Assiut Vet. Med. J.Vol. 50 No. 103 October 2004

Animal Health Research Institute, Assiut, Egypt

OCCURRENCE OF KLEBSIELLA IN SOME MEAT PRODUCTS AND THE EFFECT OF COLD STORAGE AND SOME MEAT ADDITIVES ON ITS GROWTH RATE IN ASSIUT.

(With 3 Tables and 4 Figures)

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(Received at 26/9/2004)

مدى تواجد الكليبسيلا فى بعض منتجات اللحوم وتاثير التخزين بالتبريد وبعض اضافات اللحوم على معدل نموها فى أسيوط

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تم تجميع مائة عينة عشوائية من منتجات اللحوم البيفبرجر واللانشون والسجق والبسطرمة (٢٥من كل نوع) من محلات السوبر ماركت المختلفة فى أسيوط بهدف التعرف على العدد الكلى وعزل وتصنيف ميكروب الكليبسيلا. أظهرت النتائج أن كل العزلات صنفت *Klebsiella pneumoniae*. تم عزل الميكروب بنسبة ٥٢، ٢٨و٨% من عينات البيفبرجر واللانشون والسجق بمتوسطات ٣ ,٢ × ١٠^{٤} ±١,٦ ، ٥,٩١ × ١٠^{٣} ±٤,١١ و ١,٤×١٠^{٢} ± ٠٧، خلية/جم بالترتيب. ثبت من التحليل الاحصائى وجود فروق معنوية بين متوسطات البيفبرجر والسجق وكذلك بين اللانشون والسجق بينما لا توجد فروق معنوية بين البيفبرجر واللانشون ولم يتم عزل الميكروب من عينات البسطرمة المفحوصة. تم دراسة تأثير التخزين بالبرودة عند درجتى حرارة مختلفة وهى ٤ درجة مئوية لمدة ٤ أيام و-٤ درجة لمدة ٢١ يوم على نمو الميكروب فى اللحمة المفرومة. حيث أظهرت النتائج أن تأثر الميكروب بدرجة -٤ أكبر من تاثره بدرجة + ٤ مما أدى الى تأخر نموه. وكذلك تمت دراسة تأثير بعض اضافات اللحوم مثل كلوريد الصوديوم بنسبة ١، ٢ و ٣% وكذلك نيتريت. الصوديوم بنسبة ١٠٠ و٢٠٠ جزء فى المليون فى ثلاثة درجات حرارة مختلفة وهى ٤ ، ٣٧ و٤٢ درجة مئوية على التوالى. أظهرت النتائج تأخر نمو الميكروب بزيادة درجة الحرارة وزيادة تركيز المادة المستخدمة كما ظهرت فروق معنوية فى التعداد اللوغاريتمى بين المجموعة الضابطة (control) والعينات المعاملة محل الدراسة. وقد تم مناقشة الأهمية الصحية للميكروب المعزول والاحتياطات الواجب اتخاذها لتقليل تواجدها والتخلص منه لحماية المستهلك.

SUMMARY

One hundred random samples of meat products; beef-burger, luncheon, sausage, and basterma (25 each) were collected from different

supermarkets in Assiut and subjected to enumeration, isolation and identification of Klebsiella organisms. The results showed that all the strains were identified as *Klebsiella pneumoniae*. Klebsiella organisms could be detected with percentage of 52, 28, and 8 from beef –burger, lunchon and sausage with mean values of $3.2x10^4$ ±1.6, $5.9x10^3$ ±4.11 and $1.4x10^2$ ± 0.07 cfu/g respectively .No significant difference could be detected among beef –burger and lunchon, while a signficant difference appeared between beef burger and sausage and also between sausage and lunchon at $P<0.05$. Klebsiella could not be detected in basterma. The effect of cold storage (chilling and freezing) on the growth rate of Klebsiella strains was determined at temperatures of $4C^0$ and $-4C^0$ in minced meat. The results indicated that freezing showed reduction in the growth rate and inactivation more than chilling. The effect of different cocentrations of sodium chloride (1,2 and 3%) and sodium nitrite (100, and 200 ppm) were tested against the isolated organism in minced meat at three different temperatures 4^0C, 37^0C and 42^0C respectively. Highly significant differences were recorded between the control and the treated samples by increase temperature and concentration of sodium chloride and sodium nitrite. Public health significance and suggested measures for improving the quality of meat products to protect the consumer were given.

Key words: *Klebsiella, cold storage, meat additives.*

INTRODUCTION

Meat and meat products are liable to be contaminated during processing and handling by various microorganisms thus causing spoilage and economic losses.

The genus Klebsiella was named after Edwin klebs, a late 19th. century German microbiologest .All Klebsiella species are non motile, many strains hydrolyze urea producing a light pink color in the slant urea agar, *Klebsiella pneumoniae* is indole negative and *Klebsiella oxytoca* is indole positive (Koneman *et al*, 1992).

Klebsiella species are widespread through the environment and also carried by human. Both genera are well- recognized community and nasopathogens and cause significant infections. They are a common cause of respiratory and non respiratory infections. It is responsible for 1% to 5% of all case community –acquired pneumonia, that generally occur in patients with debilitating conditions such as alcoholism,

diabetes mellitus and chronic obstructive pulmonary disease with extensive necrosis and hemorrhage. Lung abscess occur after pneumonic process or secondarily to Klebsiella species infection and have high rates morbidity and mortality (Bouza and Cercenado, 2002). *Klebsiella pneumoniae* is an opportunistic pathogen responsible for nosocomial infection and trend toward multiple antibiotic resistance (De champs *et. al*, 2004). It is also responsible for acute renal failure to acute pyelonephritis with extensive necrosis of the kidney (Creyghton *et al* 2001).

Sixty adult klebsiella meningitis patients have been identified, most cases were associated with debilitating disease and devasting metastitic septic abscesses (Lu CH *et al*, 2002). Also a histamine producing strain of *Klebsiella pneumoniae* was isolated and could be detected by (Taylor *et al* 1979).

The most popular meat products are hamburger, lunchon, sausage and basterma; For the first time Sabota *et al*, (1998) reported *Klebsiella pneumoniae* as enteroinvasive food-borne pathogen transmitted from hamburger and symptoms of gastroenteritis rapidly deteriorated to multiorgans failure. The public health significant of Klebsiella organisms in food materials particularly meat products and their role as source of Klebsiella infection have been studied by many investigators (Hechelmann *et al*, 1974 and Okolo 1985).

this study was under taken to :

1-Investigate the occurrence of Klebsiella organisms in some meat products (beef-burger, lunchon ,sausage and basterna) in Assiut.

2-Count and identification (biotyping) of the isolated of Klebsiella.

3-Study the effect of cold storage chilling ($+4C^0$) and freezing ($-4C^0$) on the growth rate of the organism in minced meat.

4-Study the effect of different concentrations of sodium chloride and sodium nitrite on klebsiella growth rate at three different temperatures, 4, 37, 42 C^0.

MATERIALS and METHODS

Collection of samples :

One hundred random samples of meat products; 25 each of beef-burger, lunchon, sausage and basterma were collected from different supermarkets in Assiut and transferred to the laboratory. All the samples were examined for enumeration, isolation and identification of Klebsiella organisms.

Preparation of samples:

The collected samples were prepared according to the technique recommended by ICMSF (1978) for enumeration of Klebsiella organisms. The surface plate technique described by Bagley and Seidler, (1978) was applied using double MacConkey- inositol- carbenicillin agar medium., The number of large mucoid and red colony with red pigment usually diffusing into the surrounding agar indicating fermentation of lactose and acid production was then calculated and recorded. The isolated Klebsiella organisms were identified according to morphological characters (Cruickshank *et. al.* 1975); biochemical characters (Edward's and Ewing, 1972) and biotyping according to Niazi *et. al.*, (1977).

Test organisms:

One strain of *Klebsiella pneumoniae* previously isolated and identified from meat products was grown in 10 ml of nutrient broth at $37C^0$/24 h. The culture was decimally diluted and plated to enumerate the organisms present. The culture was diluted to achieve an inoculum level of 10^6/g for *Klebsiella pneumoniae*. 1kg of semitendinosus muscle of cattle carcase was passed twice through meat mincer then divided and inoculated with the organisms suspension and throughly mixed to have $3x10^5$ organisms /g.

Experimental part:

1- The effect of chilling and freezing on the growth rate of Klebsiella organism in minced meat for 4day for chilling and 21day for freezing.

2-Effects of different concentrations of sodium chloride (1,2,and 3%) and sodium nitrite (100, and 200 ppm) during storage at different temperatures on the growth rate of Klebsiella organism.

1- The effect of cold storage on the growth rate of Klebsiella organisms:

a- The effect of chilling:

50 gm of the inoculated sample were stored at chilling temperature (4^0C) and examined daily for 4 days for detecting the number of Klebsiella per gram which was done by surface plate count technique.

b- The effect of freezing:

100 gm of minced meat which were inoculated with $3x10^6$/gm were stored at $-4C^0$ and examined for the aimed organisms every two days till 21 days

2- The effect of some additives and storage temperature on growth rate of klebsiella organisms:

a- sodium chloride:

600 gm minced meat previously inoculated with Klebsiella $3x10^6$ were divided into12 parts, 50gm each as the following: 3parts as control, 3parts with 1% added NaCl, 3parts with 2% added and 3parts with 3% added. Then one part of the control and one part of each concentration were stored at 4^0C, 37^0C and 42^0C. The samples were examined daily for 4 days at temperature 4^0C and for 2 days at temperature 37^0C and for one day at temperature 42^0C. The total colony count was recorded in each plate.

b- Sodium nitrite:

The used sodium nitrite concentrations were 100and 200 ppm. Three hundred grams of minced meat samples were divided into 2parts; part was inoculated with 100 ppm and divided into 3 parts (50gm each) then stored at 4^0C, 37^0Cand 42^0C. The other part was inoculated with 200 ppm and divided into 3parts according to the storage temperature 4^0C, 37^0C and 42^0C. Then the samples were examined as mentioned above.

Statistical analysis:

The data were statistically analyzed by Mann-whitney Ronk sum test (non-parametric test) according to Michigan State University (1988).

RESULTS

The results were explained in 3 tables and 4 figures.

DISCUSSION

It is evident from achieved results in table (1) and according to the biochemical identification that all strains were identified and biotyped as *Klebsiella pneumoniae*. The incidence percentages of *Klebsiella pneumoniae* recovered from different meat products were 52, 28, and 8 from beef-burger, lunchon and sausage respectively. The count ranged from $7x10^2$ to$2x10^5$,$4x10^2$ to$3x10^4$ and 7x10 to $2x10^2$ in beef-burger, lunchon and sausage respectively. The mean values were $3.2x10^4 \pm 1.6x10^4$, $5.9x10^3 \pm 4.11$ and $1.4x10^2 \pm .07$ cfu / g for the above mentioned products respectively. Klebsiella organisms could not be detected in basterma samples. The results show significant difference between the mean Klebsiella count value in case of beef-burger and

sausage and also between lunchon and sausage but no significant difference was found between mean values of beef- burger and lunchon. Sausage is liable to contamination from different sources during preparation, processing, handling storage(Chesnut *et al*, 1977). Our results for sausage is nearly similar to the results obtained by Morshdy (1991) and lower than Sedik *et al* (1988), Eldaly *et al* (1989), Khalafalla and El-Sherif (1993), and Tolba (1994), our results is higher than (Ahmed *et al* 1988 and Soriano *et al* 2000). Our results in hamburger are lower than Sedik *et al* (1988) and higher than El Mossalami *et al* (1982), Ahmed *et al* (1988) and Tolba (1994). In lunchon our findings are lower than Sedik *et al* (1988) and Refaie and Nashed (1989) and higher than Tolba (1994). Klebsiella microorganisms could not be detected in basterma and this agrees with Sedik *et al* (1988) and Tolba (1994) but Refaie and Nashed (1989) could detect klebsiella in basterma in a percentage of 11%. Basterma samples were contaminated to lesser extent than the other three products and this may attributed to the addition of nitrite which has a bacteriostatic effect (Libby, 1975) and the curing processing of the product which play a great effect in inhibiting the multiplication of the microorganisms (Fehlhaber 1981).

Klebsiella pneumoniae was reported as enteroinvasive food-borne pathogen transmitted from hamburger and has been documented to harbor heat- labile and heat stable enterotoxins (Sabota *et al,1998*). The presence of *Klebsiella pneumoniae* has a short survival time in meat product and could indicate recent contamination which turn indicated unsanitary and unhygienic handling and suspected contamination of food with feces as modes of transmission causing sever vomiting (Newton *et al*, 1977). Kleeberger *et al* (1980) reported that the higher Enterobacteriace count might be attributed to contamination of flesh used for manufacture of such products. Moreover, mincing machines, grinder, equipment and knives are considered sources of infection and contamination of meat during processing (Frazier 1967 Chesnut *et al* 1977, and Eldaly, 1983).

It is evident from the results achieved in table (2) when minced meat were inoculated with *K.pneumoniae* ($3x10^6$ /g) and stored at $4C^0$ and $-4\ C^0$ that chilling had no effect on growth till 4 days where no significant multiplication was noticed. Similar findings were reported by Hechelmann *et al*, (1974) and ICMSF (1978). While freezing showed a remarkable and gradual decrease in count during storage. Our results for freezing agree with Calcott *et al* (1976) who mentioned that cooling and warming rates affect bacterial survival like Klebsiella. Takahashi *et al*,

(1982) showed that these organisms were sensitive against freezing and thawing. Karim and yu (1980) reported a gradual decline in number of the organisms in frozen and frozen thawed meat samples 2-4 weeks after their storage at -17^{0}C. In the same sit ICMSF (1978) reported that freezing may results in death of microorganisms.

Most bacteria were sensitive to salt on freezing and thawing. Psychrotrophic bacteria can develop and multiply under refrigeration temperature and constitute the most common cause of refrigerated food spoilage Frazier and Westhoff (1978).

The effect of meat additives and storage at various temperatures on the growth rate of *K.pneumoniae* experimentally inoculated into minced meat (count in Log_{10}) is reported in table3. Storage temperature at 42C^0 showed market reduction in the count of *K.pneumoniae* in control sample and with the use of different concentrations of 1,2 and 3% sodium chloride the count was reduced from 6.48 to 3.9 Log_{10} /g and the use of sodium nitrite 100 and 200 ppm. reduced the count to Zero when incubated for 1 day.While *K.pneumoniae* at 37C^0 storage temperature the control samples showed gradual rising of the count which reach to 8.1 Log_{10}, and by addition of sodium chloride concentration the count reduced to 5.6 Log_{10}, while addition of sodium nitrite reduced the count to 5.5 Log_{10}. after 2 days. Storage temperature at 4C^0 showed no significant change until 3 days and slight increase in the 4th day and by addition of sodium chloride 1,2 and 3% the count was reduced to5.70 Log_{10} and addition of sodium nitrite 100and200ppm reduced the count to 4.85 Log_{10} after 4days of storage.

These findings showed that Klebsiella organisms are one of psychrotrophic bacteria. It survives in meat products at temperature of refrigeration, the results agree with Hechelmann *et al*, (1974) and Patterson and Gibbs (1977), that the organisms of genus Klebsiella could contribute substantially to spoilage of refrigerated meat. Our results showed that increasing sodium chloride decreased the count of *Klebsiella pneumoniae* and this may be due to the inhibitory effect of sodium chloride on microorganisms, this agree with Muralidhararao and Nandy, (1977). Also Lechowich *et al*, (1978) stated that the salt decreases water content in microorganisms and subsequently reduces the microbial growth. The effect of temperature shows that the organism count was increased to its maximum growth at 37^{0}C and this is similar to that reported by Johnson *et al* (1975) and Tsujia, *et al* (1982).

It is concluded that strict hygienic conditions should be applied and best quality of ingredients is to be used for meat processing. Food

establishment should be hygienically constructed and supplemented with good equipment's easily cleaned and disinfected. Moreover, concerned authorities should be taken an active part in controlling such products by imposing bacteriological standard to ensure a maximum safety to the consumers.

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Table 1: Statistical values of *klebsiella* count/g of the examined meat products.

(n=25 of each)

Samples	No. of postive *Klebsiella pneumoniae*	%	+ve other k. species	%	Klebsiella count/g **			
					Min.	Max.	Mean *	S.E
Beef-burger	13	52	0	0	$7x10^2$	$2x10^5$	$3.2x10^4$ E	$\pm 1.6x10^4$
Lunchon	7	28	0	0	$4x10^2$	$3x10^4$	$5.9x10^3$ E	± 4.11
Sasusage	2	8	0	0	7x10	$2x10^3$	$1.4x10^2$ F	±.07
Basterma	–	–	0	0	–	–	–	–

*Means have the same letter are not significant ($p<0.05$)
**Average of 3 trials.

Table 2: Effect of chilling and freezing on klebsiella in minced meat. (Log_{10} cfu/g.)

Storage period/day	Chilling	Storage period/day	Freezing
0	6.48	0	6.48
1	6.49	2	6.45
2	6.5	4	6.39
3	6.54	6	6.08
4	7.30	8	5.90
5	Spoilage	10	5.89
		12	5.83
		14	4.69
		16	4.60
		18	4.18
		21	4

Table 3: The Effect of sodium chloride and sodium nitrite on *Klebsiella pneumoniae* organisms in minced meat at different temperature.

Temp.	Storage Period/ day	Control	Sodium chloride			Sodium nitrite	
			1%	2%	3%	100 ppm	200ppm
42C°	0	6.48 B	6.48 B	6.48 B	6.48 B	6.48 B	6.48 B
	1	5.68 H	5.64 D	5.64 D	3.95 C	0.0 N	0.0 N
37C°	0	6.48 B	6.48 B	6.48 B	6.48 B	6.48 B	6.48 B
	1	6.69 I	5 J	5.94 A	5.70 H	6.73 I	6.15 K
	2	8.15 L	5.89 A	5.73 H	5.63 D	6.46 M	5.5 O
4C°	0	6.48 B	6.48 B	6.48 B	6.48 B	6.48 B	6.48 B
	1	6.49 B	6.46 B	6.48 P	6.30 F	6.11 Q	5.96 E
	2	6.52 B	6.32 F	6.40 E	6.15 R	6 E	5.64 A
	3	6.54 B	6.28 F	5.91 A	5.78 S	6 E	5.93 A
	4	7.30 T	6.04 E	5.90 A	5.61 D	5.70 H	4.85 U
	5	spoilage					

Log $_{10}$ cfu /g have the same letters are not significant ($p<0.05$).

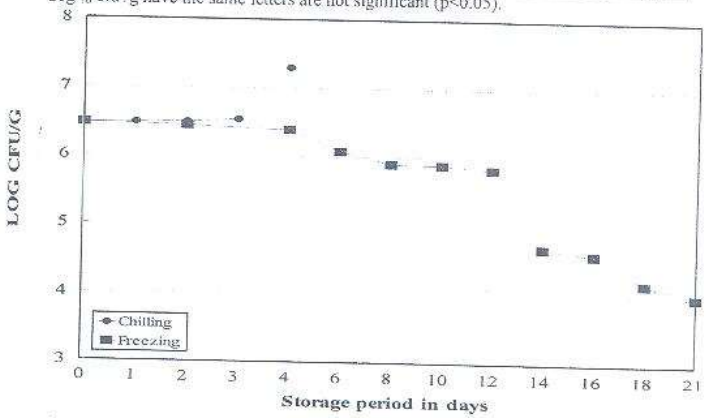

Fig. 1: The effect of chilling and freezing on *Klebsiella Pneumoniae* in minced meat.

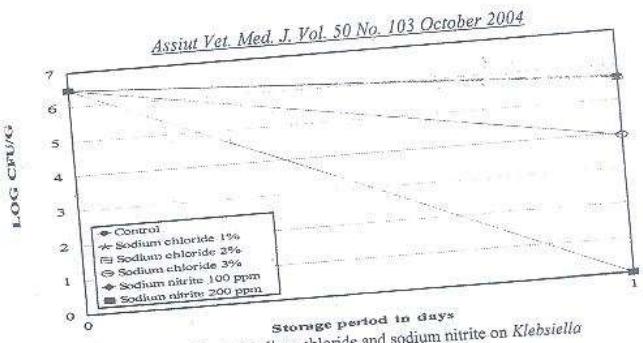

Fig. 2: The effect of sodium chloride and sodium nitrite on *Klebsiella Pneumoniae* in minced meat at 42°C.

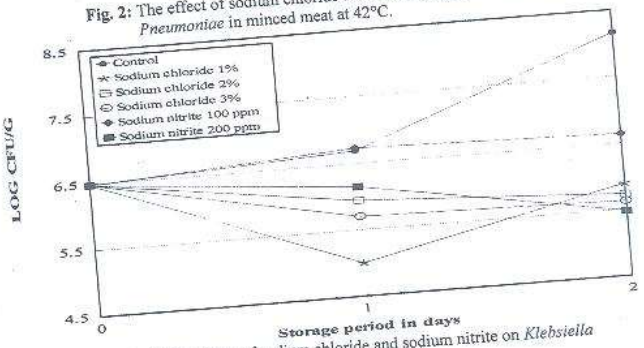

Fig. 3: The effect of sodium chloride and sodium nitrite on *Klebsiella Pneumoniae* in minced meat at 37°C.

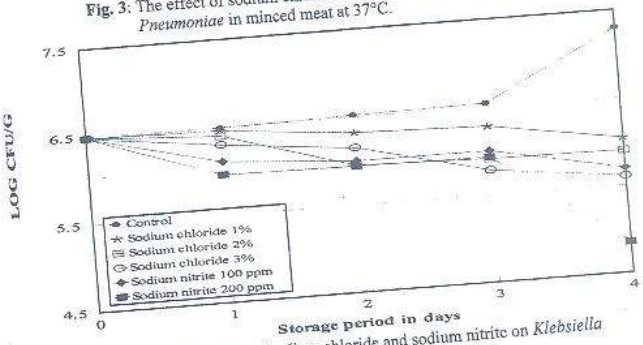

Fig. 4: The effect of sodium chloride and sodium nitrite on *Klebsiella Pneumoniae* in minced meat at 5°C.