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Investigation of Enterotoxigenic Coagulase-negative Staphylococci Isolated from Local and Imported Dairy Products; a Microbiological Study

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ABSTRACT

Coagulase negative staphylococci (CNS) have been recorded as a conveying vector for virulence genes and have been implicated in some cases of food poisoning. Research interest in CNS has increased over the past decade following their implication in infections in animals and humans. This study was aimed to detect CNS isolated from 150 dairy products (yoghurt, several types of cheese, Lork, and Serezh) in Sulaimani and Halabja governorate. Thirteen isolates out of 150 samples were identified as CNS using the VITEK® 2 system as an identification method. Results revealed that the most common isolates species including Staphylococcus saprophyticus, Staphylococcus sciuri and Staphylococcus xylosus each species have been identified in 3 samples separately (23%), followed by Staphylococcus vitulinus was in 2 samples (15%), Staphylococcus equorum found in 1 sample (8%), and Staphylococcus gallinarum also was in one sample (8%). The isolated CNS did not have enterotoxins type A to E according to RIDASCREEN kit test. Studying the growth limits of S. saprophyticus and S. vitulinus results showed that S. saprophyticus grew better at pH levels (5,6,7) at (25°C,37°C) and low NaCl concentration (5%), while low bacterial activity was observed at pH 4 at all temperatures and NaCl concentrations and also at 4°C at all pH and NaCl levels. S. vitulinus behaviour was almost the same as S. saprophyticus but, S. vitulinus was able to tolerate different NaCl concentrations and overall had higher bacterial activity in all parameter's interactions than S. saprophyticus. Investigating the effect of acetic acid and lactic acid on the growth of previous species where studied, S. saprophyticus grew better in different concentrations of L.A but

S. vitulinus showed more activity than S. saprophyticus in A.A and the growth of both species inhibited at 0.4% of L.A at the first 24 hours of incubation.

1 INTRODUCTION

The Coagulase-negative staphylococci group consists of more than 50 different species and subspecies with approximately a dozen generally found in the milk of dairy cows [1, 2]. Coagulase-negative staphylococci (CNS) are a group of opportunistic pathogens, that are not only found in humans and animals but also widely spread in the environment, such as water, soil, air, and dust [3].

CNS had little interest to scientists because it was considered non-pathogenic members of the genus, but research interest in CNS has increased over the past decade following their implication in infections in animals and humans [4]. Due to the formation of staphylococcal enterotoxins (SEs) in foods staphylococcal food poisoning (SFP) become one of the most common food-borne diseases worldwide [5]. Dairy products were well-known vehicles of staphylococcal poisoning, with raw milk and cheese being linked to outbreaks [6].

Food contamination is a major problem in all societies. It can occur at any point in the course of food production, transport, storage, or preparation [7]. Contaminated food with staphylococci due to improper handling and storage of food can be the reason behind staphylococcal food poisoning (SFP) [8]. Epidemiological studies showed that dairy products can be contaminated with CNS by using unpasteurized raw milk and also during manufacturing practices by workers. A case study of food poisoning outbreaks linked CNS strains with contaminated unpasteurized milk [9].

Although enterotoxins are produced mainly by CPS, there has been interest in some CNS involved in different infections of humans and animals [10]. Few is known about CNS growth in foods. In the past, CNS strains were rarely involved in food poisoning since they do not rapidly grow in foods. However, since humans are common carriers of these microorganisms CNS can contaminate foods and some can be associated with specific infections in humans [11].

Coagulase-negative staphylococci group is frequently considered as a positive food biota; therefore, it's generally applied in industry. CNS are widely used as an ingredient of cheese and meat starter culture because of their positive impact on sensory characteristic and fermentation processes of products [12, 13]. CNS are part of the microbiota of traditional cheeses, particularly smear soft cheese or surface mould soft cheeses, semi-hard and hard cheeses [14-16].

This study aims to determine the rate of contamination in a range of local traditional and imported (commercial) dairy products by staphylococci. To determine the rate and types of enterotoxins production by coagulase-negative staphylococci isolated from local traditional and imported dairy products. Also, to evaluate the inhibitory effects of pH, sodium chloride, temperature and preservatives such as (acetic acid and lactic acid) on coagulase-negative isolates.

2 METHODS AND MATERIALS

2.1 Sampling

A total of (150) dairy product samples including yoghurt, cheese, Serezh (A Kurdish traditional product made from buttermilk), and Lork (a product similar to local Kurdish cheese made from bovine colostrum) were collected from Sulaimani and Halabja governorates (located in the Kurdistan regional government of Iraq) from retail sale sites along with hypermarkets during November 2018 to January 2019. From these number of samples 68 samples were yoghurt of which 44 samples were from local shops, 6 samples from local factories and the rest of 18 samples were imported samples, 63 samples consisted of different type of cheese; 26 traditional handmade, 4 made by local factories and 33 imported samples. The number of Serezh and lork were 12 and 7 respectively.

2.2 Isolation and identification of CNS

All samples (5 g of each sample mixed with 45 ml peptone water) were homogenized in sterile blender cheese, Serezh and Lork required the use of 2% sodium citrate[17] to disperse the samples. The count of and isolation of presumptive staphylococci were conducted by plating tenfold serial dilutions (10^{-1} to 10^{-8} for local samples and 10^{-1} to 10^{-3} for imported samples) on nutrient agar and mannitol salt agar for 24- 48 hrs of incubation at 37 ℃ in three replicates as the total samples 100×8×3 local and 50×3×3 imported. The selected colonies were purified by streaking on mannitol salt agar and sheep blood agar. All isolates were preliminarily tested for their morphology, gram staining, mannitol fermentation, haemolysin production, catalase production, coagulase enzyme production using the traditional method [18], the isolates were further confirmed by VITEK® 2 compact system.

2.3 Detection of Coa gene

For further confirmation, all staphylococcus isolates were used for molecular testing using the PCR technique which was selected by the detection of the Coa gene. The primers that were used (F 5′-ATA GAG ATG CTG GTA CAG G-3′), (R 5′-GCT TCC GAT TGT TCG ATG C-3′). The polymerase chain reaction was done in a final volume of (25 μl) in a thermal cycler. Each reaction contained (5 μl) of DNA, (1μl) of forward and reverse primer, and (13 μl) of (2 X) amplicon master mixes. Then the nuclease-free water was used to adjust the volume of this mix too (25 μl). The PCR cycles consisted of preheating at 94°C for 45 sec, denaturation at 94°C for 20 sec, annealing at 57°C for 15 sec, and extension at 70°C for 15 sec. The amplification was performed for 30 cycles with a final extension step at 72°C for 2 min [19].

2.4 Detection of enterotoxins

All of the isolates were subjected to a procedure that enhances *in vitro* enterotoxin production using Brain heart infusion agar supplemented with phenol red. After 24 hrs of incubation at 37 ℃ the required growth to induce the production of enterotoxin in which indicated by the change of the medium's colour from yellow to red-violet (pH 8.2) [20]. For accurate results sandwich enzyme immunoassay RIDASCREEN® SET A, B, C, D, E kit (R-Bio farm, Germany) was used for determining the presence of staphylococcal enterotoxins according to the manufacture's protocol.

2.5 Survival of Staphylococcus saprophyticus and staphylococcus vitulinus

To evaluate the effects of different levels of sodium chloride concentration (NaCl), pH, and temperature on the growth of *S. saprophyticus* and *S. vitulinus*, the experiment was arranged in a factorial design in BHIB and cultured on BHI agar. This design included four levels of pH (4, 5, 6, and 7) adjusted by HCl which each level of pH applied in six levels of NaCl (0, 5, 10, 15, 20, and 25%) and three storage temperatures (4, 25 and 37°C) for (24 hrs). In each temperature level, two blanks without pH justification was applied as for each designated pH level, a set of 42 tubes were considered for a combination of different incubation temperatures

and NaCl concentrations. Each set of tubes inoculated with one of the two species of Staphylococcus isolates to reach the known bacterial concentration/ml (0.1 ml of McFarland 1 for each tube) and each set of tubes was incubated in different temperatures for (24 hrs). After incubation, the cultures were serially diluted and inoculated on sterile BHI agar using the spreading method, and the plates were incubated at (37℃) for (24 hrs). The number of colonies was counted and calculated as cfu/ml [21].

2.6 The Sensitivity of Staphylococcus saprophyticus and staphylococcus vitulinus to Different Food Additives

Two sets of flasks were prepared, each consisted of twelve (250 ml size) conical flasks containing (100 ml) BHI broth with different concentration of lactic acid and acetic acid (0, 0.05, 0.1, 0.2, 0.3 and 0.4%) v/v. Each set of flasks inoculated with one of the two CNS species and all incubated at (37℃) for 3 days. The first day of incubation (24 hours) the cultures were serially diluted and inoculated on sterile BHIA and incubated at (37℃) for (24 hrs) and the same procedure was repeated for the second day (48 hours), and the third day (72 hours) of incubation. The numbers of colonies were counted for each day and the results were calculated as cfu/ml [22].

3 RESULTS AND DISCUSSION

3.1 Enumeration of Bacterial Numbers

Bacterial growth was observed on cultured nutrient agar in (81samples; 54%), out of 150 samples while (69 samples; 46%) showed no growth on N.A. Table (1) shows the bacterial counting range on nutrient agar in different samples.

Table 1: Bacterial counting on nutrient agar medium

Origin	Type of sample	Growth range cfu g^{-1}
Local handmade	Yoghurt	3.0×10^3 - 1.1×10^{10}
	White soft cheese	4.8×10^4 - 8.0×10^9
	Block cheese	1.8×10^8 - 6.4×10^9
	Pesta cheese	5.0×10^5 - 1.9×10^7
	Cheese (High fat)	7.0×10^4 - 1.1×10^9
	Serezh	9.6×10^4 - 7.6×10^9
	Lork	1.1×10^6 - 6.0×10^9
Local factories	White cheese	1.5×10^7
Imported	Yoghurt	1.0×10^6
	Spreadable cheese	8.0×10^4
	Hard cheese	1.6×10^4 - 3.6×10^5

In a study conducted in Al-Diwaniya/Iraq to determine contamination levels in local and imported milk and its derivatives samples where 54 of (milk, cream, cheese, and yoghurt) samples were collected from markets. Their results showed that total bacterial count in local yoghurt was between (37×10^7 to 15×10^8 cfu g^{-1}) comparing with the current study which only (2 samples; 5.7%) of yoghurt had the total count above their reported range. In disagreement with this study, they found that total bacterial count in imported Iranian yoghurt samples was (62×10^8 cfu g^{-1}), while there was no bacterial growth in Iranian yoghurt samples in this study and for their tested cheese samples the level of the bacterial count of local cow and buffalo cheese was between (213×10^9 - 78×10^{11} cfu g^{-1}) was a little higher compared to this study [23]. In another study where they examined samples of Tilsit cheese and they found the total bacterial cell counts ranged between (3×10^7- 3.4×10^8 cfu/cm^3) of analyzed cheese which is comparable with the results of current study[24]. [25] found that values of Total Viable Counts (TVC) in Slovak cheeses samples after opening ranged from (1.68×10^3 CFU.g^{-1} to

2.91×10^3 CFU.g^{-1}) which is considerably lower than the average of total bacterial count (1.2×10^9 cfu g^{-1}) of cheese samples in the current study.

Staphylococci growth on MSA was only observed in a different type of cheese, Serezh, and Lork, unlike yoghurt samples which showed no growth on MSA this result could be caused by the acidity of yoghurt heat treatment during production and the salt added in yoghurt. It has been reported that yoghurt had the least attention among dairy products because of its milk pasteurization and high acidity which are effective barriers to the growth of pathogens [26] and Imported samples also were not contaminated with staphylococci. Staphylococci growth indicated in (30 samples; 20%) out of 150 samples and staphylococcal count range as shown in table (2).

Table 2: Staphylococcal count on MSA

Origin	Type of sample	Staphylococcal count on MSA
Local handmade	White soft cheese	7.2×10^3 - 2.9×10^6
	Block cheese	5.4×10^4 - 1.8×10^6
	Pesta cheese	3.1×10^5 - 5.7×10^6
	Cheese (high fat)	2.2×10^4 - 5.8×10^4
	Serezh	1.4×10^6
	Lork	2.2×10^4 - 4.4×10^5
Local factories	White cheese	7.8×10^3

Only (13 samples; 43.3%) out of 30 were able to ferment mannitol (10 samples; 76.9%) out of that 13 samples were originated from local traditional handmade cheese that includes (Pesta cheese (salted ripened), and white soft cheese (Salik), and (2 samples; 15.3%) were Lork and 1 sample (7.6%) was Serezh and these samples were chosen for further investigations. According to [27] staphylococci, cell count in 3 samples of raw goat's milk cheese on MSA was variable from (10^4-10^5 cfu g^{-1}). I similar study their results showed (10^2-10^5 cfu g^{-1}) of viable cell count of staphylococci in different European raw milk cheeses [28] the results of the two previous studies were near to the current study with an average of staphylococcal count in cheese samples (7.88×10^5 cfu g^{-1}). There are limited studies on the CNS isolated from foods in spite of being very common in food, especially dairy products. In another study [29] f where they tested 90 samples of dairy products for the presence of CNS, found that out of 60 sample of soft cheese and processed cheese only (19 samples 31.6 %) were contaminated with CNS, with a slight different with the current study in which 22 samples out of 63 cheese samples (34.9%) were contaminated with staphylococci. However, in disagreement with the current study where there was no growth indicated in yoghurt samples their results showed that 5 samples of yoghurt out of 30 samples 16%, were contaminated with CNS. Also, [30] detected that out of 200 sample of cottage cheese only (44 samples 22%) were contaminated with staphylococci which is lower than the amount of staphylococci contamination in our study (34.9%), and biochemical results found that (19 samples; 9.5%) were contaminated with CNS. But in disagreement with current study where no staphylococcal growth was indicated in yoghurt samples their results showed out of 200 yoghurt samples (13 samples; 6.5%) were contaminated with staphylococci and biochemical characterization results showed that only (4 samples; 2%) were contaminated with CNS.

In another study by [31] conducted in Turkey showed that out of 40 sample of goat cheese (23 samples; 57.5%) contaminated with CNS which is higher than the current study, and in agreement with this study, all 20 samples of salted yoghurt found free of CNS. The reason for the different ratio of contamination may be the difference between the cheese types manufactured by different production procedures and cheeses manufactured from milk obtained from animals in different species such as cow, goat and sheep.

3.2 CNS species identification

Gram-positive cocci, mannitol fermenters, catalase-positive, and coagulase-negative isolates and PCR results also showed that none of the isolates owns *Coa.* gene as shown in figure 1. The results of VITEK® 2 compact system as shown in table (3).

Table 3: Identified staphylococcal isolates using VITEK® 2 compact

Species	Type of sample
Staphylococcus saprophyticus	Pesta cheese
	Block Cheese
	Lork
Staphylococcus xylosus	2 samples of white soft cheese
	Lork
Staphylococcus sciuri	2 samples of block cheese
	Serezh
Staphylococcus vitulinus	2 samples of white soft cheese
Staphylococcus gallinarum	white soft cheese
Staphylococcus equorum	Pesta cheese

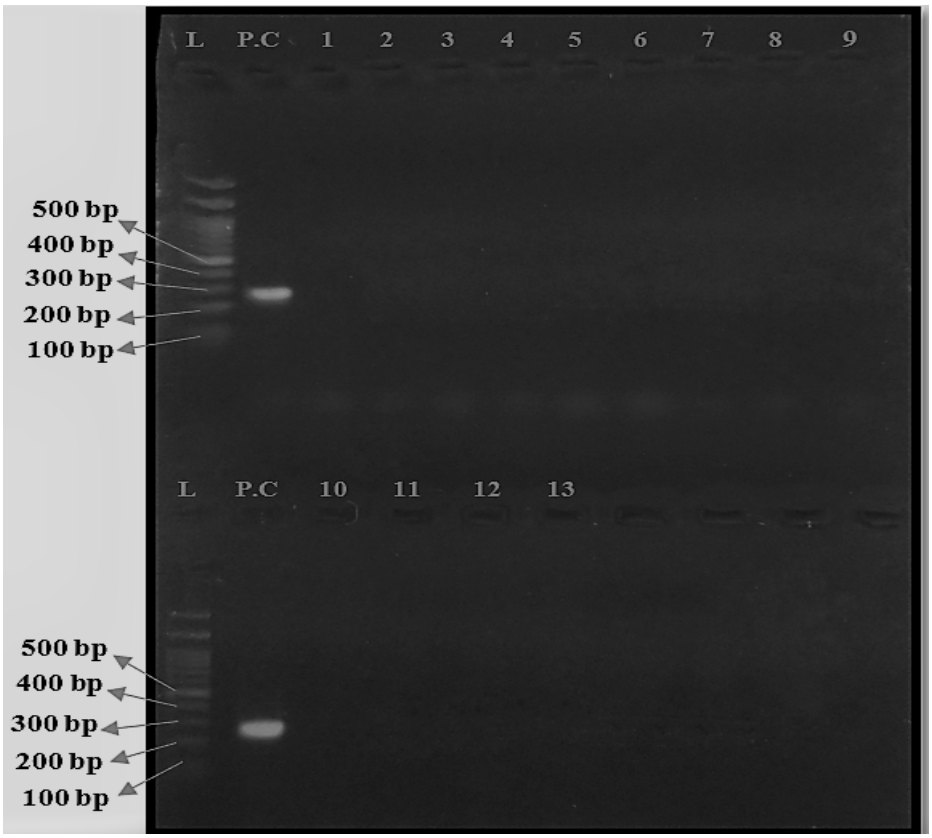

Figure 1: Uniplex PCR products on agarose gel electrophoresis for the detection of Coa. gene. L: 100 bp DNA ladder; lane P.C: Positive control (*S. aureus* ATCC 6538); Lanes 1 to13: isolates

[32] used VITEK® 2 system for identifying CNS species isolated from 72 samples of different type of cheese in Turkey reporting that only 17 samples 23.6% were CNS, where 6 (35.3%) *Staphylococcus saprophyticus*, 3 (17.6%) *Staphylococcus epidermidis*, 2 (11.8%) *Staphylococcus haemolyticus*, 2 (11.8%) *Staphylococcus hominis*, 1 (5.9%) *Staphylococcus warneri*, 1 (5.9%) *Staphylococcus xylosus*, 1 (5.9%) *Staphylococcus vitulinus*, and 1 (5.9%) *Staphylococcus lentus*. Their results compared to the current study showed that some of the

isolated species were not detected in our tested samples while other species were the same as current study such as *S. saprophyticus, S. vitulinus* and *S. xylosus* but in a higher rate. Another study reported that out of 40 samples of goat cheese 48 of CNS strains were isolated. The most dominant species in cheese were *S. saprophyticus* (29 strains 60.4%), *S. xylosus* (6 strains; 12.5%), and it was followed by *S. haemolyticus* (4 strains, 8.3%), *S. equorum* (3 strains; 6.3%) *S. capare* (2 strains; 4.2%), *S. carnosus* (2 strains; 4.2%), *S. sciuri* (1 strain; 2.1%) and *S. simulans* (1 strain; 2.1%). Their results showed that 4 CNS species were the same as the current study but in a higher range [31]. These differences in the results can be due to the samples with different sources of contamination, animal species, and applied measures of milking and production hygiene.

3.3 Detection of staphylococcal enterotoxin

The results of culturing method showed that the isolates were identified as non-enterotoxigenic staphylococci and did not change the color of the BHIA medium supplemented with phenol red from yellow to red-violet. On the other hand, using RIDASCREEN® SET A, B, C, D, E for detecting CNS enterotoxins where the detection limit of the test was 0.25 ng ml^{-1}. The absorbance value for positive controls for all samples was greater than (1.0) and the mean absorbance value for negative controls was less than (0.2) which implies that the test was performed correctly. For the entire samples, the absorbance value for all enterotoxins (A to E) was smaller than the threshold value (mean value of negative controls + 0.15) which indicates no one of samples had enterotoxins type A to E in the defined detection limits for this special kit. The overall result of enterotoxin indicated that those dairy products available in our market could be accounted for as safe and with no enterotoxigenic bacteria.

In a study conducted in south-eastern Brazil, 10 CNS strains were isolated from 6 Minas Frescal cheese, and the most dominant strains isolated were *S. saprophyticus* (40%), *S. xylosus* (30%), *S. sciuri* (20%), and *S. piscifermentans* (10%). By using RIDASCREEN® SET A, B, C, D, E for detecting CNS enterotoxins, in disagreement with current study which there was no enterotoxin detected, their results showed that out of 10 CNS strains 9 strains were able to produce SEA, SEB, SEC, SED, and SEE in concentrations ranging from 0.12 to 1.8 ng/mL and in-line with current study one strain of *S. saprophyticus* did not produce SEA to SEE enterotoxins using this essay [33]. In another study conducted in Turkey where they tested 40 samples of non-ripened white cheese where they isolated 48 CNS strains consisted of *S. saprophyticus*, *S. xylosus*, *S. haemolyticus*, *S. equorum*, *S. caprae*, *S. carnosus*, *S. sciuri*, and *S. simulans*. This study showed a little diversity to the current study with only one CNS strain of *S. sciuri* isolated from one cheese sample was able to produce enterotoxin B and the other strains were non-producers [31].

In agreement with this study [34] found that none of the CNS isolates from 121 different foodstuffs including goats' cheeses produced enterotoxins. [35] found that out of 129 CNS strains isolated from several types of cheese and dry fermented sausages only one strain of *S. saprophyticus* carried a sec gene, where the others did not have any of the classical encoding genes (i.e. sea to see). Similarly, to current study, enterotoxin genes were not detected in 87 CNS strains isolated from dairy products and meat [36]. The differences in these results may be due to the ability of CNS to produce SE or different sources of the samples from which CNS strains were isolated or such difference between studies can be because of a bias in the CNS strains studied, as well as the nature, the number and geographical origin of isolates.

3.4 Growth limits of Staphylococcus saprophyticus and Staphylococcus vitulinus as a function of pH, temperature, and sodium chloride (NaCl) concentration pH

Staphylococcus saprophyticus were isolated from Pesta cheese and *Staphylococcus vitulinus* from White soft cheese (Salik), due to the high consumption of these two types of cheese they were selected for this experiment. Most of the parameters had a significant effect on the growth of *S. saprophyticus* referred to as (B_1) and *S. vitulinus* as (B_2). The effect of pH

individually (ranged 4, 5, 6, and 7) at (37°C) for 24-hour incubation with (zero%) NaCl was performed on B_1 and B_2 which the pH was adjusted using HCl. As shown in Figure (2) no growth occurred in pH 4 for B_1 and B_2. The average bacterial growth for both species B_1 and B_2 showed a slow increase for pH 5 (3.9×10^6 and 1.2×10^5 cfu mL^{-1} for B_1 and B_2, respectively) and then more enhanced in pH 6 (2.1×10^7 and 6.5×10^6 and cfu mL^{-1} for B_2 and B_1, respectively). The pH 7 showed the highest growth for both species with significant increasing (1.3×10^8 and 1.5×10^8 cfu mL^{-1} for B_1 and B_2, respectively). There was no significant difference observed among the effect of pH 4 and pH 5 (P = 0.58) however, a significant difference was seen between pH 5 and pH 6 (p< 0.05) and also pH 7 was considerably higher and significantly different with all other pHs (p<0.001). Also, the differences between the growth amount for both B_1 and B_2 were significant (P<0.05). In agreement with this study [37] studied the effect of different pH acidic, neutral, and alkaline on *S. saprophyticus* and found that acidic situation affects the protein related to iron storage in these bacteria when exposed in pH 5.5 and also cause down-regulation of some other enzymes whilst this issue was not found in neutral and alkaline pH.

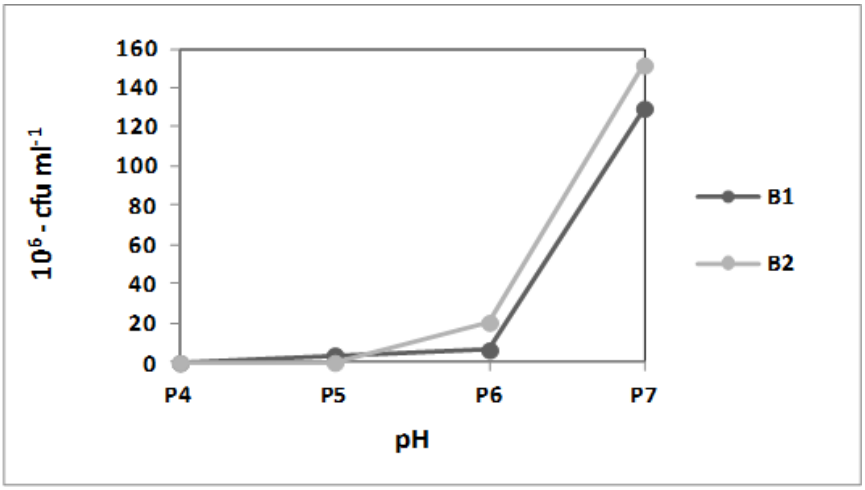

Figure 1: Effect of pH individually on *S. saprophyticus* and *S. vitulinus*

NaCl

Different concentration of NaCl was used (0, 5, 10, 15, 20, and 25%) for both bacteria B_1 and B_2 at pH 7 with incubation temperature (37°C) for 24 hours. As indicated in the figure (3) bacterial growth in both strains at (zero%) NaCl was high and gradually started to decrease with an enhancement of NaCl concentration. The highest growth point (between treatments) for both bacteria was at (5%) NaCl with an average of (7.05×10^7 cfu mL^{-1}) for B_1 and (9.93×10^7 cfu mL^{-1}) for B_2 and the lowest bacterial growth was at (25%) NaCl with an average of (3.82×10^5 cfu mL^{-1}) and (1.09×10^5 cfu mL^{-1}) for B_1 and B_2 respectively. The difference between bacterial growth at the different NaCl concentration was statistically significant (p< 0.01) except (15%) and (20%) NaCl (p = 0.872), 15%, 25% NaCl (p= 0.878), and 25% and 20% NaCl (p = 0.994) which there was no significant difference. Furthermore, there was a significant difference between the growth of both bacteria species (p< 0.01).

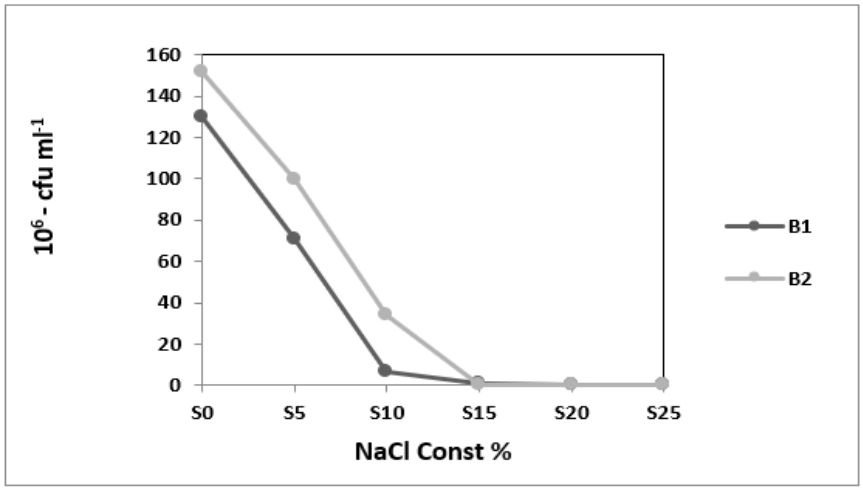

Figure 2: Effect of NaCl individually on *S. saprophyticus* and *S. vitulinus*

Temperature

For the effect of refrigerator temperature 4℃ and incubation temperature at 25℃ on the growth of B_1 and B_2 as shown in figure (4) were nearly the same with an average of (2.13×10^5 cfu mL^{-1}) and (1.23×10^8 cfu mL^{-1}) for B_1 at 4℃ and 25℃ respectively. While the average of B_2 growth during the incubation period was (6.47×10^5 cfu mL^{-1} at 4℃ and 1.24×10^8 at 25℃). At 37℃ temperature had a different effect on both bacteria. Slightly rose was observed in the growth of B_1 with an average of (1.30×10^8 cfu mL^{-1}) but, B_2 had the highest growth at (37℃) with an average (1.52×10^8 cfu mL^{-1}). There was no significant difference between B_1 and B_2 (p> 0.088). Although, there was a significant difference between all three temperatures 4℃, 25℃, and 37℃ (p < 0.0001). In another study [38] carry out the influence of different temperature (23, 30 and 37℃) on different coagulase-negative bacterial community which is important in the fermented meat industry. They found that in each temperature different kind of CNS bacterial community are predominant for example in temperature (23℃) *S.xylusus* is predominant whilst in elevated temperature *S. epidermidis* had optimum growth. In compare with our study *S.vitulinus* had better growth in lower temperature (4 ℃) than *S. saprophyticus* and with a slight difference, *S. vitulinus* showed an increased population in higher temperature (25 and 37℃) rather than *S. saprophyticus*. [39] investigated the behaviour *of S. lugdunensis, S. aureus, and S. epidermidis,* when exposed for a long time to low temperature (4 ℃), and how this factor affected amino acid composition, colony morphology, and cellular ultra-structure. The three staphylococci when it was exposed to low-temperature stress caused the formation of increasing proportions of small colony variant (SCV) phenotypes. Their results showed that SCV cells had significantly more diffuse and thicker cell-walls than their corresponding samples for *S. aureus and S. epidermidis*, however for *S. lugdunensis* the changes were not significant. The data revealed that the staphylococci responded by transforming into SCV populations during extended periods of cold-stress treatment. The observed amino acid and ultra-structural changes were suggested to represent response mechanisms for staphylococcal survival species until favourable conditions arise again. This could be a mechanism that why the population of *S. saprophyticus and S. vitulinus* were decreasing during cold temperature stress however the above study shows that this process

could be a response to survive and probably temporary and cold stress is the factor to control the bacterial population until the situation would be favourable to grow again.

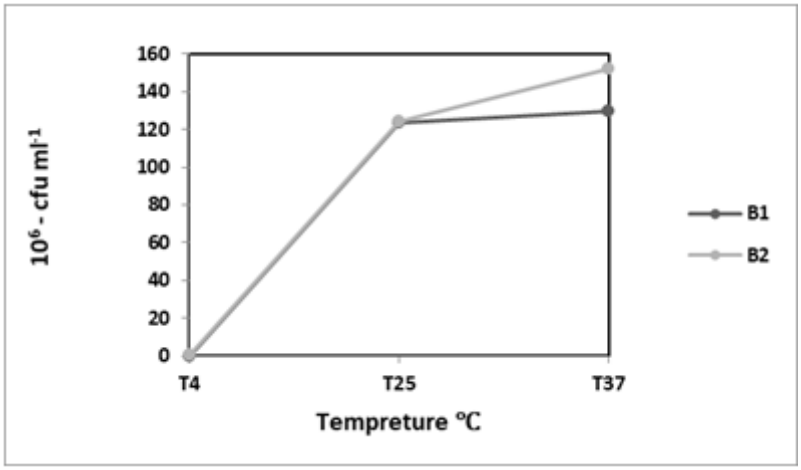

Figure 3: Effect of temperature individually on *S. saprophyticus* and *S. vitulinus*

Interactions pH and Temperature

The first interaction is between different levels of pH (4,5,6, and 7) with three temperature levels (4℃,25℃, and 37℃) and their effect on the growth of *S. saprophyticus* (B_1) as shown in figure (5 A) and *S. vitulinus* (B_2) in figure (5 B) which statistical analysis shows interaction was significant ($p< 0.01$). The lowest bacterial growth was at pH 4 at 37 ℃ for both bacteria the mean for B_1 was (5.15×10^4 cfu mL^{-1}) and B_2 no growth was indicated at pH 4 at 37 ℃. This implies that B_1 is more tolerable to the acidic situation rather than. With increasing the pH value bacterial growth in pH 5 and pH 6 was slightly increased but in pH 7 the bacterial population dramatically enhanced in both bacteria. The growth rate of bacteria under temperature (4℃) treatment remained steady with a slight change in growth in B_1 and B_2. This shows that temperature is the factor that could control growth. The highest growth for both bacteria was in pH 7 at temperature 37 ℃ and the mean for B_1 and B_2 was (3.47×10^7 cfu mL^{-1}) and (4.77×10^7 cfu mL^{-1}) respectively. There was a significant difference among all pH interaction ($p<0.01$) except between (pH 5) and (pH 4) with no significant difference B_1 ($p=0.176$) and B_2 ($p=0.528$). As well as all interactions between temperature had a significant effect on both species ($p<0.01$). As it can be seen in the figure (5 A and 5 B the general trends are correct for B_2 but statistically the difference of bacterial growth in pH (4,5 and 6) was significant ($p<0.01$) except (pH 7) which the differences in B_1 and B_2 was not significant ($p = 0.173$). Figure (5 A and 5 B) shows that the only pH that bacteria could grow in an optimum rather than other pH was (pH 7) and in all three temperatures, the other three-level of pH had lower bacterial growth. These two figures can clearly show the interaction of both two factors on each other. Overall, despite the temperature pH 4 had the lowest bacterial growth and pH 7 had the highest growth in both bacteria. However, *S. vitulinus* (B_2) can tolerate different levels of pH and temperature more than *S. saprophyticus* (B_1).

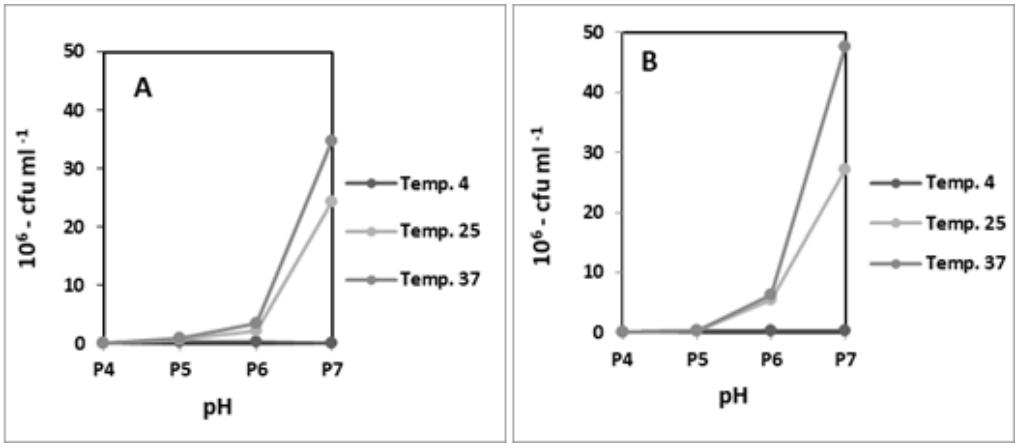

Figure 4: Effect of Interactions between pH and temperature on *S. saprophyticus* (A), *S. vitulinus* (B

NaCl and temperature

The effect of various salt concentrations (0, 5, 10, 15, 20, and 25%) at various temperature (4℃, 25℃, and 37℃) on the growth of *S. saprophyticus* (B_1) figure (6 A) and *S. vitulinus* (B_2) in figure (6 B) has been shown. The bacterial growth started at its peak with the highest growth in both bacteria at temperature (37℃) with no NaCl added. Bacterial activity gradually decreased starting with (5%) to (25%) NaCl which had the least bacterial growth. Also, figures show that the activity of bacteria starts to significantly decrease from (10%) salt for B_1 and (15%) salt for B_2 and almost remained steady in (20 and 25%) NaCl in both bacteria. While the lowest growth for B_1 was (1.45×10^5 cfu mL^{-1}) at temperature (4 ℃) with NaCl (25%) and B_2 was at temperature 37 ℃ with 25% NaCl (5.94×10^4 cfu mL^{-1}). Among all temperatures (4 ℃) mostly affected in case of bacterial growth for both bacteria in different NaCl concentrations and no significant difference was indicated. Significant difference appeared between other NaCl concentration except for (10% - 15%), (15% - 20%), (15% - 25%), and (20% - 25%) in B_1 and (15% - 20%), and (20% - 25%) for B_2. The highest bacterial growth can be achieved in no NaCl addition and lowest temperature as bacterial growth in temperature (37℃) particularly in B_2 is relatively high even in NaCl (15%) treatment. From this, it can be concluded that lower temperatures are more effective for bacterial prevention rather than NaCl addition but to reach the minimum bacterial growth occurred in less temperature and NaCl addition.

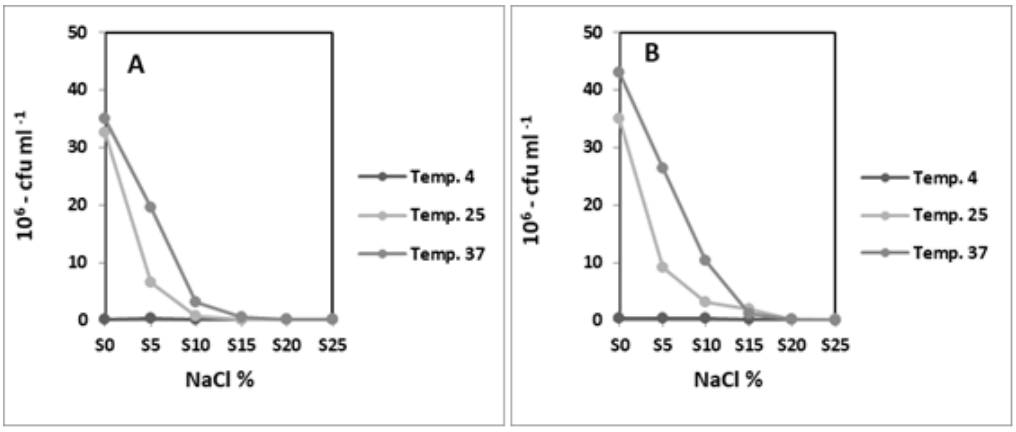

Figure 5: Effect of Interactions between NaCl and temperature on *S. saprophyticus* (A), *S. vitulinus* (B)

NaCl and pH

The interaction between different concentrations of NaCl (0, 5, 10, 15, 20 and 25%) in four levels of pH (4,5,6 and 7) and their effect on the bacterial population has shown for *S. saprophyticus* (B_1) in figure (7 A) and *S. vitulinus* (B_2) in figure (7 B). Bacterial growth for B_1 was at its highest in pH 7 where the mean was (8.43×10^7 cfu mL^{-1}) and for B_2 was (9.22×10^7 cfu mL^{-1}) without NaCl addition. Adding NaCl starting with (5%) the activity of bacteria started to drop down to (3.02×10^7 cfu mL^{-1}) and (4.22×10^7 cfu mL^{-1}) for B_1 and B_2 at pH 7, respectively. Bacterial growth gradually started to decline with increasing NaCl concentration and the growth was almost steady at (10, 15, 20 and 25%) NaCl for B_1 while bacterial activity in B_2 appeared to be approximately stable at (15, 20 and 25%) NaCl. The lowest growth for B_1 and B_2 was at (pH 4) with (25%) NaCl with the mean (3.63×10^4 and 3.70×10^4), respectively. Overall, bacterial growth in both strains was higher in (pH 7) with low NaCl concentrations and pH 4 and pH 5 had the lowest growth in all NaCl concentration for both bacteria B_1 and B_2 however, from pH 6 B_2 has started to increase gradually but for B_1 still limitation effect of pH 6 can be observed in figure (7). This again depends on the different behavior of B_1 and B_2 and a more tolerable level of B_2 rather than B_1.

In addition, [40] fulfilled experiment studying the effect of pH, temperature, glucose, and NaCl on the growth of 12 different staphylococcus strains in meat and sausage industry. The results showed with increasing temperature and pH from (10 to 26 ℃) and (4.6 to 6) respectively will increase the growth for all strains while increasing NaCl from (5 to 15%) decreases the growth but, the effect of temperature and pH was much stronger than NaCl. The results also showed that bacterial growth after 30 hours and 48 hours were similar. Also, they reported that a high concentration of NaCl has considerable negative effect at high temperature than lower temperature. The same was seen for interaction between pH and NaCl similarly to the results of this study showed increasing in growth at higher temperature and pH but, bacterial growth decreased at high NaCl concentrations. Also, general growth was lower when interactions of pH and temperature or NaCl and temperature were carried out whilst when interactions of NaCl and pH was conducted the overall growth was higher. This can show that for the 2 strains temperature had a predominant effect on bacterial growth than other factors. However, in agreement with the study of (729-2) our results show that negative effect of NaCl was greater in elevated pH (7) and temperature (37℃) from (45 cfu ml^{-1} to zero).

According to Mauriello, et al. [41] all strains *S. saprophyticus, S. xylosus and S. equorum*, grew at 10, 15 and 20 ℃, in the presence of 10% and 15% of NaCl and at pH 5.0 and 5.5. The results showed that a wide range of staphylococcal starter cultures adaptable to various sausage manufacture practice and technological conditions.

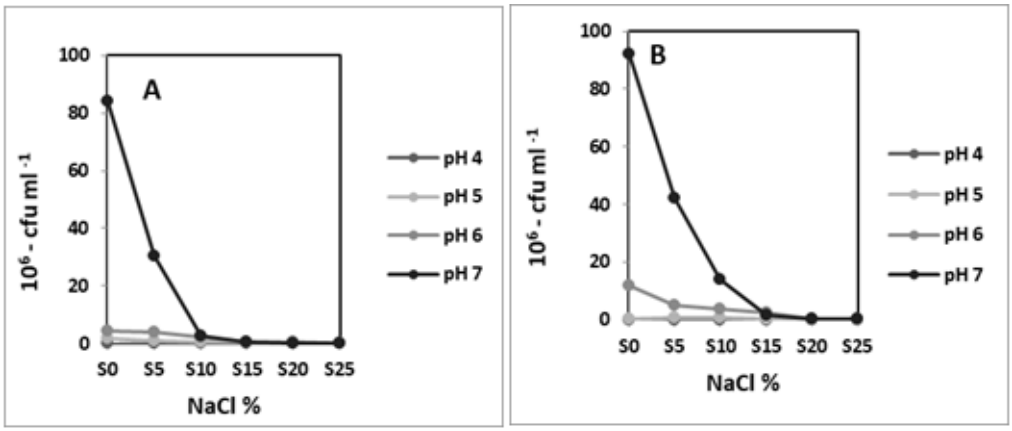

Figure 6: Effect of Interactions between NaCl and pH on *S. saprophyticus* (A), *S. vitulinus* (B)

3.5 Effect of Acetic Acid (A.A) And Lactic Acid (L.A) On S. saprophyticus (B_1) and S. vitulinus (B_2) Growth

Acid and time

The highest growth in B_1 was in lactic acid at (24 hrs) first incubation with mean (1.03×10^8 cfu mL^{-1}) at the same time maximum bacterial growth in acetic acid at (24hrs) was (8.26×10^7 cfu mL^{-1}) with the same incubation time as shown in figure (8 A and B). Furthermore, *S. vitulinus* activity was at its peak in acetic acid at (48 hrs) unlike *S. saprophyticus* with an average of (8.74×10^7 cfu mL^{-1}). One of the reasons for this could be that B_2 had to tolerate to acetic acid at first and then be able to grow in the first (24 hrs) and decreasing from (48 hrs).

S. vitulinus growth was at its highest in lactic acid at 24 hrs with a mean (8.04×10^7 cfu mL^{-1}). The activity of B_1 gradually started to drop in both A.A and L.A with expanding incubation period. The lowest growth for B_1 in acetic acid and lactic acid was at an incubation time of (72 hrs) with an average (2.79×10^7 cfu mL^{-1}) and (3.31×10^7 cfu mL^{-1}), respectively. While B_2 had its minimum growth in acetic acid with (24 hr) of incubation with a mean (2.69×10^7 cfu mL^{-1}) and in lactic acid after (72 hr) of incubation with an average (2.77×10^7 cfu mL^{-1}). This behavior for B_2 was unexpected and it implies that B_2 can tolerate and grow in acetic acid until (48 hr) and then gradually decreases slowly in a way that still the bacterial population is higher than 24h. There was a significant difference between lactic acid and acetic acid and between incubation time ($p<0.0001$) for both bacteria. Significant difference was observed among different (A.A) and (L.A) concentration ($p<0.0001$) except between (0.3%, 0.4%) there was no significant difference with value ($p=0.999$) in B_1 and ($p=0.960$) in B_2. Overall, *S. saprophyticus* activity in lactic acid was higher than acetic acid in all (24, 48, and 72hr) incubation time, unlike *S. vitulinus* that had better growth at acetic acid than lactic acid.

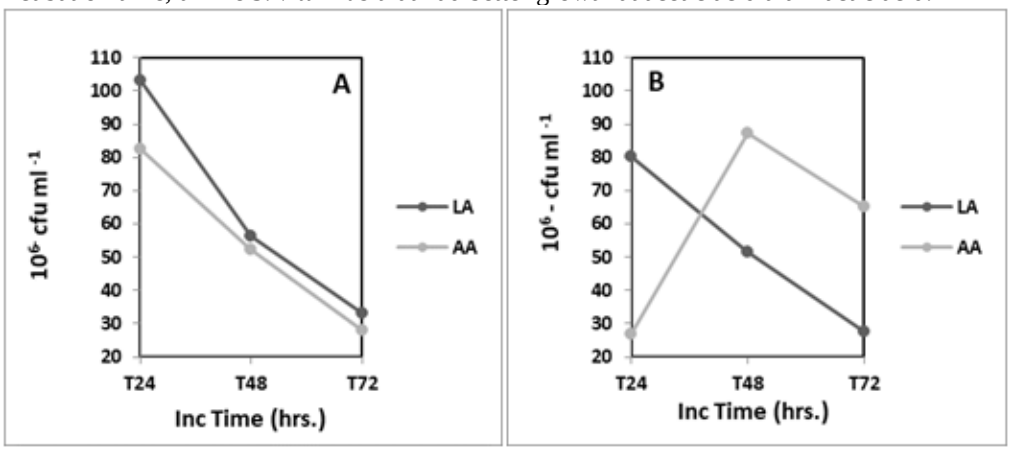

Figure 7: Effect of Interactions between Acid and Time on *S. saprophyticus* (B_1) (A), *S. vitulinus* (B_2) (B)

Acid and concentrations

Normal bacterial growth of both bacteria without adding acetic acid or lactic acid was around (2.10×10^8 cfu mL^{-1}) for B_1 and (2.08×10^8 cfu mL^{-1}) for B_2. With adding (0.05%) of A.A and L.A to both bacteria their activities started to decline continually as shown in figure (9 A and B) with (8.20×10^7 cfu mL^{-1}) as the mean of the highest growth for B_1 in the first concentration (0.05%) of acetic acid followed by (7.32×10^7 cfu mL^{-1}) for lactic acid. As the same step was repeated for B_2 and the maximum growth was in both acids with a slight difference with an average for lactic acid (9.63×10^7 cfu mL^{-1}) and acetic acid (9.59×10^7 cfu mL^{-1}). Bacterial ability to increase started to fall gradually with rising the concentration of acids in both bacteria. There was no growth in acetic acid and lactic acid (0.4%) for B_1. However, the minimum growth for B_2 in acetic acid was (7.22×10^2 cfu mL^{-1}) but there was no growth in (0.4%) lactic acid. This can imply that lactic acid has a higher ability of prevention rather than

acetic acid and also the same as other treatments B_2 acts more tolerable in acetic acid rather than B_1.

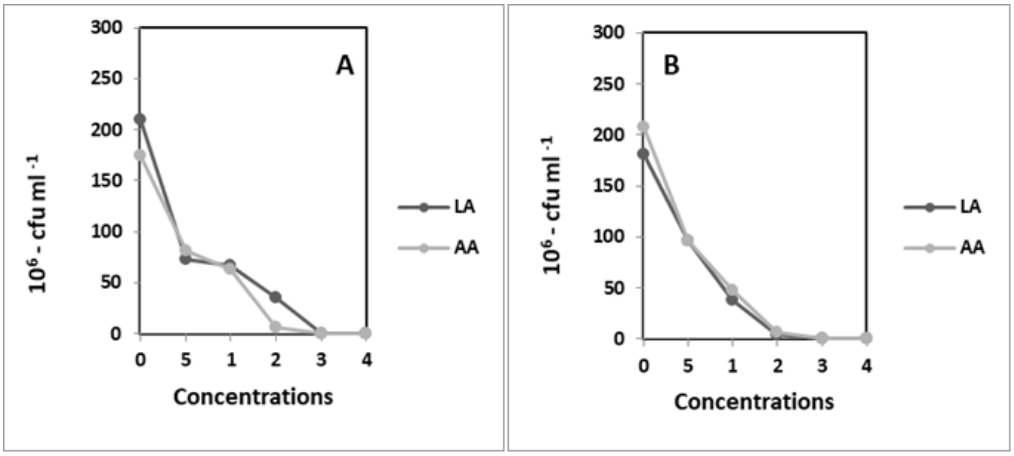

Figure 8 : Effect of Interactions between Acid and Time on *S. saprophyticus* (B_1) (A), *S. vitulinus* (B_2) (B)

Time and concentration

Bacterial activity in B_1 without adding any acids at the first incubation time for (24 hrs) with an average (2.61×10^8 cfu mL^{-1}) slowly decreased with increasing the acid concentration. But B_2, unlike B_1, had higher growth with longer incubation time rather than the first (24 hrs) with a mean (2.76×10^8 cfu mL^{-1}) for (48 hrs) of incubation at (0%) of acids (Figure 10 A and B). Starting with (0.05%) of A.A and L.A bacterial activity in B_1 decreased at first (24 hrs) and suddenly dropped to (4.40×10^7 cfu mL^{-1}) after (48 hrs) of incubation. The population of B_2 after adding (0.05%) of A.A and L.A began to decline slowly at first (24 hrs) with a mean around (1.1×10^8 cfu mL^{-1}) but after (48 hrs) the activity of the bacteria fell to an average (9.80×10^7 cfu mL^{-1}).

With rising A.A and L.A concentration, the growth of B_1 and B_2 declined gradually with almost steady growth at (0.2%), and no growth was observed after (48 hrs) of incubation with (0.3%) of A.A and L.A in both species. As for (0.4%) of A.A, no growth was indicated for B_2 after (48 hrs) of incubation. Also, a concentration (0.2%) bacterial growth of B_1 is more than B_2 unlike the behavior of B_2 which was more tolerable in all the treatments, however, B_1 appears to have more ability to tolerate at that concentration but finally, the concentration of (0.3%) and (0.4%) as mentioned above had the ability to restrict the growth of both bacteria.

The acidic pH within the cell results in damage and deformation to DNA structure, proteins, and enzymatic activities, thereby damaging the extracellular membrane [42]. Acetic acid and lactic acids are widely used sanitizers, and both are commonly recognized as safe [43]. Also, it revealed that lactic acid is less effective than acetic acid [44]. A study concluded that cells that recovered after exposure to sanitizers, were subsequently more resistance to them [45]. This is because of widespread use of them in food as in the case of antibiotics which thought to induce evolutionary changes in bacteria that helps them to survive these materials [46]. [47] studies the ability of acetic acid to inhibit the growth of various types of pathogenic bacteria by using concentration of 40mM,50 mM and 70 mM. *Streptococcus agalactiae, E. coli,* and *S. aureus* almost completely inhibited at 70 mM, although the growth of *streptococcus pneumonia, Proteus mirabilis, Streptococcus mutans,* and *Klebsiella pneumoniae* were not inhibited by the same concentration. After 24 hours of incubation in the current study, we evaluate that 70 mM considered as the minimum inhibitory concentration. The result of our study showed a high similarity as the inhibitory concentration of acetic acid found (0.4 %) which is equal to 66 mM of this organic acid.

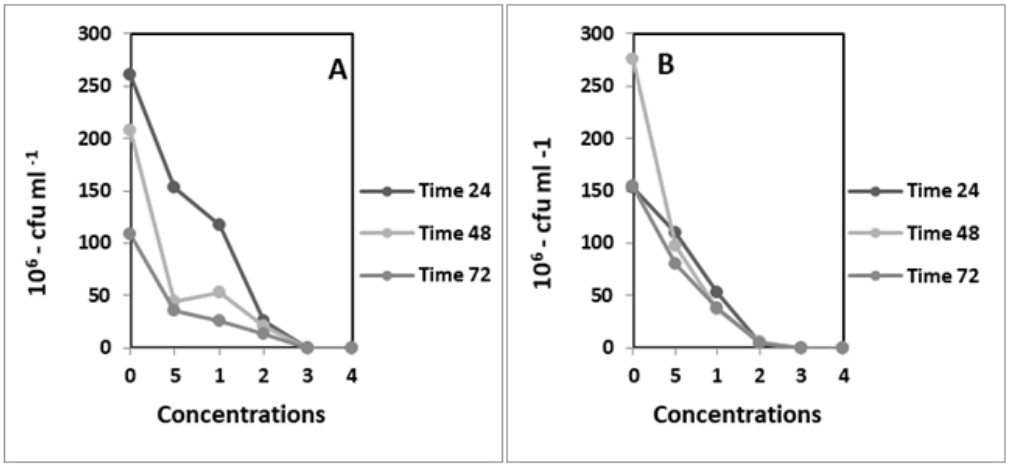

Figure 9: Effect of Interactions between Acid concentrations and Time on *S. saprophyticus* (B_1) (A), *S. vitulinus* (B_2) (B)

4 CONCLUSION

The contamination of dairy products with coagulase-negative staphylococci (mannitol fermenter) were detected in (8%) local products including cheese, Lork and Serezh and in (1%) of a cheese sample from local factories. Coagulase-negative staphylococci was absent in yoghurt samples (local and local factories) and imported samples. Local cheese samples were more contaminated than any other samples. A low rate and lowest range of bacterial growth (contamination) of local traditional handmade fermented dairy products by staphylococci mean high microbiological hygiene quality (hygienic processes) and this is important for consumer health and safety. Detection of CNS enterotoxigenicity using brain heart infusion agar supplemented with phenol red (pH 5.4) showed that all isolates were non-enterotoxin producers. Using RIDASCREEN® SET A, B, C, D, E showed that none of the samples were enterotoxigenic. *Staphylococcus saprophyticus* grew better at 37℃ and 25℃ in different pH especially (5,6 and 7) while pH 4 was more dominant than the other parameters on the growth of *S. saprophyticus* and lower bacterial activity was observed at it. Better growth was indicated in 5% NaCl while 20% and 25% NaCl had lower growth. *Staphylococcus vitulinus* had better activity in pH (6,7) at 37℃ and 25℃ with different NaCl concentrations except for 20% and 25% where lower growth was observed. As *S. saprophyticus* pH 4 had the same effect on *S. vitulinus.* Overall *S. vitulinus* had better bacterial activity in all parameters than *S. saprophyticus. S. saprophyticus* grew better in lactic acid while *S. vitulinus* had greater activity in acetic acid. Lactic acid had more inhibitory effect than acetic acid on both bacteria.

REFERENCE

[1] B. E. Gillespie, S. I. Headrick, S. Boonyayatra, and S. P. Oliver, "Prevalence and persistence of coagulase-negative Staphylococcus species in three dairy research herds," *Vet Microbiol*, vol. 134, no. 1-2, pp. 65-72, Feb 16 2009.

[2] O. C. Sampimon, H. W. Barkema, I. M. Berends, J. Sol, and T. J. Lam, "Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds," *Vet Microbiol*, vol. 134, no. 1-2, pp. 37-44, Feb 16 2009.

[3] C. E. Okoli, E. O. Njoga, S. I. Enem, E. E. Godwin, J. A. Nwanta, and K. F. Chah, "Prevalence, toxigenic potential and antimicrobial susceptibility profile of Staphylococcus isolated from ready-to-eat meats," *Vet World*, vol. 11, no. 9, pp. 1214-1221, Sep 2018.

[4] C. von Eiff, G. Peters, and C. Heilmann, "Pathogenesis of infections due to coagulasenegative staphylococci," *The Lancet Infectious Diseases*, vol. 2, no. 11, pp. 677-685, 2002.

[5] A. Paparella, A. Serio, C. Rossi, G. Mazzarrino, and C. Chaves López, "Food-Borne Transmission of Staphylococci," pp. 71-94, 2018.

[6] E. H. Marth and J. Steele, *Applied Dairy Microbiology*, 2nd Edition ed. 2001.

[7] J. M.Willey, L. M. Sherwood, and C. J.Woolverton, 7th, Ed. *Prescott, Harley, and Klein's Microbiology*. Colin Wheatley/Janice Roerig-Blong, 2008.
[8] J. F. Veras *et al.*, "A study of the enterotoxigenicity of coagulase-negative and coagulase-positive staphylococcal isolates from food poisoning outbreaks in Minas Gerais, Brazil," *Int J Infect Dis,* vol. 12, no. 4, pp. 410-5, Jul 2008.
[9] L. S. Do Carmo *et al.*, "A case study of a massive staphylococcal food poisoning incident," (in eng), *Foodborne Pathog Dis,* vol. 1, no. 4, pp. 241-6, Winter 2004.
[10] Cunha, e. Peresi, R. A. O. Calsolari, and J. P. A. Júnior, "DETECTION OF ENTEROTOXINS GENES IN COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM FOODS," *Brazilian Journal of Microbiology,* vol. 37, pp. 70-74, 2006.
[11] M. S. Bergdoll, "Importance of staphylococci that produce nanogram quantities of enterotoxin," *Zentralblatt für Bakteriologie,* vol. 282, no. 1, pp. 1-6, 1995/01/01/ 1995.
[12] M. Hugas and J. M. Monfort, "Bacterial starter cultures for meat fermentation," *Food Chemistry,* vol. 59, no. 4, pp. 547-554, 1997/08/01/ 1997.
[13] F. Irlinger, "Safety assessment of dairy microorganisms: coagulase-negative staphylococci," *Int J Food Microbiol,* vol. 126, no. 3, pp. 302-10, Sep 1 2008.
[14] E. T. Ryser, S. Maisnier-Patin, J. J. Gratadoux, and J. Richard, "Isolation and identification of cheese-smear bacteria inhibitory to Listeria spp," *International Journal of Food Microbiology,* vol. 21, no. 3, pp. 237-246, 1994/02/01/ 1994.
[15] W. Bockelmann, "Development of defined surface starter cultures for the ripening of smear cheeses," (in eng), *International dairy journal,* vol. 12, no. 2/3, pp. 123-131, 2002 2002.
[16] D. Ercolini, P. J. Hill, and C. E. Dodd, "Bacterial community structure and location in Stilton cheese," *Appl Environ Microbiol,* vol. 69, no. 6, pp. 3540-8, Jun 2003.
[17] D. Roberts and M. Greenwood, *Practical Food Microbiology*, Third ed. Blackwell, 2003.
[18] J. G. Collee, A. G. Fraser, B. P. Marison, and A. Simmons, *Practical medical microbiology*, 14th ed. New york: Churchill Livingston Inc, 1996.
[19] M. Ahmadi, S. M. R. Rohani, and N. Ayremlou, "Detection of Staphylococcus aureus in milk by PCR," *Comparative Clinical Pathology,* vol. 19, no. 1, pp. 91-94, 2009.
[20] B. T. Cenci-Goga, A. Karama, P. V. Rossitto, R. A. Morgante, and J. S. Cullor, "Enterotoxin Production by Staphylococcus aureus Isolated from Mastitic Cows," *Journal of Food Protection,* vol. 66, no. 9, pp. 1693-1696, 2003.
[21] A. Jamshidi, H. R. Kazerani, H. A. Seifi, and E. Moghaddas, "Growth limits of Staphylococcus aureus as a function of temperature, acetic acid, NaCl concentration, and inoculum level," *Iranian Journal of Veterinary Research,* vol. 9, no. 4, 2008.
[22] G. Q. Hussein, "Ecological and physiological study on Bacillus cereus isolate from local foods.," M. Sc., Colleage of Science, University of Baghdad, 1999.
[23] F. S. A. AL-Mayahy and H. H. AL-Galebi, "Bacterial contamination assessment of local and Imported diary productions in Al-Diwaniya markets," *Journal of Kerbala University,* vol. 7, no. 2, 2009.
[24] W. Bockelmann, U. Krusch, G. Engel, N. Klijn, G. Smit, and K. J. Heller, "The microflora of Tilsit cheese," vol. 4, 1997.
[25] S. Kunová, M. Kačániová, J. Čuboň, P. Haščík, and Ľ. Lopašovský, "Evaluation of microbiological quality of selected cheeses during storage," *Potravinarstvo,* vol. 9, no. 1, 2015.
[26] N. Benkerroum, H. Oubel, and W. E. Sandine, "Effect of nisin on yogurt starter, and on growth and survival of Listeria monocytogenes during fermentation and storage of yogurt," *Internet Journal of Food Safety V,* vol. 1, pp. 1-5, 11/30 2002.
[27] A. Ruaro, C. Andrighetto, S. Torriani, and A. Lombardi, "Biodiversity and characterization of indigenous coagulase-negative staphylococci isolated from raw milk and cheese of North Italy," *Food Microbiol,* vol. 34, no. 1, pp. 106-11, May 2013.
[28] V. Perreten, N. Giampà, U. Schuler-Schmid, and M. Teuber, "Antibiotic Resistance Genes in Coagulase-negative Staphylococci Isolated from Food," *Systematic and Applied Microbiology,* vol. 21, no. 1, pp. 113-120, 1998.
[29] M. A. El-Zamkan, A. G. Mubarak, and A. O. Ali, "Prevalence and phylogenetic relationship among methicillin- and vancomycin-resistant Staphylococci isolated from hospital's dairy food, food handlers, and patients," *J Adv Vet Anim Res,* vol. 6, no. 4, pp. 463-473, Dec 2019.
[30] S. Argaw, M. Addis, and H. Degefu, "Identification and Antimicrobial Resistance Pattern of Staphylococci Isolated From Cottage Cheese (Ayib) and Yoghurt (Ergo) in Selected Districts of Jimma Zone, Ethiopia," *Health Science Journal,* vol. 12, no. 1, 2018.
[31] S. P. Onen and O. Aygun, "Enterotoxin Producing Ability And Antimicrobial Susceptibility Of Coagulase Negative Staphylococci Isolated From Goat Milk, Cheese And Salted Yoghurt In Turkey," *INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY RESEARCH,* vol. 6, no. 10, 2017.
[32] C. Kurekci, "Short communication: Prevalence, antimicrobial resistance, and resistant traits of coagulase-negative staphylococci isolated from cheese samples in Turkey," *J Dairy Sci,* vol. 99, no. 4, pp. 2675-2679, Apr 2016.
[33] R. S. Casaes Nunes, C. Pires de Souza, K. S. Pereira, E. M. Del Aguila, and V. M. Flosi Paschoalin, "Identification and molecular phylogeny of coagulase-negative staphylococci isolates from Minas Frescal

cheese in southeastern Brazil: Superantigenic toxin production and antibiotic resistance," *J Dairy Sci*, vol. 99, no. 4, pp. 2641-2653, Apr 2016.
[34] J. P. Rosec, J. P. Guiraud, C. Dalet, and N. Richard, "Enterotoxin production by staphylococci isolated from foods in France," *International Journal of Food Microbiology*, vol. 35, 1997.
[35] S. Even *et al.*, "Low occurrence of safety hazards in coagulase negative staphylococci isolated from fermented foodstuffs," (in eng), *International journal of food microbiology*, vol. 139, no. 1-2, pp. 87-95, 2010/04// 2010.
[36] Blaiotta *et al.*, "PCR detection of staphylococcal enterotoxin genes in Staphylococcus spp. strains isolated from meat and dairy products. Evidence for new variants of seG and seI in S. aureus AB-8802," *J Appl Microbiol*, vol. 97, no. 4, pp. 719-30, 2004.
[37] B. S. V. Souza *et al.*, "The influence of pH on Staphylococcus saprophyticus iron metabolism and the production of siderophores," *Microbes Infect*, vol. 21, no. 10, pp. 456-463, Dec 2019.
[38] D. A. Stavropoulou, P. Filippou, S. De Smet, L. De Vuyst, and F. Leroy, "Effect of temperature and pH on the community dynamics of coagulase-negative staphylococci during spontaneous meat fermentation in a model system," *Food Microbiol*, vol. 76, pp. 180-188, Dec 2018.
[39] L. A. Onyango, R. H. Dunstan, J. Gottfries, C. von Eiff, and T. K. Roberts, "Effect of Low Temperature on Growth and Ultra-Structure of Staphylococcus spp," *PLoS ONE*, vol. 7, no. 1, p. e29031, 2012.
[40] A. K. Søndergaard and L. H. Stahnke, "Growth and aroma production by Staphylococcus xylosus, S. carnosus and S. equorum; a comparative study in model systems," *International Journal of Food Microbiology*, vol. 75, pp. 99-109, 2002.
[41] G. Mauriello, A. Casaburi, G. Blaiotta, and F. Villani, "Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy," *Meat Sci*, vol. 67, no. 1, pp. 149-58, May 2004.
[42] E. Mani-López, H. S. García, and A. López-Malo, "Organic acids as antimicrobials to control Salmonella in meat and poultry products," (in eng), *Food Research International (Ottawa, Ont.)*, vol. 45, no. 2, pp. 713-721, 2012/03// 2012.
[43] D. O. Cliver, "Microbial Decontamination, Food Safety and antimicrobial Interventions," 2007.
[44] B. Ray, *Fundamental food microbiology*, 3rd ed. CRC press, 2004.
[45] P. J. Bremer, G. C. Fletcher, and C. Osborne, "Staphylococcus aureus," *New Zealand Instit*, 2004.
[46] U. Tsegmed, "Staphylococci Isolated from Raw Milk of Yak and Cattle in Mongolia Studies on the occurrence, characterization, detection of enterotoxin and antimicrobial susceptibility profile of the isolates.," M. Sc., Agricultural Sciences, Swedish University Uppsala, 2005.
[47] M. Ewadh, H. Hasan, I. Bnyan, F. Mousa, J. Sultan, and M. Ewadh, "Antibacterial Activity of 2- (2-Hydroxy phenylimino) Acetic Acid," *Advances in Life Science and Technology*, vol. 7, 2013.