Plant Archives Vol. 20, Supplement 2, 2020 pp. 1474-1477 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

INHIBITORY EFFECTS OF EMULSIFYING SALTS STPP AND NACL ON *KLEBSIELLA PNEUMONIAE* CONTAMINATION SOFT CHEESE COW'S SAMPLES COLLECTED FROM VENDED IN BABYLON GOVERNORATE, IRAQ

Amer Jebur Obayes Al-Isawi *[1],Mohamed Ibrahem Rhma[2] and Manal Hadi Ghaffoori Kanaan[3]

[1,2] Department of Public Health, College of Veterinary Medicine, AL-Qasim Green University, Babylon, Iraq.

[3] Department of agriculture, technical institute of Suwaria, Middle Technical University, Iraq

*Corresponding author: dr.amer_42_vet@vet.uoqasim.edu

Abstract

Milk and milk products are highly vulnerable to numerous food borne chains pathogens such as *Klebsiella pneumoniae*, which make these products as possible vehicle to transfers these bacteria, so this study was carried out to detect the level of contamination of cattle product as a measurement for the exposure of consumers in one of the most common food chains in Babylon province, during the period from March to June 2019. *Klebsiella pneumoniae* .were isolated from locally cattle milk and soft cheese. A total of 81(18.7%) out of 15 of locally cattle milk and soft cheese gave positive results for *Klebsiella pneumoniae* Results of susceptibility test revealed high prevalence of resistance in MRSA isolates to four or more antimicrobial agents with which eight antibiotic resistance profiles (ARPs) were presented by these isolates. Our study has shown that locally milk and soft cheese in several markets in the province of Babylon has been highly tainted by *Klebsiella pneumoniae* throughout the period of the study and when take into consideration that the existence of multiple drug-resistant *Klebsiella pneumoniae* in milk product may constitute a serious danger for the health of the consumers, consequently these outcomes are very important from the public health viewpoint.

Keywords: Milk product, Multiple drug–resistant, *Klebsiella pneumoniae*, Babylon province.

Introduction

Food plays a vital role in our natural life for daily; billions of people are consumed milk and milk products. Milk and milk products contain good nutritional qualities and represent as a brilliant medium for growth of various type of microorganism, contamination of milk with microorganism appear as global problem (FDA, 2016 and CDCs, 2016). Problems by food borne infections have been known as a public health hazard and restricted the industry in developed and developing countries, hence, microbial food safety has begun as an important worldwide problem for customer, manufacturing, investigator and regulatory bodies. Previous two decades, diseases associated with dairy products embroiled with *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Listeria monocytogenes* and *Salmonella entertica*, as well as twenty million cases per annum result from food borne disease in the world. Spoilage of milk products leading to huge socio-economics issue worldwide, the microbial burden in foods are pointers of food quality and diverse numbers of the microbiological risks related with dairy products like butter, cheese and yoghurt. Generally, physicochemical and oppositionist feature of lactic acid bacteria plays a significant role for preserved of cheese and become safe nutritious food, but food borne disease associated with milk and soft cheese consumption and chiefly soft cheese prepared from raw milk mixed with whey have happened in numerous countries (Choi *et al*., 2016).

Materials and Methods

Handling of samples

A total of 81 locally produced bovine milk and tests of soft cheese (250 g each) were randomly collected at intervals week after week (5 tests /week) in 500ml sterile polyethylene from different areas of Babylon territories. All tests (250 g each) were transported by ice-cooled box to laboratory in college of veterinary department of public health. All tests have been done to separate and distinguish the *Klebsiella pneumoniae* From the samples.

Bacterial isolation and identification

Eleven grams portion from the surface and the center of each soft cheese samples were extracted aseptically and added to (99 ml) (40 ºC) a aqueous sodium citrate of warm (2% wt/v) and homogenized for 5 minutes in a stomacher and then the homogenates were used for isolation and identification proof of *Klebsiella pneumoniae*

Growth of culture isolates at various concentrations of NaCL

Klebsiella pneumoniae was evaluated for its ability to grow in inoculated nutrient broth supplemented with5%, 10% and 15% of sodium chloride and aerobically incubated at 37 0c for 24 h. One milliliter samples were taken every 0,3,6, hours and serially ten-fold diluted in 0.1% sterile buffered peptone water and plated in duplicates by pour plating method using the VRB media, then aerobically incubated at 37ºC for 24 h before enumeration for three replications (Amer, 2017).

Exposure to emulsifying salt

Klebsiella pneumoniae was evaluated for its ability to grow in nutrient broth supplemented with 3% of emulsifying salt (Sodium Tri Poly Phosphate or STPP. One milliliter samples were taken after (24 hours) and serially ten-fold diluted in 0.1% sterile buffered peptone water and plated in duplicates by pour plating method using the VRB agar and then incubated aerobically at 37 ºC for 24hrs before enumeration with three replications (Tenhat *et al*., 1981).

Emulsifying salts tolerance response experiment

Two liters of raw milk →milk filtration by cloth →milk pasteurization (63 ºC for 30 minutes) → milk cooling at 40 ºC for 1hrs. → *Klebsiella pneumoniae* inoculation with → Addition of commercial rennet Milk clotting by rennet

(Coagulation for 30 minutes → Curd breaking by knife and kept for 1hr at 40 ºC →Draining of whey→ mixing with (Sodium Tri Poly Phosphate or STPP) powder (3%) Cheese processing (Amer, 2017).

Antimicrobial susceptibility test

A Kirby-Bauer technique were used for screened all recovered isolates (15isolates) for antimicrobial susceptibility test on Mueller-Hinton agar according to CLSI criteria (Bauer *et al*., 1966; Clinical and Laboratory Standards Institute 2019). Discs were applied: Ampicillin (AM 25 µg), Imipenem (IPM 10 µg), Ciprofloxacin Cip (5µg), Ceftazidime (CAZ 30 µg), Cefepime (FEP 30 µg), Azteonam(ATM 30µg), Amikacin (Ak30 µg) and Gentamycin CN 10 µg.

Statistics

Data analysis were done using MedCalc Software bvba version 18 (BE, USA). Descriptive statistics such as proportion, mean and standard deviation were used. Two samples Chi-square between percent was used to compare significance between percentages, t-test between means with a 5% significant levels was used to compare (mean ± SD) for the selected antibiotics (https:// www.medcalc.org/).

Results

Tolerance of the salt:

Klebsiella pneumoniae viability during incubated in nutrient broth supplemented with 5%, 10%, 15% of sodium chloride over the three time points of 0, 3 , 6 hrs. is shown in Table (1). *Klebsiella pneumoniae* exhibited significant tolerance to 5% of sodium chloride with a decrease in the count of bacteria (log CFU / ml) of the culture through the 3 h of aerobic incubation at 37 ºC where showed a similar patterns of growth in the absence or presence of 5% sodium chloride but from hours 3. *Klebsiella pneumoniae* counts that subjected to 5% NaCl were lowered significantly than the control, supplementation of nutrient broth with different concentrations of sodium chloride had significantly ($p \leq 0.05$ impacted the inactivation degree of *Klebsiella pneumoniae* under the conditions utilized, inoculated nutrient broth supplemented with 10% NaCl produced a significant ($p \leq 0.05$) decrease of *Klebsiella pneumoniae* counts where the starting initial counts of 13×10^{-3} was reduced to 50×10^{-2} after 3 and 6 hrs. of aerobic incubation at 37 ºC respectively. Increasing the sodium chloride concentration up to15% in nutrient broth resulted a significant increase of the antimicrobial efficacy of the salt against *Klebsiella pneumoniae* where the starting initial count of 7×10^{-3} was reduced up to 10×10^{-2} after 3 and 6 hours of aerobic incubation respectively .

Table 1 : Influence of different concentrations of NaCl on the viable count of the *Klebsiella pneumoniae*

	NaCl concentration			Control	Incubation period (hrs.)
LSD Value	15%	10%	5%		
26.552*	20×10^{-4}	30×10^{-4}	80×10^{-4}	90×10^{-4}	0 hrs.
74.927*	7×10^{-3}	13×10^{-3}	20×10^{-4}	120×10^{-7}	3 hrs.
91.403*	10×10^{-2}	50×10^{-2}	30×10^{-3}	50×10^{-8}	6 hrs.

Emulsifying salts sodium tri poly phosphate (STPP) tolerance in soft cheese.

The viability of *Klebsiella pneumoniae* when incubated in soft cheese with 3% of sodium tripolyphosphates (STPP) over the three time periods of 0,3 and 6 hours are shown in table (2). Supplementation of soft cheese with 3% concentrations of STPP essentially ($p \leq 0.05$) impacted the inactivation degree of 3% of STPP after each incubation time of 3 and 6 hours. The viable count of *Klebsiella pneumoniae* were significantly ($p \leq 0.05$) reduced than the control. Inoculated in soft cheese supplemented with 3% of STPP was produced a significant ($p \leq 0.05$) lower of *Klebsiella pneumoniae* counts where the beginning introductory count of log 6.9 log cfu /ml was decreased to log 5.47 and 3.39 log cfu/ ml after 0,3 and 6 hours of incubation separately .Increasing the STPP concentration up to 3% in soft cheese resulted in a further essentially ($p \leq 0.05$) increase of the antimicrobial effectiveness of STPP against *Klebsiella pneumoniae*. (Amer,2017).

Table 2 : Effect of (STPP) 3% on the *Klebsiella pneumoniae* In soft cheese

Count of k.p cfu/ml	Time for processing (hrs)
80×10^{-5} (log 6.9)	0 time
30×10^{-4} (log 5.47)	3 hrs.
25×10^{-2} (log 3.39)	6 hrs.
1.066*	LSD Value

The influence of 3% of STPP on the count of *Klebsiella pneumoniae* in nutrient agar

The viability of *Klebsiella pneumoniae* contaminated in nutrient agar when processed with 3% sodium tripolyphosphates (STPP) over the three time points of 5,10 and 30 minutes is showed in Table (3) . Inoculated in nutrient agar supplemented with 3% STPP had essentially ($P \leq 0.05$) impacted the inactivation degree of *Klebsiella pneumoniae* Processing of nutrient agar with 3% STPP for 5 minutes produced significantly ($P \leq 0.05$) reduced of *Klebsiella pneumoniae* counts at the rate log 4.39 cfu/ ml where the beginning introductory counts of log 5.9 cfu/ ml was decreased to 4.39 cfu / ml. increasing the times up to 10 and 30 minutes resulted in a further significantly ($P \leq 0.05$) reduced of *Klebsiella pneumoniae* counts at the rate of 3.47 cfu/ ml and (0. 0) cfu/ ml respectively.

Table 3 : Effect of (STPP) 3% on *Klebsiella pneumoniae* In nutrient agar

Count of *k.p* cfu/ml	Time for processing (minutes)
80×10^{-4} (log 5.9)	0 time
25×10^{-3} (log 4.39)	5
30×10^{-2} (log 3.47)	10
0×10^{-2} (log 0. 0)	30
1.169*	LSD Value

Antimicrobial susceptibility test

A Kirby-Bauer technique were used for screened all recovered isolates (15isolates) for antimicrobial susceptibility test on Mueller-Hinton agar according to CLSI criteria((Bauer *et al*., 1966; Clinical and Laboratory Standards Institute 2019) The following antimicrobial discs were applied: Ampicillin (AM 25 µg), Imipenem (IPM 10 µg), Ciprofloxacin Cip (5µg), Ceftazidime (CAZ 30 µg), Cefepime (FEP 30 µg), Azteonam (ATM 30µg), Amikacin (Ak30 µg) and Gentamycin CN 10 µg.

Table 4 : Characteristics of Resistance, Intermediate and Susceptible recovery isolates of *Klebsiella pneumoniaeeumonia* (total 15 isolates) identified from milk samples

Antibiotic	Concentration	Resistance%	Intermediate%	Susceptible%
Ampicillin	AM 25 µg	15(100)	None(0)	none(0)
Ceftazidime	CAZ 30 µg	15(100)	None(0)	none(0)
Cefepime	FEP 30 µg	15(100)	None(0)	none(0)
Gentamycin	CN 10 µg	12(80)	2(13.33)	1(6.66)
Azteronam	ATM 30 µg	9(60)	1(6.66)	5(33.3)
Amikacin	Ak30 µg	3(20)	6(40)	6(40)
Ciprofloxacin	Cip 5µg	None(0)	3(20)	12(80)
Imipenem	IPM 10 µg	none(0)	None(0)	15(100)

Discussion

Salt tolerance

Any effect of the water activity is not important to the content of the fat. Concentrations of Sodium chloride up to 8·5% protected *Klebsiella pneumoniae* cells from inactivation by thermal in TSB (Blackburn *et al.,* 1997). Viral, enteric bacteria, and protozoal pathogens inactivation in crops, water, soil, or fertilizer may be influenced by competition, predation, water stress/osmotic potential, UV radiation, temperature, inorganic ammonia, pH, and natural supplement (Jamieson *et al*., 2002; Ferguson *et al*., 2003).

Emulsifying salts sodium tri poly phosphate (STPP) tolerance in soft cheese.

Processing of soft cheese with 3% STPP for 10 minutes as shown in Table (2) produced a significant ($p \leq 0.05$) decreased of *Klebsiella pneumoniae* counts at the rate of (5.47) log cfu/gm where the starting initial count of 6.9 log . This result are similar with Ahmed *et al.* (2012) who reported that some of STPP had cidal or static effect on *coliforms bacteria* where 2.5% of STPP to added to nutrient agar produced a significant ($p<0.01$) reduction of *E .coli O 157:H7* counts from 1.2 x 10^6 to 6.7 x10^4 cfu/gm and from 1.0 x 10^6 to 8.2 x 10^3 cfu/gm. These results indicated that processed cheese with 3% STPP having *E. coli O157:H7* at levels 6.25 log cfu/ gm was contain the levels of bacteria at that are cause sickness by the time it comes to the buyer.

The influence of 3% of STPP on the count of *Klebsiella pneumoniae* in nutrient agar:

The viability of *Klebsiella pneumoniae* was lost after 30 minutes of incubation in nutrient broth supplanted with 3% STPP (i.e. the viable cells count was zero). Several investigators studied the highly significant difference ($P \leq 0.01$) in the total count rates of coliform cfu /g before and after adding (2%, 2.5%, 3%) of emulsifying salts to the nutrient broth, that could be attributed to the difference in the pH of broth to be alkaline by emulsifying salts and became not suitable for the growth of bacteria . This led to a lack of coliform in growth in the nutrient broth. Where the emulsifying salts had a bacteria-cidal effect. (Amer, 2017).

Effect on antibiotic:

In present work, all 15 recovery isolates of *Klebsiella pneumoniae* were high resistance to Ampicillin, Ceftazidime Cefepime, Gentamycin, Azteronam and Amikacin (100%100%100%, 80%, 60% and 20%) respectively and all separates were sensitive against to Imipenem followed by Ciprofloxacin. Currently, distributed of *K. pneumoniae* in environment and contaminated of milk coming from absence of cool system that may foster the generation of pathogenic microorganisms, over and above the antibiotic resistant is reflected conduct of *K. pneumoniae* to overcome and modulation of environmental buffer, which conceder global public health concern (Mohamed and Ali Al-Shammary 2017; Abdel Hameed, 2017).

Conclusion

- High prevalence of *K. pneumoniae* in soft cheese was reflection on unhygienic practice and poor sanitation procedure, which refer to potential public health hazard.
- All separates were sensitive against to Imipenem followed by Ciprofloxacin. Currently, distributed of *K. pneumoniae* in environment and contaminated of milk coming from absence of cool system that may foster the generation of pathogenic microorganisms, over and above the antibiotic resistant is reflected conduct of *K. pneumoniae* to overcome and modulation of environmental buffer, which conceder global public health concern.
- Its lack of coliform in growth in the nutrient broth. Where the emulsifying salts had a bacteria-cidal effect.

References

Ahmed, M.S. and Shukr, M.Y. (2012). Detection of *E. coli* O157:H7 in the locally produced chesses in Baghdad & its Effect in public Health, 5-1:(1)37.

Amer (2017). The Prevalence of *E. coli O157:H7* in Locally Produced Bovine Soft Cheese Samples with Special Emphasis on its Potential Public Health Hazards Baghdad university.

Abdel Hameed, K.G. (2017). Widespread acquisition of antimicrobial resistance of *Klebsiella pneumoniae* isolated from raw milk and the effect of cinnamon oil on such isolates. International Food Research Journal, 24(2): 876-880.

Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Amer. I. Clin. Pathol., 45: 493-6.

Blackburn, Cde. W.; Curtis, L.M.; Humpheson, L.; Billon, C. and McClure, P.J. (1997).

Choi, K-H.; Lee, H.; Soomin lee, S.; Kim, S. and Yoon, Y. (2016). Cheese microbial risk assessments a review Asian-australas J. Anim Sci. Mar. 29(3): 307–314.

Clinical and Laboratory Standards Institute (CLSI). (2019). Performance Standards for Antimicrobial Susceptibility Testing.29th supplement M100S. Wayne, PA.

Food and Drug Administration (FDA). (2016). *K. pneumoniae* Food borne Pathogenic Microorganisms

and Natural Toxins Handbook. Centre for Food Safety and Nutrition.

Jamieson, R.C.; Gordon, R.J.; Sharples, K.E.; Stratton, G.W. and Madani, A. (2002). Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. Canadian Biosystems Engineering.

Mohamed Ibrahem Rahma and Ali Hassan Ahmed Al-Shammary (2017). Occurrence of extended spectrum beta lactamase resistant pneumoniae (esbl) in raw white soft cheese and its whey from Baghdad. International Journal of Current Research, 9(09): 57631-57637.

Tenhet, V.G.; Finne, R.N. and Toloday, D. (1981). Penetration of Sodium Tripolyphosphate into Fresh and Pre- frozen Peeled and Deveined Shrimp, Journal of Food Science, 46(2): 344-349.