Bacterial Biofilms in Small Scale Dairy Industry

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Abstract:

Bacterial biofilm has been incriminated as a major source of milk and dairy products contamination causing food poisoning with economic losses, therefore this study aimed to detect the possibility of cross-contamination of microorganisms from biofilms formed on manufacturing equipment and utensils surfaces to the final dairy products as rice with milk pudding and yoghurt, through detection of *Escherichia coli, Streptococcus spp., Staphylococcus aureus, and Proteus spp.* by bacteriological examination of 90 surfaces swabs from biofilms formed on the manufacturing utensils surfaces after cleaning regime, in small- scale dairy shops at Port-said Governorate, Egypt, and 45samples of each rice with milk pudding and yoghurt from the same dairy shops. The results revealed that incidence of *Staphylococcus aureus* was 53.3% in swabs samples, 62.2% in yoghurt samples and 73.3% in rice with milk pudding samples, and the incidence of *Streptococcus spp.* was 73.3% in biofilm swabs samples, 68.9 % in yoghurt samples and 71.1% in rice with milk pudding samples. While the incidence of *Escherichia coli* was 3.3% in biofilm swabs samples and 4.4% in yoghurt samples, and not detected in all examined samples of rice with milk pudding. *Proteus spp*. not found in all samples. we can conclude that the presence of biofilms and high incidence of isolated microorganisms, despite of regular cleaning reflects ineffectiveness of cleaning process and cleaning agent used for biofilm control in small dairy shops and the presence of the same microorganisms in the final dairy products may confirm cross-contamination of microorganisms from biofilms formed on the manufacture utensils and equipment surfaces to the dairy products in small-scale dairy shops.

Keywords: Biofilm, *Staphylococcus, Streptococcus spp., Escherichia coli, Proteus spp.*

Introduction

Biofilm is considered a major problem in the food industry, it is a large, complex, and organized structured bacterial community of bacterial cells that aggregate, attach and embedded in an extracellular polymeric substance which is a self-produced matrix composed of lipids, polysaccharides, nucleic acids, proteins, and other components on living or non-living surfaces. **(*Sousa et al., 2020*).**

Biofilm is considered a good environment for genetic material exchange between bacterial cells, it provides protection to the bacterial cells from changes in environmental conditions as the presence of antimicrobial substances, salinity, UV exposure, and dehydration (***Donlan, 2002***). The formation of the biofilm enhances and improves the ability of foodborne bacteria to tolerate, survive and resist stresses that could be found in food processing such as disinfection, acidity, salinity and refrigeration **(*Kumar and Anand, 1998*).** It resists sanitization and allows bacteria to spread across the food, especially via kitchen utensils **(*Kwok et al., 2022*).**

Biofilm formation consists of five steps: (a) reversible attachment (b) irreversible adhesion (c) formation of small colonies (d) biofilm maturation and (e) cell separation and diffusion **(*Stoodley et al., 2002*)**.

Extracellular polymeric substance considered the bulk of the biofilm volume and plays important roles in attachment to the surfaces, biofilm structure, cell–cell recognition, signaling, retention of water, protection of the bacterial cells and trap of nutrients, in addition to genetic exchange **(*Dogsa et al., 2005*).**

Concerning the food industry, it formed when the microorganisms didn't completely removed from food contact surfaces with the accumulation of particles and molecules on a food contact surface at the solid–liquid interface, resulting in the high concentration of nutrients, this is called conditioning causing food spoilage and foodborne infections **(*Brooks and Flint, 2008*)**, particularly in the dairy industry, milk containing non-casein protein and lactose increasing the bacterial cells number which attached to the surfaces as a result of the formation of a polymer essential to bacterial cell attachment **(*Speers and Gilmour, 1985*).**

Formation of the biofilm on dairy processing utensils and equipment surfaces can act as a constant source of pre- and post-processing contamination which affects product safety, products quality and lowers the products shelf-life, this may lead to food-borne disease with economic losses; so, increasing the frequency of cleaning must be done **(*Flint et al., 1997*).**

The most common measure of arresting the biofilm formation in the dairy industry is cleaning and disinfecting of all sites, equipment, and instruments **(*Simões et al., 2006*).**

Cleaning and sanitizing are complementary steps, and neither alone can achieve the desired outcome **(*Gibson et al., 1999*)**.
Therefore, this study aimed to detect the possibility of cross-contamination by some foodborne bacteria from biofilms formed on surfaces of manufacture equipment to the dairy products as rice with milk pudding and yoghurt with evaluation of cleaning process and detergent used in small dairy shops.

Material and Methods

1.Sampling: 90 swabs were collected from the surfaces of all pots used in manufacturing and processing of milk products, spatula, milk handling containers surfaces, and spoons in small dairy shops at Port-said Governorate, Egypt, after its cleaning and drying. Moreover 45 samples of each rice with milk pudding and yoghurt collected from the same dairy shops.
2.Preparation of the samples for bacteriological examination **(*APHA, 2004*).**
3.Detection, isolation, and enumeration of *Staphylococcus aureus* using Baird-Parker agar medium **(*Deibel and Herrttman, 1984*)**.
4. Detection, isolation, and enumeration of *Streptococci spp*. using Kanamycin Aesculin Azide medium **(*APHA, 1992*).**
5. Detection, isolation, and enumeration of *Escherichia coli* on EMB agar **(*ISO, 2001*)**.
6. Detection, isolation, and enumeration of *Proteus spp*. on standard plate count agar **(*ISO, 2001*)**.

Statistical analysis

The data was analyzed by using The GraphPad Prism10 software.

Results

The results of bacteriological examination revealed that the incidence of *Staphylococcus aureus* was 53.3% in biofilms swabs, 62.2% in yoghurt samples and 73.3%, in rice with milk pudding samples, and the incidence of *Streptococcus spp*. was 73.3% in biofilms swabs, 68.9 % in yoghurt samples and 71.1% in rice with milk pudding samples. The incidence of *Escherichia coli* was 3.3% in biofilms swabs, 4.4% in yoghurt samples and not found in rice with milk pudding samples. *Proteus spp*. not found in all samples.

Table (1): *Total Staphylococcus aureus* count/ ml*, Streptococcus spp.* count/ ml*, Escherichia coli* count/ ml *and Proteus spp.* count/ ml in examined swab samples (90 samples):

	Swab (90 swabs)				
	Positive samples		**Minimum cfu/ml**	**Maximum cfu/ml**	**Mean±SE cfu/ml**
	No.	**%**			
Staphylococcus aureus	48	53.3	5×10^{2}	3×10^{6}	$4.7\times10^{5}\pm1.0\times10^{5}$
Streptococcus spp.	66	73.3	4.2×10	7.8×10^{6}	$2.2\times10^{6}\pm0.26\times10^{6}$
Escherichia coli	3	3.3	1.2×10^{2}	2×10^{2}	$1.7\times10^{2}\pm0.26\times10^{2}$
Proteus spp.	ND	ND	ND	ND	ND

ND=Not detected.

Table (2): *Total Staphylococcus aureus* count/ gm*, Streptococcus spp.* count/ gm*, Escherichia coli* count/ gm *and Proteus spp.* count/ gm in examined yoghurt samples (45 samples):

	Yoghurt (45 samples)				
	Positive samples		Minimum (cfu/gm)	Maximum (cfu/gm)	Mean±SE (cfu/gm)
	No.	%			
Staphylococcus aureus	28	62.2	2×10^{3}	1.6×10^{6}	$3.9\times10^{5}\pm0.88\times10^{5}$
Streptococcus spp.	31	68.9	1×10^{3}	9×10^{6}	$1.4\times10^{6}\pm0.31\times10^{6}$
Escherichia coli	2	4.4	9.0×10	1.5×10^{2}	$1.2\times10^{2}\pm0.3\times10^{2}$
Proteus spp.	ND	ND	ND	ND	ND

ND=Not detected.

Table (3): *Total Staphylococcus aureus* count/ gm*, Streptococcus spp.* count/ gm*, Escherichia coli* count/ gm *and Proteus spp.* count/ gm in examined Rice with milk pudding (45 samples):

	Rice with milk pudding (45 samples)				
	Positive samples		Minimum (cfu/gm)	Maximum (cfu/gm)	Mean±SE (cfu/gm)
	No.	%			
Staphylococcus aureus	33	73.3	8.0×10^{3}	5.3×10^{6}	$9.4\times10^{5}\pm2.3\times10^{5}$
Streptococcus spp.	32	71.1	5.9×10^{2}	10.3×10^{6}	$3.2\times10^{6}\pm0.56\times10^{6}$
Escherichia coli	ND	ND	ND	ND	ND
Proteus spp.	ND	ND	ND	ND	ND

ND= Not detected.

Table (4): *Number of yoghurt and rice with milk pudding samples which confirmed the* ***Egyptian Standards (2005)***:

	Yoghurt (45 samples)		Rice with milk pudding (45 samples)	
	Samples confirm Egyptian Standards 2005/yoghurt (negative samples)		Samples confirm Egyptian Standards 2005/ UHT- Sweetened Flavored Milk (negative samples)	
	No.	%	No.	%
Staphylococcus aureus	17	37.8	12	26.7
Streptococcus spp.	14	31.1	13	28.9
Escherichia coli	43	95.5	45	100
Proteus spp.	45	100	45	100

Discussion

Results in Table (1) revealed that the incidence of *Staphylococcus aureus* in examined swabs was 53.3%, with a minimum count 5×10^2 cfu/ml and a maximum count 3×10^6 cfu/ml, while the mean value was $4.7\times10^5\pm1.0\times10^5$cfu/ml.

From Table (2), the incidence of *Staphylococcus aureus* in examined yoghurt samples was 62.2%, with a count ranging from 2×10^3cfu/gm to 1.6×10^6 cfu/gm, and the mean value was $3.9\times10^5\pm0.88\times10^5$cfu/gm. Only 37.8% examined yoghurt samples in agreement with the ***Egyptian Standards (2005) /yoghurt,*** which stated that yoghurt must be free from pathogenic microorganisms and its harmful secretions. Yoghurt *Staphylococcus aureus* count was not significantly different from swabs *Staphylococcus aureus* count (P value =0.5941), this may confirm cross-contamination of *Staphylococcus aureus* from biofilms formed on the surfaces of manufacturing equipment and utensils to yoghurt and may exclude other sources of *Staphylococcus aureus* contamination of yoghurt.

Results in table (3) revealed that in rice with milk pudding samples the incidence of *Staphylococcus aureus* was 73.3%, with a count ranging from 8.0×10^3cfu/gm to 5.3×10^6 cfu/gm, and the mean value was $9.4\times10^5\pm2.3\times10^5$ cfu/gm. *Staphylococcus aureus* count in rice with milk pudding samples was not significantly different from its count in swabs (P value =0.0769), this may confirm transmission of *Staphylococcus aureus* from biofilms formed on the surfaces of manufacture equipment and utensils to rice with milk pudding during its production. 26.7% of rice with milk pudding samples confirm ***Egyptian Standards (2005)/ UHT- Sweetened Flavored Milk.***

Results in table (1) revealed that *Streptococcus spp.* was isolated from 73.3% of swab, its count ranged from 4.2×10^1cfu/ml as a minimum value and 7.8×10^6 cfu/ml as a maximum value, and the mean value was $2.2\times10^6\pm0.26\times10^6$ cfu/ml.

From table (2) results revealed that *Streptococcus spp.* was present in 68.9 % of examined yoghurt samples with a count ranged from 1×10^3 cfu/gm to 9×10^6 cfu/gm, and the mean value was $1.4\times10^6\pm0.31\times10^6$ cfu/gm. *Streptococcus spp*. counts of swab and yoghurt were significantly different (P value=0.0492), this is most often due to some of *Streptococcus spp*. used as starter cultures in yoghurt or may be due to *Streptococcus spp.* transmission from biofilms on of the manufacture utensils and equipment surfaces used in yoghurt production. 31.1% of yoghurt samples confirm ***Egyptian Standards (2005)/ yoghurt***.

From table (3) results revealed that the incidence of *Streptococcus spp.* in rice with milk pudding samples was 71.1% , with a count ranged from 5.9×10^2 cfu/gm to 10.3×10^6 cfu/gm, and the mean value was $3.2\times10^6\pm0.56\times10^6$ cfu/gm. *Streptococcus spp*. count in swabs was non significantly different from its count in rice with milk pudding samples (P value=0.1492), this can confirm *Streptococcus spp.* transmission from biofilms formed on the manufacturing equipment surfaces to the rice with milk pudding. 28.9% of rice with milk pudding samples confirm ***Egyptian Standards (2005)/ UHT- Sweetened Flavored Milk.***

Results revealed that *Escherichia coli* isolated from 3.3% of swab, its count ranged from 1.2×10^2cfu/ml as a minimum value and 2×10^2 cfu/ml as a maximum value, and the mean value was $1.7\times10^2\pm0.26\times10^2$ cfu/ml, while in yoghurt samples *Escherichia coli* was presented in 4.4% of the samples, its count ranged from 9.0×10^1 cfu/gm to 1.5×10^2 cfu/gm, and the mean value was $1.2\times10^2\pm0.3\times10^2$ cfu/gm. Swab *Escherichia coli* count was non significantly different from *Escherichia coli* count of yoghurt (P value =0.2944), this may confirm *Escherichia coli* transmission from biofilms formed on the manufacturing utensils surfaces to yoghurt samples, and may exclude other sources of contamination by *Escherichia coli* during processing of yoghurt. 95.5% of examined yoghurt samples confirmed the ***Egyptian standards, 2005/yoghurt***.

Escherichia coli was not found in rice with milk pudding examined samples.

Concerning *Proteus spp*., the result showed that *Proteus spp.* was not found in all examined samples.

From this study, the results indicated that the cleaning regime and the detergent used in cleaning in small scale dairy shops were not efficient against biofilm formation and are not enough for controlling and removing biofilm. This is in agreement with the results of ***Gibson et al. (1991)*** and ***Öner and Ölmez (2011).***

Conclusions

The presence of biofilms and high incidence of isolated microorganisms, despite of regular cleaning reflects the ineffectiveness of the cleaning process and cleaning agent used for biofilm control in small dairy shops. The

presence of the same microorganisms in biofilms and the final dairy products may confirm cross-contamination of microorganisms from biofilms formed on the manufacture utensils and equipment surfaces to the final dairy products of small dairy shops.

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الأغشية الحيوية في صناعة الالبان على نطاق صغير

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الملخص العربي

اشتملت الدراسة على فحص بكتريولوجي لعدد 90 مسحة من الاغشية الحيوية المتكونة على اسطح ادوات التصنيع و عدد 45 عينة من كل من الزبادي وحلوي الارز واللبن المنتجة محليا في محلات تصنيع وبيع الالبان ومنتجاتها في محافظة بورسعيد في مصر .واظهرت نتائج الفحص البكتريولوجي ان نسب العينات الإيجابية للمكورات العنقودية الذهبية 53.3% ,62.2% و 73.3% لكل من مسحات من الاغشية الحيوية المتكونة على أسطح ادوات التصنيع وعينات الزبادي وحلوى الارز واللبن على التوالي , و كانت نسبة العينات الإيجابية للميكروب العقدي 73.3% ,68.9% و 71.1% لكل من مسحات من الاغشية الحيوية المتكونة على اسطح ادوات التصنيع و عينات الزبادي و حلوى الارز و اللبن على التوالي. بينما كانت النسبة المئوية للعينات الإيجابية لجرثومة الاشريشيا كولاي 3.3% و% 4.4 لكل من مسحات من الاغشية الحيوية المتكونة على أسطح ادوات التصنيع وعينات الزبادي على التوالي ولم يتم عزلها من اي عينة حلوى ارز واللبن. اما عن ميكروب البروتيوس فقد اثبتت الدراسة خلو جميع العينات المفحوصة من ميكروب البروتيوس. من النتائج السابقة نستخلص احتمالية انتقال تلك الميكروبات من أسطح الادوات الى منتجات الالبان بالإضافة الى عدم كفاءة انظمه التنظيف المتبعة في محلات تصنيع وبيع منتجات الالبان المحلية وعدم كفاية المنظفات المستخدمة للسيطرة على تواجد الاغشية الحيوية على أسطح ادوات التصنيع وغياب التطهير والتعقيم لتلك الادوات وعدم اتباع الاشتراطات الصحية.