

Animal Health Research Institute,
Assiut, Regional Laboratory

OCCURRENCE OF KLEBSIELLA SPECIES IN RAW MILK MARKET IN ASSIUT CITY AND THE EFFECT OF LOW TEMPERATURE ON ITS VIABILITY

(With 8 Tables)

By

EMAN KORASHY; H. GAD EL-RAB*

and SOHEIR ZEIN EL-ABDEIN

* Animal Health Research Institute, Sohag Regional Laboratory

(Received at 22/12/2005)

تواجد ميكروبات الكلبسيلا فى اللبن الخام المباع بمدينة أسيوط وتأثير درجات الحرارة المنخفضة على حيويتها

إيمان قرشى ، حسن جاد الرب ، سهير زين العابدين

جمعت مائة وعشرون عينة عشوائية من اللبن الخام للجاموس والأبقار والماعز والأغنام بواقع (٣٠ عينة من كل نوع) من منازل الفلاحين ومحلات بيع الألبان بمحافظة أسيوط وذلك لمعرفة مدى تلوثها بميكروبات الكلبسيلا. كذلك تم دراسة تأثير درجات الحرارة المنخفضة (التبريد والتجميد) على مدى حيوية وبقاء ميكروب الكلبسيلا نيمونى الذى تم عزله والتعرف عليه وقد تم حقنه بـ 10×17 خلية / جرام فى الزبدة المعقمة. وأيضاً تم القيام بعمل تأثير بعض المضادات الحيوية على ميكروب الكلبسيلا نيمونى المعزولة. وقد أظهرت النتائج أن ميكروب الكلبسيلا نيمونى كان له السيادة على باقى الفصائل المعزولة وذلك بنسبة (١٠ و ١٣,٣٣%) لألبان الجاموس والأبقار على التوالى بينما لم يتم عزله من ألبان الماعز والأغنام وقد كانت النسبة الكلية لميكروبات الكلبسيلا للألبان المختلفة ٢٦,٦٦% و ٢٦,٦٦% لكل من ألبان الجاموس والأبقار أما بالنسبة لفصائل الكلبسيلا المختلفة التى تم عزلها فقد كانت كالتالى: الكلبسيلا أوكسى توكا (٦,٦٦%) فى ألبان الأبقار فقط ، الكلبسيلا أوزونى (٦,٦٦% ، ٣,٣٣% و ٣,٣٣%) لألبان الجاموس والماعز والأغنام على التوالى، الكلبسيلا بلانتيكولا (٦,٦٦% ، ٦,٦٦%) فى ألبان الجاموس والأبقار فقط بينما كانت الكلبسيلا تيراجينا متواجدة فى ألبان الجاموس فقط بنسبة (١,٣٣%). أما بالنسبة لتأثير درجات الحرارة المنخفضة على ميكروب الكلبسيلا نيمونى المعزولة من ألبان الجاموس والأبقار والمحقونة فى الزبد المعقم فقد كانت النتائج كالتالى: عند درجة حرارة التبريد ($10 \pm 0^\circ$) تزايد العدد من 10×17 خلية لكل جرام كبدائية للحقن إلى 10×91 ، 10×206 ، 10×217 ، 10×224 فى اليوم الأول والثانى والثالث والسابع على التوالى بينما تدرج العدد فى التناقص إلى 10×10 ، 10×113 ، 10×220 و 10×37 عند درجة حرارة التجميد (صفر) على التوالى. بينما أختفى الميكروب تماماً بعد الأسبوع الأول عند درجتى التبريد والتجميد

وقد أعزى هذا لإرتفاع نسبة الحموضة حيث تزايدت من ٤,٣% عند البدء إلى ٤,٣ ، ٤,٣ ، ٤,٥ ، ٤,٥ و ٦,٥% في اليوم الأول والثاني والثالث والسابع على التوالي. وقد تم اختبار حساسية عترات الكليسيلا نيموني (٧) المعزولة من ألبان الجاموس (٣ عترة) والأبقار (٤ عترة) لبعض المضادات الحيوية وعددها ثمانية وقد وجد أن العترات حساسة بدرجة عالية للنورفلوكساسين وبدرجة متوسطة للجنتاميسين وبدرجة أقل للسيفوتاكزيم بينما لم يكن لباقى المضادات الحيوية أى تأثير على العترات المعزولة. هذا وقد ناقش البحث الأهمية الاقتصادية للميكروب وتأثيره على الصحة العامة والشروط الواجب اتخاذها لمنع تلوث الألبان ومنتجاتها بهذا الميكروب للحد من خطورته.

SUMMARY

One hundred and twenty random samples of raw buffalo's, cow's, goat's and sheep's milk (30 of each) were collected from different farmer's houses and dairy shops in Assiut City to be examined for the presence of Klebsiella organisms on MacConkey Inositol Carbenicillin agar. The results revealed that *K. pneumoniae* was the most prevalent species among the Klebsiella organisms isolated (10 and 13.33%) for buffalo's and cow's milks. *K. oxytoca* (66.6%) in cow's milk only, *K. ozaenae* (2.66, 3.33 and 3.33%) for buffalo's, goat's and sheep's milk respectively, *K. planticola* (6.66 and 6.66%) in buffalo's and cow's milk respectively and *K. terrigena* revealed 1.33% in buffalo's milk. Klebsiella organisms isolated from raw milk of different animals were (18) 15%, and they were 26.66% for buffalo's and cow's milks. Concerning the second part dealing with the effect of cold temperature ($5\pm 1^\circ\text{C}$) on the viability of *K. pneumoniae* in sterile butter samples revealed that, there is a gradual increase in the number of *K. pneumoniae* from 17×10^8 cells/g as an initial count to 91×10^8 , 206×10^8 , 217×10^8 and 224×10^8 cells/g in the first, second, third and seventh day respectively. While a remarkable decrease in case of freezing temperature (0°C) from 17×10^8 cells/g as an initial count to 10×10^8 , 113×10^7 , 220×10^5 and 37×10^3 in the first, second, third and seventh day respectively. *K. pneumoniae* inoculated in sterile butter completely disappeared and could not be detected after the first week in both chilling and freezing temperatures due to the high acidity percentages which reach from 4.3% at the zero time to 4.3, 4.3, 4.5 and 6.5%. Most of the isolated *K. pneumoniae* strains were highly sensitive to Norfloxacin, moderately to Gentamicin and weakly to cefotaxime but were resistant to other antibiotics used. The public health hazard and suggestive measures were discussed to prevent milk and milk products from contamination with Klebsiella organisms.

Key words: *Klebsiella, pneumoniae, ozaenae, oxytoca, planticola, terrigena, Buffalo's, cow's, goat's and sheep's milk.*

INTRODUCTION

Klebsiella is a Gram negative fecal coliform bacteria in the family Enterobacteriaceae. It could be isolated by Berry's (1933) and was named after Edin Klebs, a late 19 Century (Burnet *et al.*, 1978). It was responsible for several fatal pneumonia (Obiamiwe, 2002) and a variety of clinical syndromes in human due to consumption of milk (Koneman *et al.*, 1992).

In recent years a gradual awareness of the occurrence of *Klebsiella* organisms in both human being and animal species spread throughout the world while, a considerable interest in *Klebsiella pneumoniae* as an opportunistic pathogen responsible for nosocomial infection and trend toward multiple antibiotic resistance (Dechamps *et al.* 2004). From the side point of view *K. pneumoniae* is the most medically important species in the genus *Klebsiella*. As it is also responsible for acute renal failure to acute pyelonephritis with extensive necrosis of the kidney (Creyghton *et al.* 2001), wound infections, meningitis, endocarditis, pleuritis, enteritis and acute or chronic diarrhoeal disease (Anderson and Janoff, 1998). In addition, the organism was incriminated in causing lung infection (Lim *et al.* 1995) and mastitis in animals (Shoshani *et al.* 2000) resulting in economic losses. Thirteen epidemic outbreaks of nosocomial bacteremias attributed to *Klebsiella* organisms in USA during 1983-1991 (Goto *et al.*, 2003). Moreover, serious mortality rates from *Klebsiella* reaches 5% even with antimicrobial therapy and approaches 100% for immunocompromised patients (Al-Rabea *et al.*, 1998).

Five subspecies are related to *K. pneumoniae*: *K. ozaenae* and *K. rhinoscleromatis* (Rennie and Duncan, 1974) which are responsible for nasal cavity infections were identified as ozena and rhinoscleroma (Gamea and Tatawi, 1990 and Zohar *et al.*, 1990). Also, *K. oxytoca* which produces infections similar to those caused by *K. pneumoniae* (Mahon and Manuselis, 1995) and usually occur in newborn populations in the hospital (Monnet and Freney, 1994). *K. planticola*, *K. terrigena* and *K. ornithinolytica* (Izard *et al.*, 1981 and Al-Tarazi, 2001). *Klebsiella* species are more related to 100% of immunosuppressed individuals (Al-Rabea *et al.*, 1998) causing respiratory, intestinal, meningial infections due to heat labile and heat stable enterotoxins responsible for the virulence of *Klebsiella* species (Dhand *et al.*, 2001).

The organisms have been found in the environmental conditions surrounding the dairy animals, soil, dust, water, grass, bedding materials and fecal matter of animals and humans as the organisms are considered as normal flora of 30 to 40% of the intestinal tract of both animals and humans (Buttiaux, 1959). Also, they are found in oropharyngeal and biliary tracts (Quinn *et al.*, 1994).

Milk and milk products are liable to be contaminated by *Klebsiella* species during milking, handling, transportation, processing and storing as the organism can multiply at 5°C in refrigerator (Patterson and Gibbs, 1977).

Klebsiella organisms are implicated in many cases of food poisoning outbreaks (Hörvath *et al.*, 1964). And several investigators could isolate *K.* organisms from raw mastitic milks of different animals (Newman and Kowalski, 1973, Barkema *et al.*, 1998 and Peng *et al.*, 2003).

Because of the involvement of milk and milk products in *Klebsiella* infections this work was planned to study the following items:

- 1- Isolation of *Klebsiella* organisms from raw buffalo's, cow's, goat's and sheep's milk.
- 2- Identification of the isolated *Klebsiella*.
- 3- Studying the effect of cold storage (chilling and freezing) on the viability of isolated *K. pneumoniae* in sterile butter.
- 4- Antimicrobial susceptibility of the isolated *K. pneumoniae*.

MATERIALS and METHODS

Collection of samples:

One hundred and twenty random samples of buffalo's, cow's, goat's and sheep's milk (30 of each) were collected from different farmer's houses and dairy shops in Assiut City in clean sterile containers in an ice box and transferred to the laboratory without delay and kept in refrigerator. The collected samples were subjected to Storch's test (Lampert, 1975) to exclude the heat treated milk samples.

Isolation of *Klebsiella* organisms (Bagley and Seidler, 1978)

One milliliter of each prepared raw milk sample was inoculated into *Klebsiella* enrichment broth (Atlas and Parks, 1994) and incubated at 37°C for 24 hrs. Then loopfuls from enrichment broth were streaked on MacConkey-Inositol-Carbenicillin agar and incubated at 37°C for 24 hrs. The large mucoid and red colony with red pigment was picked up on nutrient slope tubes for further identification. The isolated *Klebsiella*

organisms were identified according to Edward's and Ewing, 1972; Niazi *et al.*, 1977 and Cruickshank *et al.*, 1978;

Experimental part:

A strain of *K. pneumoniae* previously isolated and identified from milk samples was grown in 10 ml of *Klebsiella* enrichment broth and incubated at 37°C for 24 hs. The culture was decimally diluted and plated to enumerate the organism. The dilution continues to achieve an inoculum level of 17×10^8 cells/ml as an initial count.

The effect of the cold storage of butter on the viability of *K. pneumoniae* isolated:

220 gr. of sterile butter were used to be injected with the previously isolated and identified *K. pneumoniae* (17×10^8 cells/g as an initial count). Butter was divided into two portions. One portion was stored at chilling temperature ($5 \pm 1^\circ\text{C}$) while the other was stored at freezing temperature (0°C). Growth rate and viability of *K. pneumoniae* was detected by applying the surface plating technique for the first three days then weekly on MacConkey Inositol Carbenicillin agar for 24 hs at 37°C.

Measurement of pH:

The pH of butter was determined using a pH meter (an Orion Model 701) equipped with standard electrode.

Antimicrobial susceptibility of *K. pneumoniae*:

Antibiotic sensitivity test of *K. pneumoniae* strains isolated from raw buffalo's and cow's milk was carried out according to (Baron *et al.*, 1994) using eight antibiotic sensitivity discs. Ampicillin (AM₁₀), Cefadroxil (FR₃₀), Cafotaxime (CT₃₀), Gentamicin (CN₁₀), Norfloxacin (NoR₁₀), Rifampin (RA₅), Streptomycin (S₁₀) and Tetracyclin (TE₃₀).

RESULTS

The obtained results were tabulated in Tables 1-8.

Table 1: Incidence of *Klebsiella* species in the examined raw milk samples.

Type of milk	No.	Klebsiella species	
		No.	%
Bufaloe's	30	8	26.66
Cow's	30	8	26.66
Goat's	30	1	3.33
Sheep's	30	1	3.33
Total	120	18	15

Table 2: Incidence of Klebsiella species isolated from raw buffalo's milk.

Kelbsiella species	Positive samples	
	No/30	%
K. pneumoniae	3	10
K. oxytoca	-	-
K. planticola	2	6.66
K. ozaenae	2	6.66
K. terrigena	1	3.33
Total	8	26.66

Table 3: Incidence of Klebsiella species isolated from raw cow's milk.

Kelbsiella species	Positive samples	
	No.	%
K. pneumoniae	4	13.33
K. oxytoca	2	6.66
K. planticola	2	6.66
K. ozaenae	-	-
K. terrigena	-	-
Total	8	26.66

Table 4: Incidence of Klebsiella species isolated from raw goat's milk.

Kelbsiella species	Positive samples	
	No/30	%
K. ozaenae	1	3.33
Total	1	3.33

Table 5: Incidence of Klebsiella species isolated from raw sheep's milk.

Kelbsiella species	Positive samples	
	No./30	%
K. ozaenae	1	3.33
Total	1	3.33

Table 6: Effect of cold temperature on K. pneumoniae isolated from raw buffalo's and cow's milk in sterile butter.

Time	Count/g	
	Freezing	Chilling
0	17×10^8	17×10^8
First day	10.0×10^8	91×10^8
Second day	113×10^7	206×10^8
Third day	220×10^5	217×10^8
First week	37×10^3	224×10^8
Second week	0	0

Table 7: pH in butter samples during period of storage.

Time	Acidity %	
	Freezing	Chilling
0	4.3	4.3
First day	4.3	4.3
Second day	4.3	4.3
Third day	4.5	4.5
First week	6.5	6.5
Second week	Not examined	Not examined

Table 8: Antibiotic sensitivity of *Klebsiella pneumoniae* isolated from raw buffalo's and cow's milk.

Antibiotic disc	No. of samples	Reaction
Ampicillin (AM ₁₀)	7	-
Cefadroxill (CFR ₃₀)		-
Cefotaxime (CTX ₃₀)		+
Chloramphenicol (C ₃₀)		-
Gentamycin (CN ₁₀)		++
Norfloxacin (NoR ₁₀)		+++
Rifampin (RA ₅)		-
Streptomycin (S ₁₀)		-
Tetracyclin (TE ₃₀)		-

(-) Resistant

+ Weak sensitive

++ Moderately sensitive

+++ Highly sensitive

DISCUSSION

Data obtained and recorded in Table 1 showed that the incidence of *Klebsiella* species from the examined raw milk samples of different animals was 15%. These results were completely in agreement with Vijayalakshmi *et al.*, 2001 (15%), near to Rahman *et al.*, 1992 (12.2%), higher than Kumari and Gupta, 2002 (2.46%) and Al-Ashmay, 2004 (2%). On the other hand, lower than Newman and Kowalski, 1973 (54%), while, these results were in disagreement with Grhon *et al.*, (2004) who failed to detect *Klebsiella* species from milk samples examined.

It is worth to mention that high incidence of *Klebsiella* species may be attributed to the free living of *Klebsiellae* in the external environmental conditions surrounding the dairy animals (soil, sewage, water and bedding) and faecal matter of both animals and humans that contaminate teat or udder surface (Richter *et al.*, 1992) and

consequently, contaminate milk which creates a good medium for multiplications of many microorganisms including *Klebsiella*. Also, the lack of hygiene and sanitation during handling, processing distribution as stated by Montgomerie (1979) that hands of the personal are the main factors for transmission of infection by *Klebsiella*. It is evident from Tables 2 and 3, that the incidence of *Klebsiella* species from raw buffalo's and cow's milk was 26.66% for each. That was higher giving than Horya (2005) 14.7 and 5.3% respectively. This may due to the differentiation in the culture media used, number of samples and hygienic conditions under which they are collected, also the seasons of collection. *Klebsiella* species obtained from raw buffalo's milk as shown in Table 2 were identified as *K. pneumoniae* (3 isolates) 10%, *K. ozaenae* (2 isolates) 6.66%, *K. planticola* (2 isolates) 6.66% and *K. terrigena* (1 isolate) 3.33%, while 4 isolates (13.33%) *K pneumoniae*, 2 isolates (6.66%) *K. oxytoca* and 2 isolates (6.66%) *K. planticola* in cow's milk (Table 3). It is evident from the tabulated data that *K. pneumoniae* was the most prevalent species in the examined raw buffalo's and cow's milk samples. This came in line with El-Essawy and Riad, 1989 (10%), close to Silva and Costa, 2001 (13.5%), Alonso *et al.*, 2002 (13.8%) and Seleim *et al.*, 2002 (7.3%), higher than Singh and Sharma, 1999 (3.4%) and Horya, 2005 (1.3%) and the results were lower to El-Essawy and Riad, 1989 (18%). At the same time the results were in disagreement with several investigators who failed to detect *K. pneumoniae* from raw milk samples El-Masry (1996) and Ahmed and Sotohy (2003). The high prevalence of *K. pneumoniae* in the examined raw buffalo's and cow's milk samples may be explained by the numerous outbreaks of mastitis in cattle as the organism was incriminated in causing acute and chronic mastitis and variety of infections in other domestic animals. Also, *K. pneumoniae* implicated as an etiological pathogen in human patients suffering from, gastro, respiratory and urinary infections. Also, from Tables 2 and 3 it is clear that *K. ozaenae* could be isolated from raw buffalo's milk only and it revealed 6.66%. This result was lower than El-Essawy and Riad, 1989 (14%) and higher than Horya, 2005 (3.3%). Al-Tarazi (2001) stated that *K. ozaenae* was the most frequent species among *Klebsiella*. It is responsible for an infection of the nasal cavity called Ozena which occurs in elderly persons and manifested by nasal congestion of bad smell and sinusitis (Tondo *et al.*, 2004). With regarding to Tables 2 and 3 *K. oxytoca* isolated from raw cow's milk only and could score also (6.66%). The obtained result was lower than Podshun *et al.*, 1998 (72%), near to Singh and Sharma, 1999 (5.3%) and

similar to Opsomer *et al.* (2001). It is worth to mention that *K. oxytoca* produces infections similar to those caused by *K. pneumoniae* (Mahon and Manuselis, 1995). It usually occur in new born populations in the hospital (Monnet and Freney, 1994). Also, *K. oxytoca* could be isolated from faeces and blood (Farmer, 1985). *K. planticola* among *Klebsiella* species which was obtained from raw buffalo's and cow's milk with the same percentages 6.66%. The giving result was in accordance to Podshun *et al.*, 1998 (8.7%) and Singh and Sharma, 1999 (5.3%) while, it was higher than Horya, 2005 (0.7%). *K. planticola* could be isolated from urin, blood and respiratory tract of humans causing diseases not less than that caused by *K. pneumoniae* (Mahon and Manuselis, 1995). *K. terrigena* another species of *K. pneumoniae* obtained only from raw buffalo's milk 1 (3.33%). The organism has been found in soil and water surrounding the dairy animal (Mahon and Manuselis, 1995). As shown in Tables 2 and 3 Buffalo's and Cow's milk achieved the same percentages of isolation 26.66%. This was in contrary to Sprong *et al.* (2001) and Horya (2005).

Data reported in Tables 4 and 5 varify that raw goat's and sheep's milk samples were free from *Klebsiella* species except *K. ozaenae* which revealed 1 (3.33%) for each. The result was near to Kozacinski *et al.* (2002) 0.4% from goat's milk. Rareness of goat's and sheep's milk from *Klebsiella* species may be in agreement with Hutchinson *et al.* (1985) who stated the lower of risk from unpasteurized goat's and ewe's milk due to the low evidence of contamination. At the same time the giving result was in disagreement with Muchlherr *et al.* (2003) who could isolate different *Klebsiella* species from raw goat's and sheep's milk 61.6 and 71.4% respectively, El-Battrawy *et al.*, and El-Ganzory, 2002 could isolate *K. pneumoniae* (10%) and *K. oxytoca* (16%) from ewe's milk respectively.

The aforementioned results in Table 6, showed that the effect of low temperature (chilling and freezing) on the growth of *K. pneumoniae* when inoculated into sterile butter with 17×10^8 cells/g was: in case of chilling temperature, a significant increase in the cell counts of *K. pneumoniae* from 17×10^8 as initial count to 91×10^8 , 206×10^8 , 217×10^8 and 224×10^8 cells in the first, second, third and seventh day respectively. The obtained results were in agreement with Hechelmann *et al.* (1974), Johnson *et al.* (1975) and Patterson and Gibbs (1977) and this finding represents the ability of *Klebsiella* organisms as one of psychrotrophic bacteria to survive at the temperature of refrigeration and could contribute substantially of refrigerated materials. While, in case of

freezing temperature a gradual decline in count was noticed in the first and second day from 17×10^8 cells as initial count to 10×10^8 and 113×10^7 cells respectively. On the other side, in the third and seventh day a remarkable decrease in the count of *K. pneumoniae*, reached to 220×10^5 and 37×10^3 cells, respectively. These results were in agreement with Calcott (1976). Following up to Table 6 it is worth mentioning that *K. pneumoniae* was completely disappeared after the first week at chilling and freezing temperatures and that may be due to the acidity percentages which revealed significant increase affecting the inoculated organism. From 4.3% at the zero time and reached to 6.5% at the seventh day. Similar findings were reported by several investigators on different microorganisms due to increasing of acidity percentages Huang *et al.* (1993), Rola *et al.* (1994), Abou-Eleinin (1999) and El-Gazzar (1997). It is worth to mention that the freezing temperature has been shown to cause reduction in the growth rate, inactivation and destruction of microorganisms (ICMSF, 1980).

The results in Table 7 which concern antimicrobial susceptibility pointed out that all *K. pneumoniae* strains isolated from raw buffalo's and cow's milk were found to be resistant to the most antibiotic discs used as reported by Carroll (1971), Braman *et al.* (1973) and Malinowski and Klossowska (2003), while they were highly sensitive to Norfloxacin as obtained by Grewal *et al.* (2001), Longoni *et al.* (2001) and Ozgur *et al.* (2003), moderately sensitive to Gentamycin and this is pointed out by Dhand *et al.* (2001) and Silva and Costa (2001) and weakly sensitive to Cefotaxime.

In general the excessive use of antibiotics in the treatment of *Klebsiella* infections in hospitalized patients and animals are of limited value and may lead to prolong the illness and increase the carriage of *Klebsiellae* in addition to the economic loss due to the misuse of antibiotics in veterinary practice.

In conclusion: milk represents a suitable medium for growth and multiplication of several human pathogens, including *Klebsiella*. Therefore, strict hygienic measures must be followed during all steps of milk production and its manufacture to dairy products. In addition to particular attention to the management of these dairy goat and sheep flocks in order to avoid the development of cases of clinical mastitis with different microorganisms.

REFERENCES

- Abou-Eleinin, A.M. (1999)*: Studies on *Listeria* species in milk and milk products. Ph.D. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.
- Ahmed, M.M. and Sotohy, S.A. (2003)*: Sanitary conditions of milking environment in Assiut dairy farms and the quality of their produced milk. *Assiut Vet. Med. J.*, 49: 88-112.
- Al-Ashmaway, Maha, A. (2004)*: Occurrence of verocytotoxigenic *Escherichia coli* in milk and some dairy products. Ph.D. Thesis, Fac. Vet. Med. Mansoura Univ., Egypt.
- Alonso, C.; Capita, R.; Carballo, J.; Bernrdo, A. and Garica, M.L. (2002)*: Changes in the Enterobacteriaceae poulations throughout manufacturing and ripening of Yaldetega cheese. *Dairy Sci. Abst.* 64, No. (12): 1178.
- Al-Rabea, A.A.; Burwen; D.R. and Eldeen, M.A. (1998)*: *Klebsiella pneumoniae* blood stream infections in neonates in a hospital in the Kingdom of Saudi Arabia. *Infect Control Hosp. Epidemiol.*, 19 (9): 674-679.
- Al-Tarazi, Y.H. (2001)*: Bacteriological and pathological study on pneumonia in the one humped camel in Jordan. *Revue d'Elevage et de Medecine Veterinari des pays Topicaus*, 54 (2): 93-97.
- Anderson, M.J. and Janoff, E.N. (1998)*: *Klebsiella* endocarditis report of two cases and review. *Clin Infect Dis.*; 26 (2): 468-474.
- Atlas, R.M. and Parks, L. (1994)*: *Hand Book of Microbiological Media*, CRC Press, Boca Raton, London.
- Bagley, S.T. and Seidler, R.J. (1978)*: Primary *Klebsiella* identification with MacConkey-inositol-carbenicillin agar. *App. Environ. Microbiol.* 36, 536-580.
- Barkema, H.W.; Schukken, Y.H.; Lam, T.J.; Beiboer, M.L.; Wilmink, H.; Benedictus, G. and Brand, A. (1998)*: Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *J. Dairy Sci.*, 81 (2): 411-419.
- Baron, E.J.; Peterson, L.R. and Finegold, B.M. (1994)*: *Balley and Scott's Diagnostic Microbiology*. 9th Ed., Mosby St. Louis, Baltimore.
- Berrys, J.A. (1933)*: Destruction and survival of microorganisms frozen pack foods. *J. Bacteriol.*, 26: 459-470.
- Braman, S.K.; Eberhart, R.J.; Ashbury, M.A. and Herman, G.J. (1973)*: Capsular types of *Klebsiella pneumoniae* associated with bovine mastitis. *J.A.V.M.A.* 62 (2): 109.

- Burnet, G.W.; Sherp, H.W. and Schuster, G.S. (1978):* Microbiology Oral Doencas Infecciosas. 4th ed. Guanabara Koogan, Rio de Janeiro.
- Buttiaux, R. (1959):* The value of the association Escherichia-group A streptococci the diagnosis of contamination in foods. J. Appl. Bacteriol., 22: 153-158.
- Calcott, P.H.; Lee, S.K. and Macleod, R.A. (1976):* The effect of cooling and warming rate on the survival of variety of bacteria. Can. J. Microbiol., 22 (1): 106-109.
- Carroll, E.J. (1971):* Bacterial activity of bovine serums against coliform organisms isolated from milk of mastitic udders, udder skin, and environment. Am. J. Vet. Res., 32 (5): 689-701.
- Creyghton, W.M.; Dobatto, S. and Weening, J.J. (2001):* Acute renal failure caused by Klebsiella pneumoniae pyelonephritis. Clin Nephrol., 56 (3): 391-393.
- Cruickshank, R.; Duguid, J.; Marmion, B. and Swain, R. (1978):* Medical Microbiology 12th Ed. Churchill Livingstone, Ebinbrugh, London, New York.
- Dechamps, C.; Rich, C.; Chandezon, P.; Chanal, C. and Siro, F. (2004):* Factors associated with antimicrobial resistance among clinical isolates of Klebsiella pneumoniae. 1- Year survey in a French University Hospital Eur. J. Clin. Microbiol.
- Dhand, N.K.; Saini, S.S.; Sharma, D.R. and Sandhu, K.S. (2001):* Acute bacterial pneumonia due to Klebsiella pneumoniae in Angora rabbits. Indian J. of Comparative Microbiol. Immunol. and Infect. Dis., 22 (2): 177.
- Edward's, P.R. and Ewing, W.H. (1972):* Identification of Enterobacteriaceae, 3rd Ed. Burgess Publ. Co. Minneapolis.
- El-Battrawy, N.; Zaki, M.S. and Bayoumi, F.S. (2002):* Some microbiological and clinico-pathological studies on ewes suffering from sub-clinical mastitis. Bulletin of the National Research Center (Cairo) 27 (2): 211-219.
- El-Essawy, H.A. and Riad, A.A.M. (1989):* Enteropathogenic in raw market and farm milks. Alex. J. Vet. Sci., 5 (2): 209-217.
- El-Ganzory, H.H. (2002):* Chemical and microbiological quality of Ewes milk. SCVMJ, V (1): 43-50.
- El-Gazzar, F.E. (1997):* Proceeding of the First Scientific Conference of Agricultural Science. Faculty of Agri., Assiut, December 13-14, 1997, Vol. II.

- El-Masry, M.A.L. (1996):* Dairy house hygiene in relation to incidence of mastitis. M.V.Sc., Fac. Vet. Med. Zagazig Univ.
- Farmer, J.J.III; Davis, B.R. and Hickman-Brenner, F.W. (1985):* Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol. 21: 46-76.
- Gamea, A.M. and Tatawi, F.A. (1990):* The effect of rifampicin on rhinoscleroma: an electron microscopic study. J. Laryngol. Otol., 104 (10): 772-777.
- Goto, Y.; Murakami, T.; Koike, A.; Haga, T. and Shinjo, T. (2003):* Bacteriological examination of umbilical infections in calves. J. of the Japan Vet. Med. Assoc. 56 (8): 528-530.
- Grewal, K.D.; Gupta, M.P.; Srivastava, A.K. and Randhawa, S.S. (2001):* Disposition pattern of enrofloxacin in blood and milk of buffaloes suffering from clinical mastitis. Indian J. of Animal Sci. 71 (4): 347-34.
- Grhon, Y.T.T.; Wilson, D.J.; Gonez, R.N.; Hertle, J.A.; Schulte, H.; Bennelt, G. and Chukken, Y.H. (2004):* Effect of pathogen specific clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 87: 3358-3374.
- Hechelmann, H.; Bom, Z.; Uchidd, K. and Lesstner, L. (1974):* Vorkommednes tribus Klebsielleae eikuhlge lagertemflesh und Fleischwaren. Fleischwirtschaft, 54: 1515-1517.
- Hörvath, J.; Hanny, J. and Pethes, C. (1964):* Massenauftreten von Lebensmittelvergiftungen verursacht durch Bakteien der Gruppe Klebsiella. Zbl. Bakt. Hyg., I. Abt. Org. 193, 191-195.
- Horya, A.G. (2005):* Prevalence of Klebsiella species in milk and some milk products in Assiut Governorate. M.V.Sc. Faculty of Veterinary Medicine, Assiut University.
- Huang, J.; Lacroix, C.; Daba, H. and Simard, R.E. (1993):* Inhibition of growth of Listeria strains by mesenterocin 5 and organic acids. Lait. 73: 357-370.
- Hutchinson, D.N.; F.J. Botton, W.C. Jelly, W.G. Mathews, D.R. Telford, D.E. Counter, E.G. Jessop and S.D. Horsley (1985):* Campylobacter enteritis associated with consumption of raw goat's milk.
- ICMSF (1980):* International Commission on Microbiological Specification Microbiology ecology of food, Vol. 2. Academic Press, New York, London, pp. 522-552.

- Izard, D.; Ferragut, C.; Gavini, K.; Kesters, J. and Leclerc, H. (1981): Klebsiella terrigena, a new species from soil and water. Int. J. Syst. Bacteriol., 31: 116-127.*
- Johnson, R.; Colwell, R. and Tamura, K. (1975): Numerical taxonomy study of enterobacteriaceae. Int. J. Syst. Bact., 25 (1): 12-37.*
- Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schreckenberger, P.C. and Winn, W.C. (1992): Color Atlas and Textbook of Diagnostic Microbiology 4th Ed. R. Winter (ed) J.B. Lippincott Company, Philadelphia, PA.*
- Kozacinski, M.; Hadziosmanovic, M.; Mayic, T.; Krodjole, I. and Cvrtila, Z. (2002): Relationships between the results of mastitis testes, somatic cell counts and the detection of mastitis agents in goat's milk. Tierarztliche Umschau.*
- Kumari, P.M. and Gupta, B.J.R. (2002): Diagnosis and therapy of subclinical mastitis in post-parturient cows. Indian Vet. J. 79 (1): 79-89.*
- Lampert, L.M. (1975): Modern Dairy products. 3rd., Chemical Pub. Co., Inc., New York.*
- Lim, B.; Wang, J.Y.; Halmshav, U.; Hoppe, H. and Reid, K.B. (1995): Expression of the carbohydrate recognition of its binding to lipopolysaccharide of Gram negative bacteria. Biochem. Biophys. Commun., 15, 202 (3): 1674-1680.*
- Longoni, H.; Plugá, M.E.; Domingues, P.F. and Silva, A.V. (2001): Effectiveness of enrofloxacin in the treatment of environmental bovine mastitis during an outbreak in a dairy herd. Napgama, 4 (1): 19-22.*
- Mahon, C.R. and Manuselis, G., Jr. (1995): Text Book of Diagnostic Microbiology Chapter (16), Philadelphia, London.*
- Malinowski, E. and Klossowska, A. (2003): Cow mastitis pathogen resistance to antibiotics, Medycyna weterynaryjna 59 (2): 230-235.*
- Monnet, D. and Freney, J. (1994): Method for differentiating Klebsiella planticola and Klebsiella terrigena from other Klebsiella species. J. Clin. Microbiol. 32: 1121-1122.*
- Montgomerie, J.Z. (1979): Epidemiology of Klebsiella and hospital associated infections. Rev. Infect. Dis., 1: 736-753.*
- Muchlherr, J.E.C.; Zweifel, S.; Corti, J.E. Blanco and Stephan, R. (2003): Microbiological quality of Raw Goat's and Ewe's Bulk-Tank Milk in Switzerland. J. Dairy Sci. 86: 3849-3856.*

- Newman, L.E., and Kowalski, J.J. (1973):* Fresh sawdust bedding a possible source of Klebsiella organisms. *Am. J. Vet. Res.*, 34 (7): 979-980.
- Niazi, Z.; Kirpal, G.; Amtsberg, G. and Refait, M. (1977):* Biochemistry, serology, pathogenicity to mice and the resistance to antibiotics of Klebsiella strains of various kind of animals. *Berl. Munch. Lievarzt. Wcsnr.* 90 (22), 435-440.
- Obiamiwe Umeh, M.B.B.S. (2002):* Klebsiella infections center for AIDS Research and Education, David Geffon School of Medicine at UCLA.
- Olsson, C.; Ahrne, S.; Pattersson, B. and Molin, G. (2004):* DNA based classification of food associated Enterobacteriaceae previously identified by Biolog GN Microplates. *Syst. Appl. Microbiol.*, 27 (2): 219-228.
- Opsomer, G.; Vlieghe, S.D.E.; Loureyns, J.; Hoflack, G.; Beeckman, D.; Kruif, A.D.E. (2001):* Evaluated number of coliform bacteria in the bulk milk due to chronic Klebsiella oxytoca mastitis. *Valams Diergeneeskundig Tijdschrift* 70 (1): 50-53.
- Ozgun, N.Y.; Bagcigil, A.F.; Ikiz, S.; Kilicarslan, M.R.; Carioglu, B. and Ilgaz, A. (2003):* Isolation of Klebsiella pneumonia from mares with metritis and stallions, detection of biotypes and capsule types. *Turk Veterinerik ve Hayvancilik. Dergisi* 27 (1): 241-247.
- Patterson, J.T. and Gibbs, P.A. (1977):* Incidence and spoilage potential of isolates from vacuum-packaged meat of high value. *J. Appl. Bact.*, 43: 25-38.
- Peng, X.; Zeng, Z. and Chen, Z. (2003):* Screening of therapeutic drug of clinical mastitis in dairy cattle and their efficacy Chinese. *J. of Vet. Sci.* 23 (2): 193-195.
- Podshun, R.; Acklurn, H.; Okpara, J.; Olinderkamp, Ullmann, U. and Borneff-Lipp-Lipp, M. (1998):* Isolation of Klebsiella planticola from....
- Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G. (1994):* Clinical veterinary microbiology. Mosby-year Book Europe Limited – London, England. 1st Ed.
- Rahman, H.; Nath, N.C. and Boro, B.R. (1992):* Bacterial flora and insecticidal residue in raw milk marketed in Guwahati city, Assam. *Indian J. of Comparative Microbial. Immunol. And Infect. Dis.* 13 (3 & 4): 105-108.

- Rennie, R.P. and Duncan, J.B.R. (1974): combined biochemical and serological typing of clinical isolates of Klebsiella. *Appl. Microbiol.* 28: 534-539.
- Richter, R.L.; Ledford, R.A. and Murphy, S.C. (1992): Milk and milk products. In: *Compendium of Methods for the Microbiological Examination of Foods*, 3rd Ed. For Venderzant, and D.F. Splittstoesser (eds.). American Public Health Association, Washington, D.C.
- Rola, J.; Kwialek, K.; Wojton, B. and Michalski, M. (1994): Incidence of *Listeria monocytogenes* in raw milk and dairy products. *Medycyna Wet.* 50: 323-325.
- Seleim, R.S.; Rashed, A.Y.M. and Fahmy, B.G.A. (2002): Mastitis pathogens: attachment-related virulence features, whey protein markers and antibiotic efficacy in cows. *Vet. Med. J.*, 50 (3): 405-418.
- Shoshani, E.; Leitner, G.; Hanochi, B.; Saran, A.; Shpigel, N. and Berman, A. (2000): Mammary infection with *Staphylococcus aureus* in cows: progress from inoculation to chronic infection and its detection. *J. Dairy Res.*, 67: 155-169.
- Silva, N. and Costa, G.M. (2001): An outbreak of acute bovine mastitis caused by *Klebsiella pneumoniae* in a dairy herd. *Arquivo Brasileiro de Medicina Veterinariae Zootecnia*, 53 (4): 401-405.
- Singh, B.R. and Sharma, V.D. (1999): Characterization of brood spectrum Klebacin produced by *Klebsiella pneumoniae* subspecies aerogenes. *Indian J. of Comparative Microbiol. Immuno. And Infect. Dis.* 20 (2): 116-120.
- Sprong, R.C.; Hulstein, M.F.E. and Meer, R.V. (2001): Bactericidal activities of milk lipids. *Antimicrobial agents and chemotherapy* 45 (4): 1298-1301.
- Tondo, E.C.; Lakus, F.R.; Oliveria, F.A. and Brandelli, A. (2004): Identification of heat stable protease of *Klebsiella oxytoca*. *Microbiol.*, 38 (2): 146-50.
- Vijayalakshmi, P.; Prathahan, S. and Dhanapalan, P. (2001): Comparative study on the efficacy of diagnostic tests in the field diagnosis of bovine mastitis. *Indian Vet. J.*, 78: 4-6.
- Zohar, Y.; Talmi, Y.P. and Strauss, M. (1990): Ozena revisited. *J. Otolaryngol*, 19 (5): 345-349.