0.11	0.22
D16		CO.1	3.09	0.08	0.18	0.30
D24		CO.3	0.78	0.16	0.08	0.26
D26		CO.2	0.89	0.20	1.21	0.07
D28		CO.2	2.45	0.70	0.08	0.22
D30		CO.1	0.76	0.11	ND	0.07
C7*	Cream cake and other additives (Fourth group)	CO.12	3.11	11.96	0.03	0.30
C11		CO.4	13.69	21.30	0.06	0.34
C12		CO.10	93.28	1.45	0.66	0.12
C13		CO.1	1.25	0.75	3.50	0.88
C17		CO.2	2.55	0.14	1.21	0.69
C20		CO.2	0.34	0.13	0.29	0.53
C21*		CO.11	0.28	0.18	0.93	0.27
C23		CO.1	1.77	0.09	0.04	0.04
C29		CO.3	2.05	1.24	0.87	0.14

Table 2. *Continued.*

Sample No.	Examined cake samples	production company Code	Total count of examined microbial groups by (cfu/g)×10^3			
			Enteric bacilli	Coliform group	*Bacillus cereus*	*Vibrio* spp.
A1	Chocolate cake (Frist group)	CO.1	0.06	-	ND	ND
A2		CO.1	0.16	+	ND	0.03
A3*		CO.11	0.09	-	ND	0.02
A4**		CO.5	0.15	+	ND	0.06
A5*		CO.11	0.14	+	0.14	0.07
A8		CO.6	0.08	+	0.66	0.18
A14		CO.7	ND	-	0.05	ND
A15		CO.2	ND	-	0.15	ND
A25		CO.8	ND	-	0.11	ND
B6*	Chocolate cake and other additives (Second group)	CO.11	0.03	+	0.03	0.06
B10		CO.4	ND	+	ND	0.06
B18		CO.1	ND	-	1.28	ND
B19		CO.2	0.02	+	0.75	ND
B22		CO.9	ND	-	0.02	ND
B27		CO.2	0.02	-	0.04	ND
D9	Cream cake (Third group)	CO.3	ND	-	1.54	0.46
D16		CO.1	0.03	+	ND	ND
D24		CO.3	0.14	-	0.05	ND
D26		CO.2	ND	-	0.33	ND
D28		CO.2	0.02	+	0.14	ND
D30		CO.1	ND	-	0.05	ND
C7*		CO.12	ND	-	0.03	ND
C11		CO.4	0.06	+	ND	0.58
C12		CO.10	ND	+	0.18	2.92
C13	Cream cake and other additives (Fourth group)	CO.1	0.23	-	0.20	ND
C17		CO.2	ND	-	0.02	ND
C20		CO.2	ND	-	0.10	ND
C21*		CO.11	ND	-	0.02	ND
C23		CO.1	ND	-	0.02	ND
C29		CO.3	ND	-	0.12	ND

ND: not detected

* These products were imported abroad.

**Expired sample

3.1.2. Thermophilic Bacteria

All samples listed in Table2 showed to be contaminated with thermophilic microorganisms. The highest value of thermophilic bacterial count of chocolate cake (16.05×10^3) cfu/g for sample A8 while sample A5 was the lowest one (0.07×10^3) cfu/g. Results of the second group (chocolate cake and other additives) proved that B10 sample was the highest contaminated one while B18 was the lowest sample to be 2.96×10^3 and 0.10×10^3cfu/g, respectively.

Results of the third group (cream cake samples exhibited that the highest contaminated sample was D9 showing 2.33×10^3cfu/g and the lowest sample was D16 which gave 0.08×10^3cfu/g. Results of cream cake with other additives (the fourth group) showed that, the total count of thermophilic bacteria ranged between 21.30×10^3 and 0.09×10^3cfu/g for C11 and C23, respectively. Generally, samples of C11 (21.30×10^3) and A5 (0.07×10^3) cfu/g were the highest and the lowest values of thermophilic bacterial count, respectively. Fillings may be fully cooked by baking with the casing or cooked separately in bulk and filling into a baked casing or cream pies or spread onto a baked cake, cream cake.

The main microorganisms of those products are fungi and spore-forming bacteria as reported before [7]. Therefore, it is not fully cooked fillings other contamination from the bacteria was presented in cake samples. Preservatives in an aqueous ingredient may migrate to a fatty phase when mixed with a high fat ingredient, which may subsequently allow growth of spoilage microbiota or pathogens as reported before [2].

3.1.3. Staphylococci Bacteria

Results of staphylococcal count are presented in Table 2. Staphylococci presented in all examined cake samples except A1, A2, A3, A4, A5, B6 and D30 samples. The highest value was 3.50×10^3cfu/g in C13 sample followed by 2.90×10^3cfu/g in A14 sample. In Belgian study on the cake, aerobic plate counts were higher than about 12% of examined samples contained 10^1 to 10^2cfu/g of *Staphylococcus aureus* [7].

The obtained results are in line with those [15] who found that *Staphylococcus aureus* in examined chocolate cake was 26.6% of the total examined samples (>10^2cfu/g). The interference between a low pH, high a_W of filling and a low a_W cake may support growth and toxins production by *Staphylococcus aureus* even when the individual ingredients do not support growth. Similarly, preservatives in an aqueous ingredient may migrate to a fatty phase when mixed with a high fat ingredient, which may subsequently allow growth of

spoilage microbiota or pathogens [2].

The obtained results are in line with those [16] who reported that, enterococci was 5.9% of cream cake samples at level of ≥ 10^6cfu/g, micrococci/staphylococci were found in (23.5%) cream cake samples at level of ≥ 10^4cfu/g.

3.1.4. *Aeromonas* spp.

Aeromonas spp. was presented in all examined cake samples except A4 sample as shown in Table 2. The highly contaminated sample with *Aeromonas* spp. was in case of A5 being 2.73×10^3cfu/g.

Similar results were obtained [17] who isolated *Aeromonas* spp. from bakery products samples obtained from the Salem, Erode, Tiruppur, Namakkal and Coimbatore districts of Tamil Nadu, India. Detection of enterotoxigenic *Aeromonas* sp. from cream is not uncommon. In India, 285 food samples were examined comprising fish (100), milk (85) and cream (100). *Aeromonas* sp. was found in 40 (14%) samples examined with predominance of *A. hydrophila*. More than 50% of isolated *Aeromonas* produced enterotoxin [18].

3.1.5. Pathogenic Enteric Bacilli

The pathogenic enteric bacilli were counted and obtained results are listed in Table 2. It was observed that, the pathogenic enteric bacilli were found in all examined cake samples except A14, A15, A25, B10, B18, B22, D9, D26, D30, C7, C12, C17, C20, C21, C23 and C29 which gave negative results. Results in positive samples were between 0.02×10^3 to 0.23×10^3cfu/g in D28 and C13, respectively. Microorganisms in foods such as *E. coli* have been found in real and limitation cakes and pastries from a variety of manufacturers. Cooked foods such as cakes, and angel cake could be cross-contaminated in the preparation area from a contaminated egg hazards from *Salmonellae ingredient*, particularly from a dried egg product [7]. *Salmonella* spp. have been found in cream and chocolate cakes [19]. People have been poisoned (111) by the bacterium *Salmonella* sp. through the cake [20]. A wide range of contaminated foods is associated with *Salmonella* food poisoning, including eggs, milk and dairy products, cake mixes, cream-filling desserts and chocolate as explained before [21].

3.1.6. Detection of Coliform Bacteria

Eleven samples were obtained negative results and nineteen were positive for coliform bacteria (Table 2). The obtained results are in line with those of [22] who reported that, coliform bacteria were abs /0.1 g of chocolate and cream cake samples. Also, chocolate cake samples were examined for coliform bacteria [15] who found their presence in ratio of 83.3% of the total count (>10^2cfu/g). Untreated cream posses similar risks to untreated milk, possibly increased by the additional handling in its preparation. Untreated cream was implicated in an outbreak of *E. coli* 0157 phage type 2 food poisoning in the United Kingdom [18]. Similar results were obtained [16] who found that, coliform was presented in 11.8% of cream cake samples at level of ≥ 10^4cfu/g. The bacterial counts for cake, meat pie and egg roll on MacConkey agar gave comparable results when considered with counts obtained on deoxycholate agar, and ranged from 4.1–4.2 $\log_{10}$cfu/g and 4.18–4.34 $\log_{10}$cfu/g, respectively [23].

3.1.7. *Bacillus cereus*

Among all samples which examined for *Bacillus cereus*, seven of them gave negative results such as A1, A2, A3, A4, B10, D16 and C11 while other samples gave positive results ranged between 0.02×10^3 and 1.54×10^3cfu/g in C21 and D9, respectively. In Belgian study on cake, aerobic plate count were high, since 22% of examined samples contained >10^5cfu/g of *B. cereus*. Many fillings support the growth of spoilage bacteria especially if they have a high a_w, near to neutral pH, and contain high protein ingredients such as meat, egg or milk. Cooked fillings spoil from spore formers that survive the cooking, other bacteria introduced after the cooking step, or those that survive inadequate cooking [7]. On this respect, [16] reported that, *B. cereus* was detected in 10 (59%) of cream cake samples at level of ≥ 10^2cfu/g. *B. cereus* can be isolated from a remarkable range of different foods and food ingredients, dairy products and dried foods [24].

3.1.8. *Vibrio* spp.

Twenty examined samples have did *Vibrio* spp. as shown in Table 2. Results proved that C12 was the highest count being 2.92×10^3cfu/g. The witness of these results that many researchers believe high-sugar icings or low-pH toppings (i.e. fruit) will not support growth of spoilage bacteria but will eventually permit fungi to grow [7]. The highest load of *Vibrio cholerae* was found in bakery products. Cake and bread collected from a bakery food shop in Siddeswari area showed higher load of contamination being (2.6×10^6cfu/g) and (1.1×10^6cfu/g), respectively [25]. All samples were not in conformity with the standard specifications of the Egyptian cake (ES: 4037/2005) [14].

3.2. Identification of Bacteria Isolated from Cake Samples

Forty-two bacterial isolates were obtained from twelve different cake producing companies as shown in Table 3. The obtained bacterial isolates from either cream or chocolate cake were divided into different groups according to the microscopic and biochemical examination.

3.2.1. Gram-Positive, Endospore Forming Rods Bacteria

The morphological and biochemical of this group characteristic (G1) showed that the cells were bacilli in shape, positive to Gram stain, endospore forming cells, motile, catalase positive, M. R. positive. As shown in Table 4, bacterial isolates of G1 (No. 4, 7 and 39) oxidase negative, indole negative, V. P. test positive, and citrate utilization positive. These isolates were identified and designated as *Bacillus cereus* according to the protocol of [4 and 9]. The morphological and biochemical tests of the other bacterial isolates of G2 showed that the cells were bacilli in shape, positive to Gram stain, endospore forming cells, motile,

catalase positive, M. R. positive. Three isolates of G1 (No. 26, 27 and 34) oxidase positive (Table 4), indole negative, V.P. test positive, and citrate utilization positive. From these results, these isolates can be designated as *Bacillus sublilis* according to the identification protocol [4 and 9].

Table 3. *Bacterial isolates obtained from cake samples and their sources.*

Isolates Group	Isolates No.	Cake Production Companies						
		Co1	Co2	Co3	Co4	Co5	Co6	Co7
G1	4, 7, 39	*		*			*	
	26, 27, 34				*	*		*
	8, 9, 21, 22, 23, 28, 2948, 49, 50	**			*		*	*
	12, 18, 19, 20, 35, 38	*			*		*	
G2	30, 45, 51		*	*				
	36, 44		*	*				
	13, 37, 52		*	*				
	15, 46, 47			*		*		
G3	2, 3, 10, 31, 32	**						*
	1, 16, 17, 33		*					
	∑	6	4	5	3	2	3	3

Table 3. *Continued.*

Isolates Group	Isolates No.	Cake Production Companies					∑
		Co8	Co9	Co10	Co11	Co12	
G1	4, 7, 39						3
	26, 27, 34						3
	8, 9, 21, 22, 23, 28, 2948, 49, 50			*	****		10
	12, 18, 19, 20, 35, 38	*		*	*		6
G2	30, 45, 51	*					3
	36, 44						2
	13, 37, 52				*		3
	15, 46, 47					*	3
G3	2, 3, 10, 31, 32		*		*		5
	1, 16, 17, 33				*	**	4
	∑	2	1	2	8	3	42

* The isolates which chosen for the identification.

3.2.2. Gram Negative, Rods Bacteria

The morphological and biochemical tests of the bacterial isolates of G3 showed that the cells were rods, negative to Gram stain, non spore forming, motile, catalase positive. Ten isolates (No. 8, 9, 21, 22, 23, 28, 29, 48, 49 and 50) were oxidase positive. These isolates were identified and designated as *Aeromonas* spp. [9].

The morphological and biochemical tests of the bacterial isolates of G2 showed that the cells were rods, negative to Gram stain, non spore forming, motile, catalase positive, M.R. negative. Six isolates of G2 (No. 12, 18.19, 20, 35 and 38) were oxidase positive, indole negative, V.P. test negative, and citrate utilization positive. From these results, these isolates were designated as *Pseudomonas* spp. according to the identification protocol [4 and 9].

The morphological and biochemical tests of the bacterial isolates of G2 showed that the cells were rods, negative to Gram stain, non spore forming, motile, catalase positive, M.R. positive. Three isolates of G2 (No. 30, 49 and 51) were oxidase negative, indole negative, V.P. test negative, and citrate utilization positive (Table 4). From these results, these isolates designated as *Salmonella* spp. according to the identification protocol [4 and 9].

The morphological and biochemical tests of the bacterial isolates of G2 showed that the cells were rods, negative to Gram stain, non spore forming, motile, catalase positive, M.R. positive. Isolates of G2 (No. 36 and 44) were oxidase negative, indole negative, V.P. test negative, and citrate utilization negative (Table 4). These isolates were designated as *Shigella* spp. according to the protocol of [4 and 9]. The morphological and biochemical tests of the bacterial isolates of G2 showed that the cells were rods, negative to Gram stain, non spore forming, motile, catalase positive, M.R. positive. Three isolates of G2 (No. 13, 37 and 52) were oxidase negative, indole positive, V.P. test negative, and citrate utilization negative (Table 4). From these results, these isolates can be identified and designated as *Escherichia coli* according to the identification protocol [4 and 9]. Bacterial isolates of G2 were Gram-negative, curved rods which were motile. Three isolates of G2 (No. 15, 46 and 47) catalase and oxidase-positive (Table 4). These isolates were designated as *Vibrio* spp. [26].

3.2.3. Gram Positive, Cocci Bacteria

The morphological and biochemical tests of the bacterial

isolates showed that the cells were cocci, positive to Gram stain, non spore forming, non motile, catalase positive. Five isolates of G3 (No. 2, 3, 10, 31 and 32) were oxidase negative (Table 4). These isolates were identified and designated as *Staphylococcus aureus* [9]. The morphological and biochemical tests of the bacterial isolates showed that the cells were cocci, positive to Gram stain, non spore forming, non motile, catalase positive. Four isolates of G3 (No. 1, 16, 17 and 33) were oxidase negative (Table 4). These isolates were designated as *Micrococcus* spp. [9].

Table 4. *Biochemical tests of the obtained bacterial isolates.*

Isolates Group	Isolates No.	Morphological tests				
		Shape	Gram stain	Motility	Spore formation	Catalase
G1	4, 7, 39	Rod	+	+	+	+
	26, 27, 34	Rod	+	+	+	+
G2	8, 9, 21, 22, 23, 28, 2948, 49, 50	Rod	-	+	-	+
	12, 18, 19, 20, 35, 38	Rod	-	+	-	+
	30, 45, 51	Rod	-	+	-	+
	36, 44	Rod	-	+	-	+
	13, 37, 52	Rod	-	+	-	+
	15, 46, 47	Curved	-	+	-	+
G3	2, 3, 10, 31, 32	Cocci	+	-	-	+
	1, 16, 17, 33	Cocci	+	-	-	+

Table 4. *Continued.*

Isolates Group	Isolates No.	Biochemical tests					Bacteria suspected
		Oxidase	Indole	R.M.	V.P.	Citrate	
G1	4, 7, 39	-	-	+	+	+	*Bacillus cereus*
	26, 27, 34	+	-	+	+	+	*Bacillus sublilis*
G2	8, 9, 21, 22, 23, 28, 2948, 49, 50	+	+	-	+	+	*Aeromonas*spp.
	12, 18, 19, 20, 35, 38	+	+	-	-	+	*Pseudomonas*spp.
	30, 45, 51	-	-	+	-	+	*Echerichia*spp.
	36, 44	-	-	+	-	-	*Salmonella* spp.
	13, 37, 52	-	+	+	-	-	*Shigella*spp.
	15, 46, 47	+	ND	ND	ND	ND	*Vibrio* spp.
G3	2, 3, 10, 31, 32	-	ND	ND	ND	ND	*Micrococcus* spp.
	1, 16, 17, 33	-	ND	ND	ND	ND	*Staphylococcus aureus*

* +: positive, -: negative, ND: Not detected.

4. Conclusion

All samples were not in conformity with the standard specifications of the Egyptian cake ES: 4037/2005 [14] and that is considered a very dangerous, because bacteria are significant spoilage microorganisms of food, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing toxins. Bacterial growth on cake is a common problem for the cake manufacture. Species of *Bacillus, Aeromonas, Pseudomonas, Echerichia, Salmonella, Shigella, Vibrio,* and *Staphylococcus* are common contaminants of cake. By the searching in the medical references, it was observed that, most of this bacteriahad the ability to human and animal pathogenicity or produced toxins. The growth of toxigenic bacteria on cake samples must be considered as a problem of safety for human consumption [27 and 28].

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