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Detection of some Food Poisoning Bacteria from Milk Utensils and Dairy Products in Port-said Governorate, Egypt

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Abstract

The aim of this study is to determine the possibility of transmission of some food poisoning bacteria (*Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli* and *Proteus* spp.) from the surfaces of equipment and utensils used in small dairy shops for production of dairy products to the final dairy products through microbiological examination of 90 swabs which were collected from the surfaces of equipment and utensils in small dairy shops from different localities at Port-said governorate, Egypt, in addition to 45 of each commercial small scale yoghurt and rice with milk pudding samples collected from the same small dairy shops. The results showed that the mean values for *Staphylococcus aureus* counts were $4.7\times10^5\pm1.0\times10^5$ cfu/g, $3.9\times10^5\pm8.8\times10^4$ cfu/g and $9.4\times10^5\pm2.3\times10^5$ cfu/g in swabs, yoghurt and rice with milk pudding samples, respectively, and The mean values for *Streptococcus* spp. were $2.2\times10^6\pm2.6\times10^5$ cfu/g,$1.4\times10^6\pm3.1\times10^5$cfu/g and $3.2\times10^6\pm5.6\times10^5$ cfu/g in swabs, yoghurt and rice with milk pudding samples, respectively, While the mean values of *Escherichia coli* were $1.7\times10^2\pm2.6\times10^1$ cfu/g and $1.2\times10^2\pm3\times10^1$ cfu/g in swabs and yoghurt samples, respectively, with absence in examined rice with milk pudding samples. *Proteus* spp. couldn't be detected in any examined samples. It could be concluded that the microorganisms which isolated from yoghurt and rice with milk pudding samples have already found on equipment and utensils used in its manufacture, this may confirm cross-contamination from equipment and utensils surfaces to yoghurt and rice with milk pudding as a final dairy product of small dairy shops.

KEYWORDS
Escherichia coli, *Staphylococcus*, *Streptococcus*.

INTRODUCTION

Bacterial biofilm is an aggregation of bacterial cells that stick to each other forming structured communities of cells on living or non-living surfaces encased within a self-produced polysaccharide matrix of extracellular polymeric substance (EPS) (Costerton *et al.*, 2003), These communities provides a good environment for the exchange of genetic material between cells in addition to protection against several environmental conditions such as dehydration, UV exposure and salinity (Donlan, 2002), it is a survival strategy employed by bacteria to become less sensitive to antibiotics and disinfectants (Van Houdt and Michiels, 2010).

EPS contributes the bulk of the volume of a biofilm and is primarily responsible for its slimy macroscopic properties. The main constituents of EPS include polysaccharides, proteins, DNAs, lipids, and other polymeric compounds (Myszka and Czaczyk, 2009). Within this matrix there are channels for the circulation of nutrients and water (Flemming and Wingender, 2010). These channels also provide interspecies bacterial exchange or sharing of different metabolic substrates in biofilm (Kokare *et al.*, 2009).

Biofilm formation is a multi-step process (Stoodley *et al.*, 2002), it begins when free-floating bacterial cell come in contact with a suitable surface and begin to put down roots, this process occurred through a several steps leading to its adaptation under stress and diverse nutritional and environmental conditions (Hentzer *et al.*, 2005).

In food industrial sectors, the presence of biofilms causes food spoilage which resulting in several food-borne infections (Brooks and Flint, 2008), especially in the dairy processing industry, biofilms on the surfaces of milk processing equipment have been considered as a major source of milk recontamination (Flint *et al.*, 1997).

Foodborne pathogens can grow as biofilms in their normal habitats causing severe hygienic problems and economic loss as a result of spoilage of food (Nyenje *et al.*, 2012). *Staphylococcus aureus* and in general *Staphylococcus* genera established a high-density biofilm, it can produce biofilms on both biotic and abiotic surfaces along the food production chain (Kukhtyn *et al.*, 2017), *Streptococcus* spp. is among the bacteria most frequently isolated from surfaces in the food industry, it could persist in various parts throughout milk production and can form predominantly mono-species biofilms on heat exchanger plates in the downstream side of the sections of pasteurizers (Flint *et al.*, 1999). While *Escherichia coli* use its membrane proteins, flagella, and pili to initiate attachment to the surfaces. Acid-adapted *Escherichia coli* O157:H7 has shown enhanced survival and prevalence in biofilms on stainless steel surfaces (Venkitanarayanan and Doyle, 2003), *Proteus* mirabilis has been found to produce biofilms on a wide range of surfaces (McLean *et al.*, 1991).

Outbreaks originating from fermented dairy products have been reported in several countries (Mungai *et al.*, 2015) and their contamination occurred by contaminated tools, surfaces, and packaging material sources (Gonfa *et al.*, 2001). Consumption of yoghurt may predispose the consumer to *Escherichia coli* in-

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fections (Gulmez and Guven, 2003). In Rice with milk pudding production, it was found that the equipment which was used in its production and the employees working in enterprises were among the sources of its contamination (Alisarli *et al*., 2002).

Therefore, this study was conducted for detection of *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli* and *Proteus* spp. from the surfaces of the dairy equipment used in small scale dairy shops and in yoghurt and rice with milk pudding produced in the same shops to determine the possibility of cross-contamination from the surfaces of utensils to the final products of yoghurt and rice with milk pudding.

MATERIALS AND METHODS

Sampling

A total of 90 swabs were collected from small dairy shops in different localities at Port-said governorate, Egypt. Swabbing was done on the milk handling containers, small and large pots, spatula, and spoons after they had been cleaned and ready to be used in handling milk and its products. In addition, 45 samples of each commercial small-scale yoghurt and rice with milk pudding were collected from the same small dairy shops. All collected swabs, yoghurt and rice with milk pudding were transferred directly to the laboratory.

Preparation of the samples for microbiological examination

Ten-fold serial dilutions up to 10^{-10} were prepared from each sample according to APHA (2004).

Enumeration and isolation of *Staphylococcus aureus* were performed on Baird-Parker medium plates at 37°C for 48 hours (Deibel and Herrttman, 1984).

Enumeration and isolation of Streptococci spp. was done by using Kanamycin Aesculin Azide Agar plates at 36±1°C for 48 hours (APHA, 1992).

Enumeration and isolation of *Escherichia coli* was performed by using Eosin Methylene Blue agar plates at 37°C for 24 hours (ISO, 2001).

Enumeration and isolation of *Proteus* spp. was done by using standard plate count agar plates at 37°C for 24 hours (ISO, 2001).

Statistical analysis

The unpaired t-test with Welch's correction by the Prism software was used to calculate P values between treatment groups. There is a significant difference at the 95% confidence level ($P \leq 0.05$).

RESULTS

Results showed that the mean values for total *Staphylococcus aureus* counts (Table 1) were $4.7\times10^5\pm1.0\times10^5$ cfu/g, $3.9\times10^5\pm8.8\times10^4$ cfu/g and $9.4\times10^5\pm2.3\times10^5$ cfu/g in examined swabs, yoghurt samples, and rice with milk pudding samples, respectively, with incidence 53.3%, 62.2% and 73.3%, respectively. The mean values for total *Streptococcus* spp. (Table 2) were $2.2\times10^6\pm2.6\times10^5$ cfu/g,$1.4\times10^6\pm3.1\times10^5$ cfu/g and $3.2\times10^6\pm5.6\times10^5$ cfu/g in examined swabs, yoghurt samples, and rice with milk pudding samples, respectively, with incidence 73.3%, 68.9 % and 71.1%, respectively. While the mean values of *Escherichia coli* (Table 3) were $1.7\times10^2\pm2.6\times10$ cfu/gm and $1.2\times10^2\pm3\times10$ cfu/g in examined swabs and yoghurt samples,

Table 1. Comparison between Statistical analytical results of total *Staphylococcus aureus* count/ g in examined swab samples (n=90), yoghurt and rice pudding (n=45 of each).

Type of examined samples	Samples Number (NO)	Positive samples (incidence)		Cfu/g		
		No	%	Minimum	Maximum	Mean ±SE
Swab [a]	90	48	53.3	5×10^2	3×10^6	$4.7\times10^5\pm1.0\times10^5$
Yoghurt [b]	45	28	62.2	2×10^3	1.6×10^6	$3.9\times10^5\pm8.8\times10^4$
Rice with pudding samples [c]	45	33	73.3	8.0×10^3	5.3×10^6	$9.4\times10^5\pm2.3\times10^5$

a: P value between a and b =0.5941 considered not significant; b: P value between a and c =0.0769 considered not significant.

Table 2. Comparison between Statistical analytical results of total *Streptococcus* spp. count/ gm in examined swab samples (n=90), yoghurt and rice pudding (n=45 of each).

Type of examined samples	Samples Number (NO)	Positive samples (incidence)		Cfu/g		
		No	%	Minimum	Maximum	Mean ±SE
Swab [a]	90	66	73.3	4.2×10^1	7.8×10^6	$2.2\times10^6\pm2.6\times10^5$
Yoghurt [b]	45	31	68.9	1×10^3	9×10^6	$1.4\times10^6\pm3.1\times10^5$
Rice with pudding samples [c]	45	32	71.1	5.9×10^2	10.3×10^6	$3.2\times10^6\pm5.6\times10^5$

a: P value between a and b =0.5941 considered not significant; b: P value between a and c =0.0769 considered not significant.

Table 3. Comparison between Statistical analytical results of total *Escherichia coli* count/ gm in examined swab samples (n=90), yoghurt and rice pudding (n=45 of each).

Type of examined samples	Number of examined samples. (NO)	Positive samples (incidence)		Cfu/gm		
		No	%	Minimum	Maximum	Mean ±SE
Swab [a]	90	3	33	1.2×10^2	2×10^2	$1.7\times10^2\pm2.6\times10^1$
Yoghurt [b]	45	2	4.4	9.0×10^1	1.5×10^2	$1.2\times10^2\pm3\times10^1$
Rice with pudding samples [c]	45	0	0	0	0	0

P value between a and b =0.2944 considered not significant.

respectively, with incidence 33% and 4.4%, respectively, with absence of *Escherichia coli* in examined rice with milk pudding samples. *Proteus* spp. couldn't be detected in all examined swabs, yoghurt, and rice with milk pudding sample.

DISCUSSION

The results of the microbiological examination revealed that 53.3% of the examined swab samples were contaminated with *Staphylococcus aureus* with a mean value of $4.7\times10^5\pm1.0\times10^5$cfu/g. Nearly similar results were reported by Öner and Ölmez (2011), higher result obtained by Marques *et al*. (2007). While Lower results were recorded by Malek *et al*. (2012). Our results in agreement with Lee *et al*. (2014) who found that 45% of examined *Staphylococcus aureus* strains had the ability to produce biofilm on microplates and stainless steel.

From Table 1, 62.2% of examined yoghurt samples were contaminated with *Staphylococcus aureus* with a mean value of $3.9\times10^5\pm8.8\times10^4$cfu/g. No significant difference between swab *Staphylococcus aureus* count and yoghurt *Staphylococcus aureus* count. This may indicate that the high incidence of *Staphylococcus aureus* in yoghurt samples could be attributed to cross-contamination from bacteria formed on equipment surfaces to yoghurt. All positive samples (62.2%) are failed to confirm the Egyptian Standards (2005) /yoghurt, which reported that yoghurt must be free from pathogenic microbes and its harmful secretions. Nearly similar results were reported by Attalla *et al*. (2018). Higher results were recorded by Nwamaka and Chike (2010), while Lower results were recorded by Aman *et al*. (2021). In contrast to our findings Eissa *et al*. (2010) could not detect *Staphylococcus aureus* in examined samples.

73.3% of examined rice with milk pudding samples were contaminated with *Staphylococcus aureus* bacteria with a mean value of $9.4\times10^5\pm2.3\times10^5$cfu/g. No significant difference between swab *Staphylococcus aureus* count and rice with milk pudding *Staphylococcus aureus* count. This may indicate that most of *Staphylococcus aureus* count in rice with milk pudding samples come from the equipment surfaces during the production. Nearly similar results were reported by Abdel-Latif and Saad (2016) and Higher results were recorded by Sotohy *et al*. (2022), while Lower result by Ertas *et al*. (2010).

Presence of enterotoxigenic *Staphylococcus aureus* strains in dairy products indicating unhygienic conditions during production, processing, storage, and handling of milk products (Thaker *et al*., 2013) and can induce foodborne intoxications in humans (Sergelidis and Angelidis, 2017).

Concerning *Streptococcus* spp., results revealed that 73.3% of examined swab samples were contaminated with *Streptococcus* spp. with a mean value of $2.2\times10^6\pm2.6\times10^5$cfu/g. Higher results were recorded by Bassi *et al*. (2017), Our results in agreement with Sharma and Anand (2002) and Gunduz and Tuncel (2006). and 68.9 % of examined yoghurt samples were contaminated with *Streptococcus* spp. with mean value of $1.4\times10^6\pm3.1\times10^5$cfu/g.

There is significant difference between swab *Streptococcus* spp. count and yoghurt *Streptococcus* spp. count, the Presence of *Streptococcus* spp. in high incidence in yoghurt samples, is most often due to some Streptococci used as starter cultures in yoghurt production, poor hygiene, handling of the food by infected people, the use of raw (unpasteurized) milk, or could be due to its transmission from the surfaces of the equipment and utensils used in manufacturing of the dairy products. all positive yoghurt samples for *Streptococcus* spp. are failed to confirm Egyptian Standards (2005)/ yoghurt.

Nearly similar results were reported by Eissa *et al*. (2010), Higher results were recorded by Motawee and Saleh (2016), and Lower result recorded by Nwamaka and Chike (2010). While 71.1% of examined rice with milk pudding samples were contaminated with *Streptococcus* spp. with a mean value of $3.2\times10^6\pm5.6\times10^5$cfu/g. No significant difference between swab *Streptococcus* spp. count and rice with milk pudding *Streptococcus* spp.

The obtained results in agreement with Nayem and Ahmed (2013) who isolated *Streptococcus* spp. from examined milk-based sweets. our results indicating that swab *Streptococcus* spp. count and rice with milk pudding *Streptococcus* spp. count are non-significantly different this may indicate that the high incidence of *Streptococcus* spp. in rice with milk pudding samples could be due to cross-contamination from surfaces of dairy processing utensils and vats to rice with milk pudding samples.

Results in Table (3) revealed that 3.3% of examined swab samples were contaminated with *Escherichia coli* with a mean value of $1.7\times10^2\pm2.6\times10^1$cfu/g. Higher result was reported by Lajhar *et al*. (2018), our results revealed the presence of *Escherichia coli* on the surfaces of equipment used in the manufacture of dairy products, this is in agreement with Sharma and Anand (2002) and Kukhtyn *et al*. (2017). While 4.4% of examined yoghurt samples contaminated with *Escherichia coli* with a mean value of $1.2\times10^2\pm3\times10^1$cfu/g.

No significant difference between swab *Escherichia coli* count and yoghurt *Escherichia coli* count. This may indicate that *Escherichia coli* in yoghurt samples come from surfaces of dairy processing equipment and there is no contamination of yoghurt with *Escherichia coli* during manufacturing and handling. 4.4% of examined yoghurt samples contaminated with *Escherichia coli* bacteria failed to confirm Egyptian standards. 2005/yoghurt. Nearly similar results were reported by Shittu *et al*. (2016) and higher results were recorded Aman *et al*. (2021), while Lower results were recorded by Motawee and Saleh (2016).

In contrast to our findings Salisu *et al*. (2016) could not detect *Escherichia coli* in the examined samples. *Escherichia coli* was not detected in all examined rice with milk pudding samples. Similar results were recorded by Mamun *et al*. (2020) who could not detect *Escherichia coli* in examined rice with milk pudding samples. In contrast to our findings, Sotohy *et al*. (2022) showed that *Escherichia coli* was detected in 16% (8 samples) of rice with milk pudding samples.

Proteus spp. not detected in any examined samples. In contrast to our findings Gunduz and Tuncel (2006) found that most of the biofilm isolates were *Proteus*, Jerry *et al*. (2016) isolated *Proteus* spp. from the examined yoghurt samples and Chatli *et al*. (2014) found that *Proteus* spp. was detected in 12.5% of burfi samples as a milk-based deserts.

With respect to the present data, it is observed that hygienic measures are not applied during production of yoghurt and rice with milk pudding sold in Egyptian public markets.

CONCLUSION

With respect to the present data, it is observed that the microorganisms which isolated from yoghurt and rice with milk pudding samples (*Staphylococcus aureus*, *Streptococcus* spp. and *Escherichia coli*) have already been formed on surfaces of equipment and utensils used in small dairy shops for production of dairy products, there is no significant difference between counts of these microorganisms in swab samples and counts of them in yoghurt and rice with milk pudding samples, except swab *Streptococcus* spp. count and yoghurt *Streptococcus* spp. count were significantly different. This may confirm cross-contamination from equipment and utensils surface to yoghurt and rice with milk pudding as a final dairy product of small dairy shops. Cross-contamination has been shown to be a risk that causes food poisoning outbreaks and economic losses. So that we can conclude that bacteria formed on dairy-processing equipment surfaces can act as a persistent source of milk products contamination by spoilage and pathogenic microorganisms which affect its quality, safety, and shelf life.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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