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ORIGINAL ARTICLE

Incidence of *Stenotrophomonas* Species in Milk and Some Dairy Products in Beni-Suef Province, Egypt

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Abstract
Stenotrophomonas maltophilia is an emerging worldwide, multi-drug resistant, opportunistic respiratory tract pathogen causing severe economic losses in milk production as well as deterioration of dairy products. So we collected a total of 210 samples of farms milk, dairy shops milk, Damietta cheese, ice cream, yoghurt, cooking butter and cream, the highest incidence was in ice cream (80%) followed by ice cream (66.67%), while the lowest incidence was in Damietta cheese (3.33%). S. maltophilia was inoculated in milk to examine its survival in cream, butter, and cheese. The obtained results revealed that the pathogen can survive for 30, 30, 28, 30, and 8 days in the inoculated ice cream, butter 0% salt, butter 3% salt, cheese 0% salt, cheese 6% salt respectively, and measure count, pH, salt for 30 days.

Keywords
Incidence, inoculation, *Stenotrophomonas*

1. Introduction
Milk and dairy products represent an essential part of human diet. They act as source of proteins, minerals as calcium, phosphorus, magnesium and vitamins D, A, B2, B12, so dairy foods improve the immune system and bone functions **(Verruke et al. 2019).** However, they are considered a high risk category for potential microbial contamination due to insufficient animal health control, as well as inadequate training of farmers and dairy processing employees about milk hygiene and weakness in the cold chain during production and storage **(Chizari et al. 2008).**

The quality of raw milk and dairy products has been improved by refrigeration on farms and in processing plants. So, the present practices for collection and storage of raw milk favored the growth of psychrotrophic bacteria including *Stenotrophomonas* which gain access to bulk tank milk via several pathways as infected mammary gland, contaminated udder, milking machine and dairy farm environment **(Hayes and Boor 2001).**

The recently known *Stenotrophomonas* was originally classified as a member of the genus *Pseudomonas* **(Hugh and Ryschenkow 1961)** and Xanthomonas **(Swing et al. 1983),** finally coming to rest in *Stenotrophomonas* **(Palleroni and Bradbury 1993).** The genus *Stenotrophomonas* is wide spread in the environment and may be isolated from materials used in clinical laboratories and medical practice, hemodialysis water and dialysate samples, cannulae, prosthetic devices, dental unit water lines **(Hoefel et al., 2005),** foods **(Qureshi et al. 2005),** water, soil, plants, animals, raw and micro filtrated milk **(Rasolofo et al., 2010).** Recently, it is emerged as an opportunistic pathogen due to resistance to wide range of antibiotics, formation of biofilm on biotic and a biotic surfaces (**Di Bonaventura et al. 2007),** secretion of extracellular enzymes e.g. DNase, lipase, protease, lecithinase and hyaluronidase enzymes **(Ryan et al. 2009),** evasion of the host immune system **(Waters et al. 2007).** These enzymes (protease, lipase and lecithinase) cause deterioration in flavor, texture of milk as well as the final product **(Eneroth et al. 1998; Cleto et al. 2012).**

S. maltophilia is the only species that is known to cause human disease (**Ryan et al. 2009).** The rate of infection has been increased over the past several years causing septicemia, bacteremia **(Denton and Kerr, 1998),** urinary tract infections **(Vartivarian et al. 1996),** Pneumonia, chronic obstructive pulmonary disease **(Brooke 2012; Adegoke et al. 2017),** endocarditis **(Mehta et al. 2000)**, meningitis **(Platsouka et al. 2002)** infections of bones and joints, eye infections (keratitis, scleritis and dacryocystitis) **(Sefcick et al. 1999).** Transmission of *S. maltophilia* may occur through direct contact with the different sources of infection. The hands of health care personnel have been reported to transmit nosocomial *S. maltophilia* infection in an intensive care unit **(Schable et al. 1991).**

The importance of this study was the successful isolation of *Stenotrophomonas* from milk and some dairy products as well the as the determination of the extent of the survival of *S. maltophilia* after inoculation in some dairy products.

2. Materials and methods

2.1. Collection of samples

A total of 210 samples including farm milk (collected from 6 farms), dairy shops milk (from local markets), Damietta cheese (from 30 dairy shops), small scale yoghurt (from 30 dairy shops), small scale ice cream (from 30 different street vendors), cooking butter (from 30 different farmers houses) and cream (30 of each) were collected from different localities in Beni Suef governorate, Egypt. The collected samples were delivered as soon as possible to the laboratory in an insulated ice box and examined in the same day.

2. 2. Preparation of Samples (APHA, 1992)

2.2.1. Milk samples: 250 ml from each sample were thoroughly mixed by inversion several times to mix milk and cream layer. .

2.2.2. Cheese samples: 25 g of each cheese sample were stomached for 2 min. with the enrichment broth.

2.2.3. Ice cream samples: the samples were left to melt in a thermostatically controlled water bath at 44°C for not more than 15 min. Each sample was then thoroughly mixed using a sterile stirrer before being examined.

2.2.4. Cream samples: 250 samples from each raw cream sample were thoroughly mixed before being examined.

2.2.5. Cooking butter: 10g of each raw cooking butter sample were left in a thermostatically controlled water bath at 44°C for not more than 15 min. Then the sample was thoroughly mixed and homogenized by a sterile stirrer before being examined.

2.3. Enrichment procedure (Bollet et al. 1995)

One ml /g of the milk samples / the prepared sample of milk products was aseptically inoculated into sterile cotton plugged test tube, containing 9 ml of nutrient broth and incubated at 37°c for 24- 48 hrs.

2.4. Plating on selective agar media (Goncalves-Vidigal et al. 2011)

A loop full from the incubated broth was streaked on plates of steno medium agar (blood agar base supplemented with Impenium+ Vancomycin and Amphotericin B). Plates were incubated at 37°c for 24-48 hrs. The colonies were smooth, glistening, with entire margins (the outer part of the colonies was rounded) and white to pale yellow in color **(Denton and Kerr 1998).**

2.5. Identification of the isolated *Stenotrophomonas Spp.*

Presumptive colonies of *Stenotrophomonas spp*. were subjected to standard biochemical tests including, catalase test **(Land et al. 1991) ,** Oxidase test **(Baron et al. 1994),** Sugar fermentation test **(Speck 1976),** Arginine dihydrolase medium **(Collins and Lynes 1989),** Oxidation Fermentation (OF) test **(Hugh and Leifson 1953)** were applied.

2.6. Detection of *S. maltophilia* by using PCR

DNA was extracted following the manufacturer's recommendations using QIAamp DNA mini kit instructions (cat. No. 51304) (AppliChem GmbH, Darmstadt, Germany). The DNA concentration was measured using a spectrophotometer (DU530; Beckman Coulter, Brea, CA). An average of 10 µg of DNA was obtained.

Cycling conditions of the primers during PCR Oligonucleotide primer of the gene 23S rRNA were obtained from Metabion (Planegg-Steinkirchen, Germany) with a sequence (forward 5 GCTGGATTGGTTCTAGGAAAACGC 3, and reverse, 5 ACGCAGTCACTCCTTGCG 3) as reported by Gallo et al., 2013. DNA (5µl) was assayed in a 25 µl reaction mixture containing 12.5 µl Emerlad Amp GT PCR master mix (code no. RR310A; Takara Bio, Kusatsu, Japan), 1µl of each primer of 20 pmol concentrations, and 5.5 µl of RNA-free water. The reaction was performed in a thermal cycler model 2720 (Applied Biosystems, Foster City, CA). The primary denaturation was at 94 °c for 5 min, then secondary denaturation at 94°c for 30s (35 cycles), and final extension at 72°c for 7 min.

Gel electrophoresis was run of 2o µl of each reaction PCR product; negative control and positive control were loaded in a 1.5% agarose gel (AppliChem GmbH) at 1-5 V/cm of the tank length for 30 min and the gel was transferred to UV Cabinet (Thermo Fisher, Waltham, MA). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra, San Francisco, CA) and the data were analyzed using computer software **(Sambrook et al. 1989).**

2.7. Survival of *S. maltophilia* in dairy products

2.7.1. Preparation of culture

S. maltophilia isolates recovered from raw milk in this study were used to examine its survival in cream, butter, and cheese. *S. maltophilia* was inoculated in in trypticase soya broth (Oxoid, Ltd.) and incubated at 35°c for 48 h. The resultant culture containing about 9 log_{10} cfu/ml was used to inoculate the milk used for preparing cream, butter, and cheese to give an initial count of (7.2 log_{10} cfu/ml).

2.7.2. Manufacturing of cream

Twenty kilograms of buffalo's milk were used, where the milk was heated to 85 °c, cooled to 35- 40 °c, then *S. maltophilia* was added to give an initial concentration of 7.2 log_{10} cfu/ml. The milk was refrigerated for 24 h. for cream formation **(Ma and Barbano 2000).** The obtained cream was divided into two parts: the first was stored at 4°c for 30 days. The second part was used for butter manufacturing. Cream samples were collected daily for one week then every 2 days up to 30 days for enumeration and counting of *S. maltophilia*, for acidity according to AOAC **International (2000)** and for PH determination using Corning 240 PH meter (Corning, Suffolk, United Kingdom).

2.7.3. Butter manufacturing

The second part of the cream obtained by gravity method was used in the manufacturing of butter. The cream was cooled to a temperature of 5-7 °c then mixed in a sterile blender for 10 min. The obtained butter was divided into two parts: the first part without salt, whereas the second part had 3% salt. Both parts were kept at –18 °c for 30 days. Butter samples were collected daily for one week then every 2 days for enumeration and counting of *S. maltophilia* daily for 1 week then every 2 d up to 30 d and for determination of acidity and PH as above, and salt % according to **Aakanchha et al. (2020).**

2.7.4. Cheese manufacturing

This followed the method of **Hamad (2015)** with a little modification. The obtained milk remaining after cream separation (with a culture concentration of 6.2 log_{10} cfu/ml) was heated at 30°c in a thermostatically controlled water bath, calcium chloride solution 0.02 % and the rennet was added at the rate of 1.5 g/100 kg milk (Chr. Hansen rennet) were added. At this point and before curdling the milk was equally divided into two portions for producing cheese with 0 % salt and 6% salt. The curd was ladled in rectangular frames (20 X 20 cm) lined with sterilized cloth and the resulting functional white soft cheeses were cut into cubes and packaged into plastic containers, which filled with cooled whey of the same lot of the resulting cheeses and stored under refrigeration (4 °c) for 30 days. Samples were taken at zero time, after curdle formation and every 2 days until 30 d for enumeration and counting of *S. maltophilia* and for determination of acidity, PH, and salt % as above.

2.7.5. Examination of samples

Collected samples were examined for 1- Count of *S. matophilia*

Acidity %: acidity of cream: **(AOAC 2000),** acidity of butter **(Aakanchha et al. 2020),** acidity of cheese **(AOAC 2000).**

Determination of salt %: salt % of butter **(Aakanchha et al. 2020),** salt % of cheese **(AOAC 2000),** determination of PH, by using PH meter **(coming EEL, model 5).**

2.7.6. Statistical analysis

We calculated the sample size according to Raosoft and All statistical calculations were done using SPSS (statistical package for the social science version 26.00) statistical program at 0.05, 0.01 and 0.001 level of probability **(Snedecor and Cochran 1982),** Quantitative data with non-parametric distribution were done using Analysis of variance Mann Whitney test to compare between the two groups. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non-significant (NS) at the level of > 0.05, significant at the level of < 0.05, 0.01 and highly significant at the level of < 0.001, **(Hardle and Simar 2007).**

3. Results

Table (1): Incidence of *Stenotrophomonas spp.* in milk and dairy products.

	Number of samples	Positive No. (%)	***Stenotrophomonas* species acc. to biochemical examination**					PCR examination
			S. nitritireducens	*S. maltophilia*	*S. acidominiphila*	*S. africana*	*S. rhizophila*	
Farm milk	**30**	7 (23.33%)	4 (13.33%)	-	1 (3.33%)	2 (6.67%)	-	-
Dairy shops milk	**30**	18 (60%)	11 (36.67%)	1 (3.33%)	1 (3.33 %)	2 (6.67%)	3 (10%)	1 (3.33%)
Damietta cheese	**30**	1 (3.33%)	1 (3.33%)	-	-	-	-	-
Small scale ice cream	**30**	24 (80%)	4 (13.33%)	2 (6.67%)	5 (16.67%)	6 (20%)	7 (23.33%)	2 (6.67%)
Small scale yoghurt	**30**	2 (6.67%)	-	-	1 (3.34%)	-	1 (3.33%)	-
Cooking butter	**30**	7 (23.33%)	2 (6.67%)	3 (10%)	-	1 (3.33%)	1 (3.33%)	3 (10%)
Cream	**30**	20 (66.67)	3 (10%)	4 (13.33%)	6 (20%)	3 (10%)	4 (13.34%)	4 (13.33 %)
Total	**210**	79 (37.62)	25 (11.9%)	10 (4.76%)	14 (6.67%)	14 (6.67%)	16 (7.62 %)	10 (4.76%)

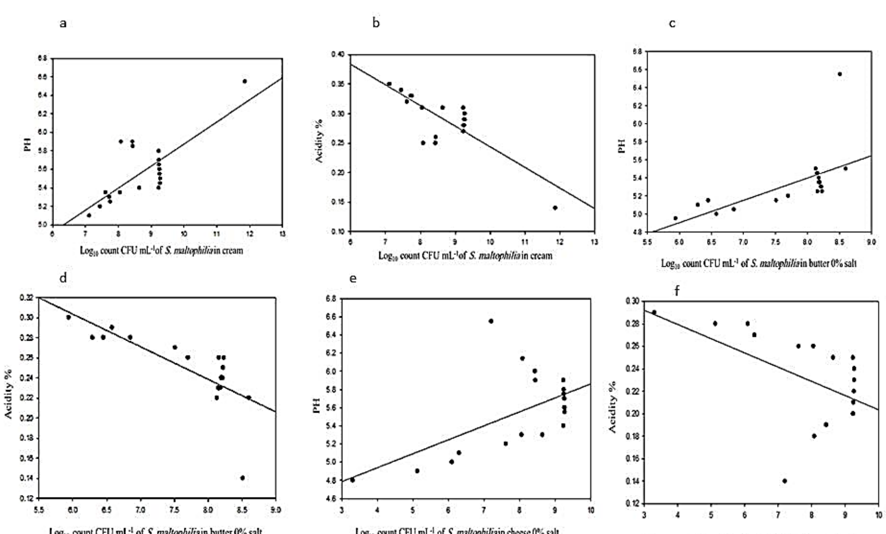

Figure 1. Correlations between Log_{10} count of *S. maltophilia* and PH& acidity in cream, butter 0% salt and cheese 0% salt. (a-b): correlation between Log_{10} count of *S. maltophilia* and PH and acidity in cream respectively, (c-d): correlation between log10 count of *S. maltophilia* and PH and acidity in butter 0% salt respectively. (e-f): correlation between log10 count of *S. maltophilia* and PH and acidity.

Table (2): Survival of *S. maltophilia* in dairy products.

Storage periods	Cream			Butter 0% salt			Butter 3% salt				Cheese 0% salt			Cheese 6% salt			
	Count log10 cfu/ml	PH	Acidity %	Count log10	PH	Acidity %	Count log10	PH	Acidity %	Salt %	Count log10	PH	Acidity%	Count log10 cfu/ml	PH	Acidity%	Salt%
Zero time	**7.2**	**6.55**	**0.14**	**7.2**	**6.55**	**0.14**	**7.2**	**6.55**	**0.14**	**2.3**	**6.2**	**6.55**	**0.14**	**6.2**	**6.55**	**0.14**	**4.85**
1	8.1	5.9	0.25	7.2	5.50	0.22	6.9	5.50	0.22	2.35	6.6	6.14	0.18	5.2	6.2	0.18	4.9
2	8.4	5.90	0.25	8.1	5.50	0.22	6.8	5.50	0.22	2.35	6.7	6	0.19	4.2	6.1	0.18	4.95
3	8.4	5.85	0.26	8.1	5.45	0.23	6.3	5.50	0.22	2.4	6.5	5.9	0.19	4	6.1	0.18	5
4	9.2	5.80	0.27	8.1	5.45	0.23	6.1	5.45	0.23	2.45	6.3	5.9	0.2	3.4	6	0.19	5.05
5	9.2	5.70	0.27	8.1	5.40	0.23	6.1	5.45	0.23	2.45	6.3	5.8	0.2	3.2	5.9	0.19	5.1
6	9.2	5.65	0.28	8.1	5.35	0.23	6.0	5.45	0.23	2.5	5.9	5.75	0.21	3.0	5.8	0.2	5.1
7	9.2	5.60	0.28	8.1	5.35	0.24	5.6	5.40	0.24	2.5	5.9	5.7	0.21	2.3	5.8	0.2	5.15
8	9.2	5.60	0.29	8.2	5.35	0.24	5.3	5.40	0.24	2.55	5.9	5.7	0.22	2.0	5.8	0.21	5.15
10	9.2	5.55	0.29	8.2	5.30	0.24	5.2	5.40	0.25	2.55	5.9	5.6	0.22	BLN	5.7	0.21	5.2
12	9.2	5.50	0.29	8.2	5.30	0.25	5.2	5.35	0.25	2.6	5.9	5.6	0.23				
14	9.2	5.45	0.3	8.2	5.25	0.26	5.2	5.35	0.26	2.65	5.9	5.55	0.24				
16	9.2	5.40	0.31	8.1	5.25	0.26	5.2	5.30	0.26	2.7	5.9	5.4	0.25				
18	8.6	5.40	0.31	7.6	5.20	0.26	5.0	5.30	0.27	2.7	5.9	5.3	0.25				
20	8.0	5.35	0.31	7.5	5.15	0.27	4.5	5.25	0.27	2.75	5.8	5.3	0.26				
22	7.6	5.35	0.32	6.4	5.15	0.28	3.6	5.25	0.28	2.75	5.7	5.2	0.26				
24	6.2	5.30	0.33	6.2	5.10	0.28	3.6	5.25	0.28	2.8	4.4	5.1	0.27				
26	6	5.25	0.33	5.6	5.05	0.28	3.5	5.20	0.28	2.8	4.1	5	0.28				
28	5.1	5.20	0.34	5.4	5.00	0.29	3.1	5.15	0.29	2.85	3.6	4.9	0.28				
30	3.3	5.10	0.35	4.9	4.95	0.30	BLN	5.10	0.29	2.9	3.3	4.8	0.29				

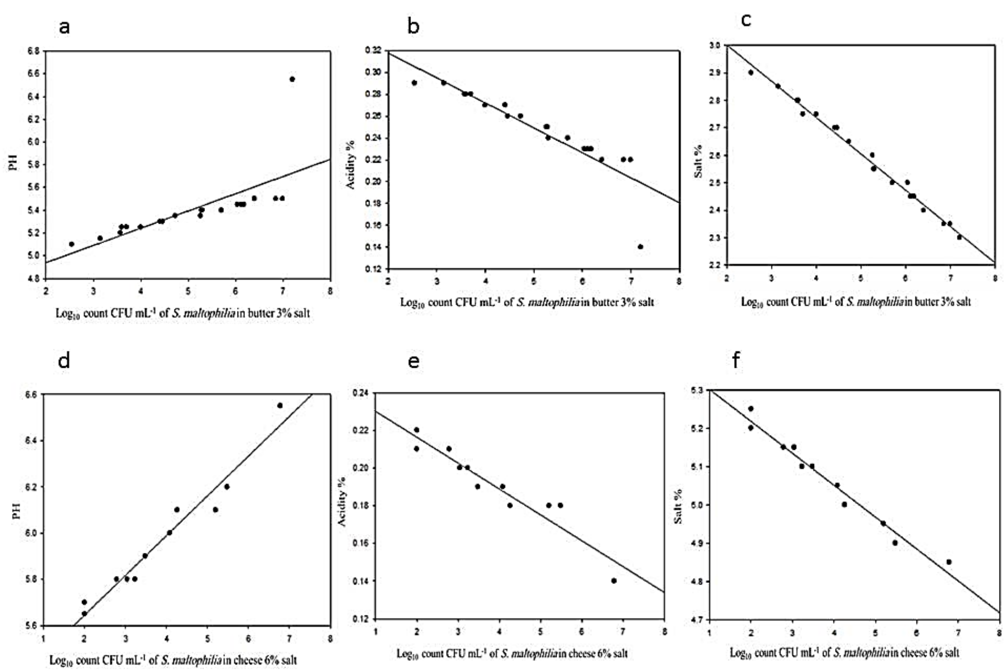

Figure 2. Correlations between *S. maltophilia* and PH, acidity and salt in butter 3% salt and cheese 6% salt. (a, b, c): correlations between log 10 count of *S. maltophilia* and PH, acidity and salt. (d, e, f): correlations between log 10 count of *S. maltophilia* and PH, acidity and salt.

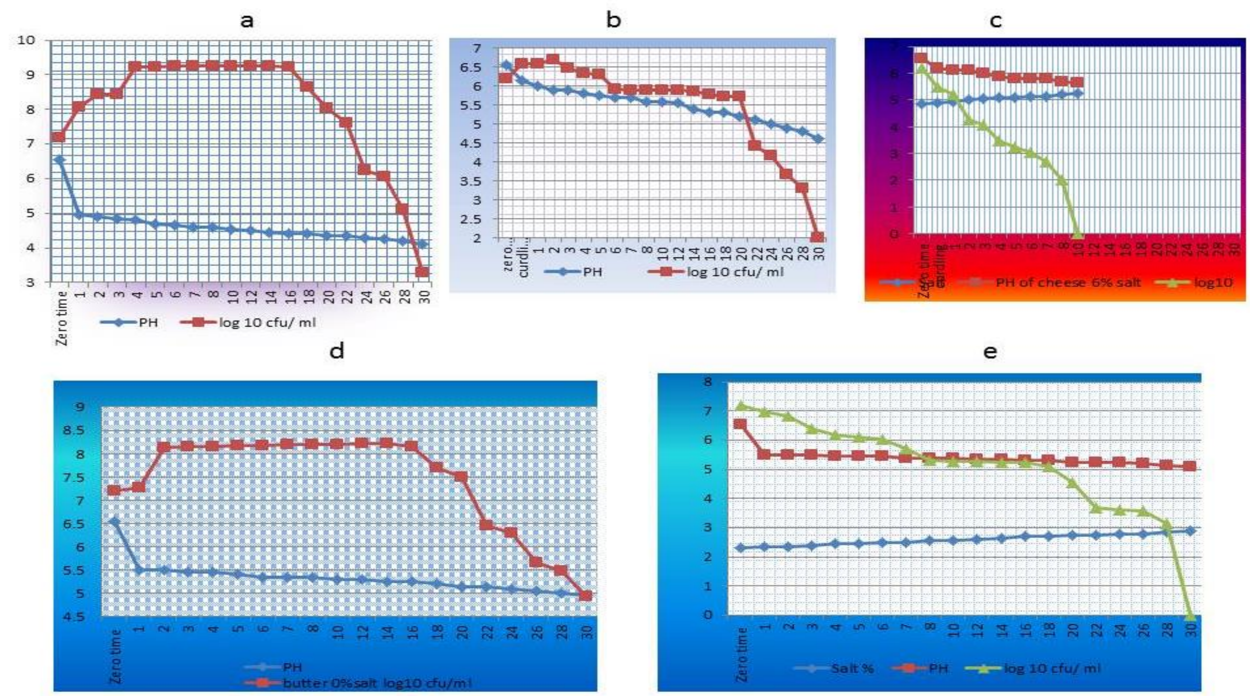

Figure 3. Survival of *S. maltophilia* in cream (a), survival of *S. maltophilia* in cheese 0 and 6% salt (b, c), survival of *S. maltophilia* in butter 0 and 3% salt (d, e).

Table (3): Comparison between butter 0 and 3% salt and cheese 0 and 6% salt.

		Median	Mann-Whitney	P value
Log Count of *S. maltophilia*	**Butter 0% salt**	8.14	30.5	0.000 HS
	Butter 3% salt	5.27		
	Cheese 0% salt	5.67	5.598	0.000 HS
	Cheese 6% salt	2.87		
PH	**Butter 0% salt**	5.30	151	0.187 ns
	Butter 3% salt	5.37		
	Cheese 0% salt	5.57	108.5	0.013 S
	Cheese 6% salt	5.67		
Acidity%	**Butter 0% salt**	0.24	200	0.987 ns
	Butter 3% salt	0.25		
	Cheese 0% salt	0.23	107.5	0.011 S
	Cheese 6% salt	0.21		
Salt%	**Butter 0% salt**	0	210	0.000 HS
	Butter 3% salt	2.57		
	Cheese 0% salt	0	210	0.000 HS
	Cheese 6% salt	5.20		

S= significant at p value ≤ 0.05, HS= highly significant at p value ≤ 0.001

5- Discussion

The results shown in **Table (1)** revealed that *Stenotrohomonas* spp was isolated from 79 out of 210 examined milk and dairy products. The highest incidence strain was *S. nitritireducens* followed by *S. africana* then *S. acidaminiphila* and finally *S. maltophilia* with an incidence rate 11.9%, 7.62%, 6.19%, 4.76% respectively.

In farm milk, *Stenotrophomonas* spp was isolated at percent of 23.33%.The highest incidence was 13.33% for *S. nitritireducens* followed by 6.67% for *S. africana* then 3.3% for *S. acidaminiphila* while *S. maltophilia* and *S. rhizophila* failed to be detected. These results may be due to poor hygienic measures during milking, wet bedding which agreed with **Finkmann et al. (2000)** who reported that *Stenotrophomonas* spp could be isolated from water, soil, sludge and plant rhizophere. On the other hand, dairy shops milk showed incidence rates of 36.67%, 3.33%, 3.33%, 6.67% and 10% for *S. nitritireducens*, *S. maltophilia*, *S. acidaminiphila*, *S. africana* and *S. rhizophila*, respectively. This may be attributed to contamination of milk with water, soil **(Ryan et al. 2009).**

Damietta cheese, the only contaminated sample (3.33%) was identified as *S. nitritireducens*. The low incidence in Damietta cheese may be the results of high salt content which interferes with the growth of *stenotrophomonas spp*. **(Martinez 2011).**While in ice cream, the highest incidence was 23.33% for *S. rhizophila* followed by *S. africana* was 20%, *S. acidamniphila* was 16.67% then *S. nitritireducens* was 13.33% and finally *S. maltophilia* was isolated at percent of 6.67%. The high incidence may be attributed to unhygienic ice making machine (Qureshi et al., 2005), poor handling and unsanitary conditions during frozen storage **(Mathews et al. 2013).**

Regarding yoghurt, the isolated strains were *S. acidaminiphila* and *S. rhizophila* with percentages of 3.34%, 3.33%, respectively. The low results in yoghurt may be due to high acidity % that agreed with **Gallagher et al. 2019** who reported that *Stenotrophomonas spp.* require PH 6-7 for growth. On the other hand, cooking butter had incidence rates of 23.33%, 6.67%, 10%, 3.33% and 3.33% for *S. nitritireducens, S. maltophilia, S. africana* and *S. rhizophila* while *S. acidaminiphila* was failed to be detected. These high results may be attributed to contamination with human derived aerosols to the butter during processing, handling of butter with persons suffer from skin lesions, respiratory disorders **(Vartivarian et al. 1996; Wainwright et al. 2009).**

In cream, the highest incidence was 66.67% for *Stenotrophomonas spp* among all examined products. *S. rhizophila* and *S. maltophilia* were isolated at percentage of (13.33%) for each then, *S. acidamnphila* and *S. africana* were isolated at percent of 3.33% for each one. The high results may be the result of the ability of *Stenotrophomonas* to form biofilm in the dairy utensils which is difficult to be cleaned **(Di Bonaventura et al. 2007).** The results in table (1) showed that all *S. maltophilia* identified in the biochemical examination were confirmed by PCR examination with an incidence of 3.33%, 6.67%, 10% and 13.33% for dairy shops milk, ice cream, cooking butter and cream respectively.

Survival of S. maltophilia

a. Survival of *S. maltophilia* in cream

The initial population of *S. maltophilia* at zero time was 7.2 $\log_{10}$ cfu/ml with a PH of 6.55 and 0.14% acidity %, there was a progressive rise the count of *S. maltophilia* to a peak of 9.27 $\log_{10}$ cfu/ml at the 14^{th} day, gradually decreasing to 3.3 $\log_{10}$ cfu/ml at the end of the storage period. In addition, the PH decreased to 5.1 with acidity 0.35% at the end of storage period **(Table 2).**

It is obvious from results shown in **Fig. (1a, b)** that there was a significant positive correlation (r = 0.76) between the count of *S. maltophilia* in liquid cream and PH (P = 0.00), which reflects the fact already mentioned that *S. maltophilia* growth is impaired at low PH **(Gallagher et al. 2019).**

b. Survival of *S. maltophilia* in butter

The initial population of *S. maltophilia* in butter 0% and 3 % salt at zero time was 7.2 $\log_{10}$ cfu/ ml with PH 6.55 and 0.14% acidity. A gradual increase occurred in the count of *S. ma ltophilia* in butter 0% salt to 8.23 $\log_{10}$ cfu/ ml at the 14^{th} day of storage with PH 5.25 and 0.26% acidity, then decreasing toward the end of storage period to reach 4.94 $\log_{10}$ cfu/ ml with PH 4.95 and 0.3% acidity **(Table 2).** There are no previous reports on the survival of *S. maltophilia* in butter. On the contrary, there was a gradual decrease in the count of *S. maltophilia* in butter 3% salt from the 1^{st} day of storage (6.9 $\log_{10}$ cfu/ml) until it disappeared completely at the end of the storage period (30^{th} day) with PH 5.1 and 0.29% acidity with the salt at 2.9% **(Table 2).** Taking these results together in consideration, the disappearance of *S.*

maltophilia in butter 3% salt indicates that salt content interferes with its growth **(Martinez 2011)** as shown in **Table (3).**

It is concluded from the results illustrated in **Fig. (1c, d)** that there were significant positive correlations (r = 0.61, P = 0.004 and r = 0.70, P = 0.001) between the count of *S. maltophilia* and PH in butter 0% and 3% salt, respectively. Moreover, there were significant negative correlations (r = -0.76, P = 0.000 and r = -0.90, P = 0.000) between the counts of *S. maltophilia* and acidity % in butter 0% and 3% salt, respectively. These findings confirm that growth of *S. maltophilia* is impaired at low PH **(Gallagher et al. 2019).**

Regarding butter 3% salt, there was a negative correlation (r = -0.99, P = 0.000) between the count of *S. maltophilia* and salt **(Fig. 2a, b, c).** These results agreed with Martinez, 2011, who found that the growth of *S. maltophilia* is impaired with salt > 2% and at 7% salt there was no observed growth, indicating that high salt concentration products will have reduced risk of infection with this harmful pathogen **(Table 3)** which confirms these findings and shows that there was a difference between butter 0% and 3% salt regarding the count of *S. maltophilia*. Therefore, it is highly recommended to add > 2% salt during butter making.

c- Survival of S. maltophilia in cheese

The initial population of *S. maltophilia* during cheese making (0% and 6% salt) at zero time was 6.2 $\log_{10}$ cfu/ ml with a PH 6.55 and 0.14% acidity. For cheese 0% salt, a slight increase occurred by day 2 of storage reaching 6.7 $\log_{10}$ cfu/ml with PH 5.9 and 0.19% acidity, after that there was a gradual decrease till reach 2 $\log_{10}$ cfu/ml at the end of storage period (30th day), with PH 4.6 and 0.3% acidity **(Table 2).** Concerning cheese 6% salt, one log reduction in the count of *S. maltophilia* occurred on the 1st day of storage, followed by a continuous reduction until complete disappearance on the 10th day of storage, at which the reading for the PH, acidity, and salt were 5.65, 0.22% and 5.25% respectively **(Table 2).**

The results illustrated in **Fig. (1e, f)** showed that there were significant positive correlations (r = 0.58, P = 0.008 and r = 0.98, P = 0.000) between the count of *S. maltophilia* and PH in cheese 0% and 6% salt, respectively. Taken together, all this evidence demonstrates that *S. maltophilia* is suppressed by lowering PH **(Gallagher et al. 2019).** For cheese 6% salt, there was a strong negative correlation (r = - 0.99, P = 0.000) between count of *S. maltophilia* and salt **(Fig. 2d, e, f).** The data presented in **Table 3** showed a highly statistically significant difference between cheeses 0% and 6% salt regarding the count of *S. maltophilia* (P < 0.001). These findings are similar to the above results of butter 3% salt; therefore, confirming the advisability of adding salt above 2% during cheese manufacturing to reduce the risk of *S. maltophilia* contamination **(Martinez 2011).**

5. Conclusion

In this study, we concluded that the presence of *Stenotrophomonas* in milk and dairy products represents an indicator for poor hygienic measures during processing and handling. *S. maltophilia* is lipophilic organism, so; cream and butter were the most contaminated products with this pathogen. The growth of *S. maltophilia* is impaired by high acidity so, it couldn't be isolated from yoghurt. Also, high salt % suppress *S. maltophilia*, growth and multiplication in dairy products so, it can be used as a method for dairy products preservation.

6. Authors Contributions

All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

7. Conflict of interest

The authors declare no conflict of interest

8. References

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