

PREVALENCE OF KLEBSIELLA SPECIES IN MARKET RAW MILK AND CREAM

Maha A. M. Al-Ashmawy and Arwa H. M. Nassar

Food Hygiene and Control Dep., Faculty of Vet. Med., Mansoura University

ABSTRACT

A total of one hundred random samples, of market milk and cream (50 samples each) were collected from different localities in El-Dakhalla Governorate to be examined for the incidence of *Klebsiella* species and its sensitivity towards some antibiotics.

Klebsiella on VRB and MacConkey agar media were detected in percentage of 52% and 48% from market raw milk and in percentages of 30% and 42% from cream using the two selective media respectively.

K. oxytoca, *K. ozaenae*, *K. pneumoniae* and *K. rhinoscleromatis* could be isolated in percentages of 28, 22, 18 and 10 respectively from market raw milk on MacConkey agar. While on VRB medium they were isolated in percentages of 26, 22, 30 and 8 respectively.

K. pneumoniae, *K. oxytoca*, *K. ozaenae* and *K. rhinoscleromatis* were isolated from cream at varying percentages ranging from 4-28% on MacConkey agar medium and from 4-20% on VRB medium.

The biogram of *Klebsiella pneumoniae* was assessed towards different antibiotics, *K. pneumoniae* was resist to Amoxacyllin, Ampicillin/Sulbactam, Cefoperazone, Erythromycin, Nalidixic acid and Norfloxacin. While the susceptibility of *K. pneumoniae* to Neomycin was intermediate but highly susceptible to Amikacine, Cefotaxime, Ciprofloxacin, Chloramphenicol and Nitrofurantoin.

The public health and economic importance of isolated *Klebsiella* spp. as well as recommendation for preventing and minimizing the microbial contamination of market milk and cream were also discussed.

INTRODUCTION

Milk and its products are exposed to contamination with several types of microorganisms from different sources. Under suitable circumstances, these contaminants find their way to grow

and multiply in milk and its products leading to undesirable changes that rendering it of an inferior quality or even unfit for human consumption beside it may constitute a public health hazard.

Klebsiella is among the oldest genera in the Enterobacteriaceae established in 1885 (Orskov, 1984 and Grimont et al., 1991), the four most commonly encountered species of Klebsiella had all been described by the turn of the century. This list included *K. pneumoniae*, *K. oxytoca*, *K. ozaenae* and *K. rhinoscleomatis* (Janda and Abbott, 1998).

The presence of Klebsiella in market raw milk and/or cream does not necessarily mean that there was fecal contamination as it carried in intestinal tract of the human and animals also in plant, soil as well as water (Weissfeld et al., 1994) and carried by 6% of healthy people in nose and throat, approximately one third of people carry Klebsiella in their stool (Grimont et al., 1991). Their significance in foods depends upon the circumstances to which market raw milk and cream have been exposed.

As milk and its products represent potential hazardous source of virulent Klebsiella (El-Sukhon, 2003) which not only threatened the public health but also the dairy products so this work was conducted to study prevalence of Klebsiella and antibiotic sensitivity of *K. pneumoniae*.

MATERIAL AND METHODS

One hundred samples of market raw milk and cream (50 samples of each) were collected randomly from dairy shops, supermarkets and groceries then transferred to the laboratory as rapidly as possible in El-Dakahlia Governorate, Egypt.

All samples are thoroughly mixed in their retail containers, then starch test was applied for detection of raw milk.

* Bacteriological examination:

(A) Preparation of serial dilutions (APHA, 1992).

(B) Isolation of Klebsiella species:

(1) Using solid medium (Violet Red Bile Agar Medium):

The technique recommended by APHA (1992) was applied. One ml of market raw milk or

cream and their decimal dilutions were transferred into sterile plates. To each plate 10-15 ml of Violet Red Bile Agar Medium (VRB) at 45°C were added. An agar control plate for each flask of medium used was poured. The contents of the plates were thoroughly mixed by tilting and rotating then allowed to solidify on a level surface. The plates were inverted and incubated at 35°C for 24 hrs.

Five representative colonies appeared on uncrowded plates described by APHA, (1992) as convex, moist, mucoid may almost ooze across the agar which is considered to be characteristic of *Klebsiella* species, other genera may appear mucoid were picked up and seeded on nutrient agar plates for purification and identification.

(2) Using Liquid medium:

Fermentation tubes of 2% Brilliant Green Lactose Bile Broth were inoculated with one ml from each of previously decimal serial dilutions thereof to obtain positive and negative tubes. The tubes after mixing were incubated at 35°C for 48 hrs. Loopfuls from the positive fermentation tubes (positive acid and gas) were streaked onto the surface of MacConkey agar plates and inoculated at 35°C for 24 hrs. The suspected colonies were streaked onto slope agar for further identification.

* Identification of Isolates:

The isolated organisms were identified according to Holt et al., (1994) by the morphological characters (gram staining and motility test) and biochemical reactions (catalase t., oxidase t., methyl red, citrate utilization, indole, urase, voges-proskauer, gelatin liquefaction and carbohydrate fermentation tests).

*Antimicrobial sensitivity testing of *K. pneumoniae* (Using disc diffusion method) (Gulian et al., 1994):

The antibiotic discs used are (Amikacine, Amoxacyllin, Ampicillin sulbactam, Cefoperazone, Cefotaxime, Chloramphenicol, Ciprofloxacin, Erythromycin, Neomycin, Nitrofurantion, Norfloxacin and Nalidixic).

RESULTS AND DISCUSSION

The results given in table (1) showed that *Klebsiella* spp. were isolated from 48% of examined market milk samples on MacConkey agar and from 25% of samples on VRB medium.

Klebsiella spp. detected on MacConkey agar medium (Table, 2) were identified as *K. pneumoniae* (18%), *K. oxytoca* (28%), *K. ozaenae* (22%) and *K. rhinoscleromatis* (10%). While, on VRB medium were 30%, 26%, 22% and 8% respectively.

K. oxytoca was the predominant (36.06%) one of the *Klebsiella* isolated, followed by *K. ozaenae* (31.14%), *K. pneumoniae* (21.30%) and *K. rhinoscleromatis* (11.47%) on MacConkey agar medium. While, on VRB medium were 31.7%, 30.0%, 31.7% and 6.7% respectively (Table, 3).

Data in table (1) also pointed out that *Klebsiella* spp. were isolated from 42% of examined cream samples on MacConkey agar and from 30% on VRB medium.

K. pneumoniae (4%), *K. oxytoca* (12%), *K. ozaenae* (28%) and *K. rhinoscleromatis* (14%) were isolated on MacConkey agar medium. While, on VRB medium were 6%, 14%, 20% and 4% respectively (Table, 2).

The percent of identified isolates of *Klebsiella* spp. were *K. pneumoniae* (8.1%), *K. oxytoca* (16.2%), *K. ozaenae* (45.9%) and *K. rhinoscleromatis* (29.7%) on MacConkey agar medium while, on VRB medium were 6.45%, 25.80%, 28.06% and 9.60% respectively. (Table, 3).

The results declared that MacConkey agar medium was more effective in isolation of *K. pneumoniae* and *K. rhinoscleromatis*. On the other hand, VRB medium was recommended in case of *K. oxytoca* and *K. ozaenae*.

Table (4) declared that *K. pneumoniae* was resistant to Amoxycillin, Ampicillin sulbactam, Cefoperazone, Erythromycin, Nalidixic acid and Norfloxacin. While, susceptibility of *K. pneumoniae* to Neomycin was intermediate. However, it is susceptible to Amikacin, Cefotaxime, Ciprofloxacin, Chloramphenicol and Nitrofurantoin.

Bacterial contamination by *Klebsiella* species lead to faults in milk including ropiness and sliminess.

Klebsiella species are frequently incriminated in causing mastitis and subclinical mastitis which leads to economical losses as reduction in milk production from affected quarters and a need to cull cows with badly indurated quarters, production of low quality milk renders it unsuitable for human consumption or interferes with manufacturing process. highly cost of treatment due to adhesion of *Klebsiella* to host tissues which has been attributed to the expression of arrange of fimbrial and nonfimbrial adhesions (Greenwood et al., 2002). Heat stable protease of psychrotrophic strains of *K. oxytoca*, isolated from milk, is characterized by high thermal resistance leading to unpleasant properties in UHT processing (Tondo et al., 2003).

To overcome these economic losses which caused by *Klebsiella* we should know how this pathogen reach udder and subsequently affect milk to prevent losses, the possible ways of en-

trance are through hands of milkers by using machine cups. *Nes* (antibiotic resistant *K. pneumoniae* was isolated from cockroaches) (El-Soubaty, 2002).

Klebsiella associated with water, so washing udder or utensils with contaminated water can pollute milk. While, air-born spread of *K. pneumoniae* is possible since the organism colonize the upper respiratory tract of humans who handle milk (Hollander et al., 2001).

It is worth mentioning that milk and milk products that are improperly handled provide a ready medium for transmission of *K. pneumoniae* that may causing different types of infection to consumers.

Periodical examination of dairy animals at the farm levels for mastitis, personal hygiene, application of strict legislative standards of hygiene at the chain of milk and products production and selection of the highly susceptible antibiotics towards *Klebsiella* for the treatment are considered as a recommendations should be undertaken to prevent contamination of milk and cream by *Klebsiella* organisms.

RESULTS AND DISCUSSION

Table (1): Incidence of Klebsiella organisms in the examined samples

Examined samples	Media used	MacConkey		VRB	
	Total No. of samples	No. of positive samples	%	No. of positive samples	%
Market raw milk	50	24	48	26	52
Cream	50	21	42	15	30

Table (2): Incidence of isolated Klebsiella spp. from market raw milk and cream on MacConkey agar and VRB medium

Type of Media	Source of Samples	No. of total samples	No. of positive samples							
			<i>K. pneumoniae</i>		<i>K. oxytoca</i>		<i>K. ozaenae</i>		<i>K. rhinoscleromatis</i>	
			No	%	No	%	No	%	No	%
MacConkey	Market raw milk	50	9	18	14	28	11	22	5	10
	Cream	50	2	4	6	12	14	28	7	14
VRB	Market raw milk	50	15	30	13	26	11	22	4	8
	Cream	50	3	6	7	14	10	20	2	4

Table (3): Frequency distribution of Klebsilla isolates from market raw milk and cream samples on MacConky agar medium and VRB medium

Type of Media	Source of Samples	No. of total isolates	No. of identified isolates							
			<i>K. pneumoniae</i>		<i>K. oxytoca</i>		<i>K. ozaenae</i>		<i>K. rhinoscleromatis</i>	
			No	%	No	%	No	%	No	%
MacConkey	Market raw milk	61	13	21.30	22	36.06	19	31.14	7	11.47
	Cream	37	3	8.1	6	16.2	17	45.9	11	29.7
VRB	Market raw milk	60	19	31.7	19	31.7	18	30.0	4	6.7
	Cream	31	2	6.45	8	25.80	18	58.06	3	9.68

Table (4): Antibiotic sensitivity of *K. pneumoniae* isolated from samples of market raw milk and cream

Types of antibiotic	Resistant	Intermediate	Susceptible
Amikacine	-	-	+
Amoxacyllin	+	-	-
Ampicillin sulbactam	+	-	-
Cefoperazone	+	-	-
Cefotaxime	-	-	+
Ciprofloxacin	-	-	+
Chloramphenicol	-	-	+
Erythromycin	+	-	-
Nalidixic acid	+	-	-
Nitrofurantoin	-	-	+
Norfloxacin	+	-	-
Neomycin	-	+	-

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الملخص العربي

مدى إنتشار الكليبسيلا فى لبن الأسواق والقشدة

مها عبده محمد العشماوى أروى حسن محمد نصار

قسم الرنابة الصحية على الأغذية - كلية الطب البيطرى - جامعة المنصورة

أجريت الدراسة على مائة عينة (خمسون عينة من لبن الأسواق الحام الطازج وخمسون عينة من القشدة) جمعت من أماكن متفرقة من محافظة الدقهلية، وقد تم عزل وتصنيف ميكروبات الكليبسيلا فى اللبن الحام بنسبة ٤٨٪ وذلك باستخدام مستنبت MacConkey، بينما تم العزل بنسبة ٥٢٪ باستخدام مستنبت VRB. أما فى حالة القشدة وجدت النسب كالآتى (٤٢٪) باستخدام مستنبت MacConky و (٣٠٪) عندما استخدم مستنبت VRB.

وتد أسفرت النتائج على تلوث لبن الأسواق الحام بميكروبات *K. pneumoniae* (١٨٪)، *K. oxytoca* (٢٨٪)، *K. ozzaenae* (٢٢٪)، *K. rhinoscleromatis* (١٠٪) وذلك باستخدام مستنبت MacConkey وكانت النسب هى ٣٠٪، ٢٦٪، ٢٢٪، ٨٪ على التوالي باستخدام مستنبت VRB. وكذلك أمكن عزل نفس الميكروبات من القشدة ولكن بنسب تتراوح بين ٤ إلى ٢٨٪ باستخدام مستنبت MacConkey ولكن عندما استخدم مستنبت VRB كانت النسب تتراوح بين ٤٪ إلى ٢٠٪.

وقد بنيت النتائج تلوث لبن الأسواق الحام بميكروب تلوث لبن الأسواق الحام بميكروب الكليبسيلا بصورة أكبر من اللبن فى حالة القشدة وقد أرجع التلوث إلى عدم إتباع الاشتراطات الصحية أثناء نقل وبيع اللبن.

وباستخدام إختبار الحساسية للمضادات الحيوية وجد أن ميكروب *K. pneumoniae* يقاوم كلا من الأموكسيسيلين والامبسللين وسينبرازون والأيرثروميثين وناليديكسيك ونوروفلوكساسين، وقد أظهرت النتائج حساسية متوسطة لانيوميسين، ووجد أن المضادات الفعالة ضد ميكروب *K. pneumoniae* هى سيفوتكسيم وسيبروفلوكساسين وكلورمفينيكول وتيتروفبرانيتون.

هذا وقد تم شرح الأهمية الصحية والاقتصادية لميكروب الكليبسيلا ومدى تأثيره على الإنتاج الحيوانى وكذلك النصائح الهادفة لمنع أو تقليل التلوث الجرثومى بميكروب الكليبسيلا للبن الحام بالأسواق والقشدة إلى أدنى مستوى له لضمان أقصى درجات الأمان الصحى للمستهلكين وأطول عمر تخزينى للألبان.