Journal of Al Azhar University-Gaza (Natural Sciences), 2013, 15 : **79-88**

Antibiogram of Klebsiella spp. isolated from ice cream samples in Gaza Strip

Abdelraouf A. Elmanama[1], Hashem Arafa [2]

[1] *Medical Laboratory Sciences Department, Islamic University, Gaza, Palestine*
[2] *Public Health laboratories, Ministry of Health*

elmanama_144@yahoo.com

Received 3/4/2013 Accepted 21/5/2013

Abstract: *Ice cream containing milk making it perishable food and good medium for the growth of a wide variety of potential pathogens. Klebsiella pneumoniae is an emerging infectious agent that has been clearly linked with the consumption of contaminated foods especially ice cream. This study examined one hundred locally produced ice cream samples. Out of 100 ice-cream samples analyzed for Fecal coliform and Klebsiella spp., 42 samples were positive for Fecal coliform and 37 samples were Klebsiella spp. positive. Klebsiella spp. was examined for susceptibility to eight antimicrobial agents. Minocycline showed the lowest activity (11%) followed by Doxycycline (24%). Klebsiella spp. isolates were highly sensitive to Norfloxacin (100%) and Gentamicin (95%). In conclusion, locally manufactured ice cream is a potential source for acquiring Klebsiella spp. Therefore, a more strict surveillance system should be implemented to ensure adherence to good manufacturing practices.*

Keywords: *Klebsiella* spp.*,* Antibiotic sensitivity, Ice cream, Gaza strip.

Introduction

Ice cream, which is a milk product, is consumed globally and most consumption occurs outside homes and amongst children during the summer months in temperate countries and all year round in tropical nations **(Anil *et al.*, 2012).** Ice cream is a nutritionally enriched dairy product produced by freezing pasteurized mixture of milk solids other than fat, sugar, emulsifier and stabilizer. Flavor enrichment of ice cream is an optional addition of fruit nuts, candies, syrups and other flavoring ingredients **(Mohammad and Habib, 2011).**

The protein and vitamin contents of ice cream are fairly low, but as a source of calcium it is as valuable as milk. The unfrozen ice cream mix is oil -in- water emulsion. The aqueous phase in which the fat globules are dispersed also serves as a dispersion medium for proteins

in colloidal dispersion, sugars and salts in true solution **(Osamwonyi *et al.*, 2011).**

Ice cream is a nutritious food for man and also an excellent medium for the growth of many microorganisms including some which may be pathogenic to man. Although ice cream is a frozen product, it has been documented to be contaminated by bacterial pathogens such as *Salmonella* spp., *Listeria* spp., *Yersinia* spp., *Staphylococcus aureus*, *Escherichia coli*, coliforms, *Bacillus* spp. amongst others in many countries **(Javade, 2011).**

Possible sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream mix such as separated milk and milk powder cream, flavoring, coloring, substances, stabilizers and from air during processing **(Ojokoh, 2006).**

In humans, *Klebsiella pneumonia* is an important cause of nosocomial infection lower respiratory tract like pneumonia, septicemia, urinary tract and soft tissue infections **(Ashik & Aitabh, 2010, Cryz *et al.*, 1986)**. *Klebsiella* can lead to wide range of disease as notably pneumonia, urinary tract infection, septicemia. They are opportunistic pathogens found in the environment and in mammalian mucosal surfaces **(Kumar and Talwar, 2010).**

Antibiotic resistance has been classified by the World Health Organization as one of the three major public health threats of the 21st century **(Lachmayr *et al.*, 2009).** Antibiotic resistant strains of the Coliform bacterium *Klebsiella* becomes an increasingly serious complication in the treatment of human and animal diseases caused by this organism **(Talbot *et al.*,1980).**

Klebsiella pneumoniae has been isolated from different sources including lung, tonsillitis gland, respiratory tract, urine tract and food. Although, *K. pneumoniae* caused disease in all age groups, the majority of cases are infants. While the reservoir for *K. pneumoniae* unknown, a growing number of reports suggested that food such as ice-cream and milk were the major sources of *K. pneumonia*e. This study examined the possibility that locally sold ice cream may carry *K. pneumoniae*.

Materials & Methods

Collection of the Samples

In the present study, a total of 100 samples of ice-cream were collected from different markets of Gaza City between May and June 2011 were analyzed for the presence of *K. pneumoniae &* Fecal coliform. All samples were produced by local manufacturers (no imported samples). Samples were categorized into four groups; 1. ice-cream with artificial flavors, 2. with chocolate, 3. with coconut & 4. with fresh fruits. Samples were collected in sterile containers and transported to Public Health Laboratories (Ministry of Health) in an icebox within 2 hours of collection. The samples were then examined for the presence of *K. pneumoniae* and Fecal coliform according to Bacteriological Analytical Manual **(FDA, 1995)**.

Analysis of the Samples

Twenty five grams of each sample were homogenized with 225 ml peptone water (0.1 %). Decimal dilution (10^{-2}, 10^{-3}) of ice cream samples were prepared then a 0.1 ml of each dilution was transferred onto to the surface of mFC agar & HiCrome *Enterobacter sakazakii* agar (HiMedia, India) and spread with a sterile L shaped glass road. mFC agar was incubated at 44 ºC for 24 hours and the HiCrome *Enterobacter sakazakii* agar was incubated at 37ºC for 24-48 hours. After the incubation period, the blue colonies on mFC agar and the yellow mucoid colonies with diameter 4–5 mm on HiCrome *Enterobacter sakazakii* agar were counted. All suspect colonies were then examined using API 20 E system to confirm *K. pneumoniae* isolates.

Antimicrobial susceptibility testing

Eight antibiotic were evaluated for their ability to inhibit the growth of *Klebsiella* spp. The antibiotic sensitivity test was performed by disc diffusion method with commercially available antibiotic discs **(HiMedia, 1998)** on Mueller Hinton agar (MHA). The discs used were Gentamicin (GM 10 µg), Minocycline (Mi 30 µg), Doxycyline (DO 30 µg), Cefuroxime sodium (CXM 30 µg), Cephalexin (CL 30 µg), Chloramphenicol (C 30 µg), Norfloxacin (NOR 10 µg) and Nalidixic acid (NA 30 µg). Standardized inoculum was spread on Mueller Hinton agar, using a sterile swab and the disc of the antibiotic were placed on it using sterile forceps. After incubation at 37ºC for 24

hours, the diameter of zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate, susceptible or susceptible according to Clinical and Laboratory Standard Institute **(CLSI, 2009)** and Himedia **(HiMedia, 1998).**

Results

A total of 100 ice-cream were tested for the presence of fecal coliforms & *Klebsiella* spp. (ice cream with various flavors, ice cream with chocolate, ice cream with coconut & ice cream with fresh fruit). The results showed that 42 % of the samples were positive for fecal coliforms and 37% were positive for *Klebsiella* spp. **(Figure 1).**

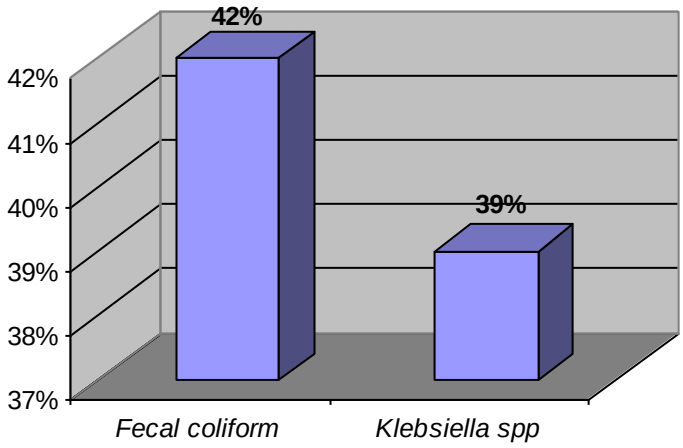

Figure (1): The percentage of contaminated ice-cream by fecal coliform & *Klebsiella* spp.

The highest incidence of fecal coliform was exhibited by ice cream samples containing fresh fruits (80%) while those containing coconut showed the lowest incidence (12%). A statistically significant difference was found between the different types of ice-cream samples (Table 1).

Table (1): Fecal coliform positive samples distributed by ice cream type (N= 25 each)

Ice cream type	Fecal coliform

Antibiogram of Klebsiella spp. isolated from ice cream samples.

		Positive	Negative
Various flavors	N	8	17
	%	32	68
Chocolate	N	11	14
	%	44	56
Coconut	N	3	22
	%	12	88
Various fruits	N	20	5
	%	80	20
Total	N	42	58
	%	42	58

Chi Square p = 0.001

Again, ice cream samples containing fresh fruits showed the highest incidence of *Klebsiella* isolation rate. In fact all samples that are positive for fecal coliform were positive for *Klebsiella* spp. A statistically significant difference was found between the different types of ice cream samples (Table 2).

Table (2): *Klebsiella* spp. positive samples distributed by ice cream type (N= 25 each)

Ice cream type		*Klebsiella* spp.	
		Positive	Negative
Various flavors	N	5	20
	%	20	80
Chocolate	N	11	14
	%	44	56
Coconut	N	3	22
	%	12	88
Various fruits	N	20	5
	%	80	20
Total	N	39	61
	%	39	61

Chi Square p = 0.001

None of the negative fecal coliform samples harbored *Klebsiella*, while 93% of the positive samples showed positive results for *Klebsiella*. (Table 3)

Table (3): *Klebsiella* spp. isolation from ice cream samples

contaminated with fecal coliform

Fecal coliform		*Klebsiella* spp.		Total
		Positive	Negative	
Positive	N	39	3	42
	%	93	7	100
Negative	N	0	58	58
	%	0	100	100
Total	N	39	61	100
	%	39	61	100

A total of 37 isolates of *Klebsiella* spp. from various ice cream samples were examined for their susceptibility to 8 antimicrobial agents used to treat infections caused by gram-negative bacteria. The results obtained are presented in **Table 4.** The highest resistance percentage was found against Doxycycline (59%) followed by minocycline (49%). Norfloxacin followed by Gentamicine showed the highest activity against the tested isolates (100% and 95% respectively).

Table (4): The antibiogram of the isolated *Klebsiella* spp. (N=37)

Antimicrobial	**Abbreviation**	**Potency**	**%**		
			Sensitive	**Intermediate**	**Resistant**
Gentamicin	GM	10 µg	95	0	5
Minocycline	Mi	30 µg	11	40	49
Doxycycline	Do	30 µg	24	17	59
Cefuroxime	CXM	30 µg	70	5	24
Cephalexin	CL	30 µg	68	11	22
Choramphenicol	C	30 µg	76	3	22
Norfloxacin	Nor	10 µg	100	0	0
Nalidixic acid	NA	30 µg	68	27	5

Discussion

The results of this study may be considered as a reflection of the microbiological quality of the locally sold ice cream. 42% of the tested samples were positive for the presence of fecal coliforms. This

is clearly a public health concern that should be taken seriously. An additional concern that should also be considered is the source of contamination by these fecal bacteria.

Although high percentage of samples are contaminated with fecal coliforms, higher rates were found in a similar study in Pakistan **(Ahmed *et al.*, 2009)** where the authors found 100% of the samples were contaminated with *E. coli*. On the contrary, only 20% of samples contained *E. coli* in a study done in Nigeria **(Ojokoh, 2006).** Others researchers found that 56.7% of commercial ice-cream was contaminated by Fecal coliforms **(Windrantz and Arias, 2000).**

Klebsiella spp. was isolated from 37% of the tested samples. The reasons for searching for this bacteria in ice cream samples were, earlier un-published reports showing high isolation rates and the increase in resistance to antibiotics from hospital *Klebsiella* isolates **(1 Elbayoumi, 2012). Vaishnovi et al, (2003)** found that *K. pneumoniae* was the second predominating organisms after *S. aureus* when they examined 141 ice-cream samples**.**

The epidemiology of foodborne disease is changing. New pathogens have emerged, and some have spread worldwide. Many pathogens including *Salmonella*, *E. coli O157:H7*, *Campylobacter*, and *Yersinia enterocolitica*, have reservoirs in healthy food animals, where they spread to an increasing variety of foods. These pathogens cause millions of cases of sporadic illness and chronic complications, as well as large and challenging outbreaks over many states and nations **(Tauxe, 1997).** *K. pneumonia* is a new emerging bacteria in food. **Sabota et al, (1998)** reported *K. pneumonia* as an enteroinvasive food-borne pathogen transmitted from a hamburger**.**

In summer ice-cream is very popular delicacy in Gaza strip and is consumed most commonly by children. Ice-cream frequently associated with tonsillitis and respiratory tract infection due to their improper handling and serving practices **(Barro *et al.*, 2006 and WHO, 2003).** *K. pneumonia* cause a series of human health problems as tonsillitis, urine infection and respiratory tract. Many of strains are antibiotic resistance where they increase the virulence of illness. The emergence of multiply-resistant *Klebsiella* spp. has greatly complicated the treatment of *Klebsiella pneumonia*, as evidenced by mortality rates which can exceed 50% **(Cryz *et al.*, 1986).**

Regarding ice-cream additives, the results showed that 80% of ice-cream samples with fruit are contaminated. This could be due to contaminated fruits used. Fruit can be contaminated with members of Enterobacteriaceae family at harvest. **Tambekar *et al.*, (2009)** indicated that environmental exposure is the first source of microorganisms**.**

The results showed that *Klebsiella* spp. susceptibility to Doxycycline & Minocycline was 24% & 11% respectively. Gentamicin and Norfloxacin were effective in inhibiting the growth of *Klebsiella* spp. These results indicate that community isolates of *Klebsiella* are much less resistant than hospital isolates **(Elbayoumi, 2012)**. **Mahami *et al.*, (2001)** found that the resistance of *Klebsiella* to Gentamicin, Chlcoramphenicol & Cefuroxime each accounted for 12 %**.**

Conclusions

A high percentage of the locally sold ice cream samples contained fecal coliform as well as *Klebsiella* suggesting that ice-cream may contribute to transmission of intestinal pathogens. Finding of potential pathogens in the tested ice-cream samples should be viewed with concerns by the concerned authorities, manufacturers as we as consumers. Continuous close monitoring of locally produced food should be implemented and raising public awareness of the possible dangers of consuming contaminated products should also be considered.

References

Ahmed K, Hussain A, Imran, Qazalbash MA, Hussain W. Microbiological quality of ice cream sold in Gilgit town. Pakistan Journal of Nutrition. 2009; 8 (9); 1397-1400.

Anil P, .S; Karla, .G and Abiodun, A. Evaluation of the bacteriological quality of ice cream sold in Trinidad. Journal of Food, Agriculture & Environment. 2012; 10(2): 39-45.

Ashik K, & Aitabh, T. Antimicrobial Resistance Patterns of *Klebsiella* spp. Isolated from Raw Milk of Doon Valley. Developmental Microbiology and Molecular Biology. 2010;1(1):21-35.

Barro N, Bello A, Savadogo A, Ouattara C, Iiboudo A and Traoré A. Hygienic status an assessment of dishwashing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou. African

Journal of Biotechnology. 2006; 5(11):1107-12.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement M100-S19. Wayne, PA, USA: CLSI; 2009.

Cryz S, Fürer E and Germanier R. Immunization against fatal experimental *Klebsiella pneumoniae* pneumonia. Infection and Immununity. 1986; 54(2):403-7.

Elbayoumi, Y. Epidemiology of Septicemia at Neonatal Intensive Care Units in Gaza City Hospitals. 2012; Islamic university-Gaza (unpublished master thesis).(

FDA. Bacteriological Analytical Manual. FDA. 1995;8.

HiMedia. The HiMedia Manual for Microbiology Laboratory Practice. HiMedia Laboratories Pvt Limited. A-406, Bhaveshwar Plaza, L B S Marg Mumbai 400086, India1998.

Javade A, Safarmashaei, S. Fecal Coliforms and Fecal Streptococci Contamination of Traditional Ice Cream in Tabriz. American-Eurasian J Agric & Environ Sci, 2011;11(6):812-4.

Kumar A. and Talwar A. Antimicrobial Resistance Patterns of *Klebsiella* spp. Isolated from Raw Milk of Doon Valley. International Journal of Developmental Microbiology Molecular Biology. 2010; 1(1) 21-35

Lachmayr K, Kerkhof L, Dirienzo A, Cavanaugh C, Ford T. Quantifying nonspecific TEM beta-lactamase (blaTEM) genes in a wastewater stream. Applied Environmental Microbiology. 2009 75(1):203-11.

Mahami T, .S; Yaro M & Adu-Gyamfi A. Prevalence of antibiotic resistant bacteria in milk sold. International Research Journal of Microbiology. 2011;2(4):126-32.

Mohammad M, .M & Habib, M. Microbiological Contamination of the Traditional Chocolate Ice Cream Sold in the Northwest Region of Iran. Global Veterinaria. 2011: 6(3): 269-71.

Ojokoh O. Microbiological Examination of Ice Cream Sold in Akure. Pakistan Journal of Nutrition. 2006;5(6):536-8.

Osamwonyi O, Obayagbona O, and Olisaka F.. Evaluation of the Bacteriological Quality of Ice Creams Sold in Some Locations Within Benin City. Continental J Food Science and Technology. 2011;5 (3):6-11.

Sabota J, Hoppes W, Ziegler J, DuPont H, Mathewson J, Rutecki G. A new variant of food poisoning: enteroinvasive *Klebsiella pneumoniae* and

Escherichia coli sepsis from a contaminated hamburger. American Journal of Gastroenterology. [Case Reports]. 1998; 93(1):118-9.

Talbot H, Yamamoto D, Smith M and Seidler, R. Antibiotic Resistance and Its Transfer among Clinical and Nonclinical *Klebsiella* Strains in Botanical Environments. Applied & Environmental Microbiology. 1980 97-104.

Tambekar D, Jaiswal V, Dhanorkar D, Gulhane P, and Dudhane M. Microbial Quality and safety of street vended fruit juices: A case study of Amravati city. Internet Journal of Food Safety. 2009;10:72-6.

Tauxe R. Emerging foodborne diseases: an evolving public health challenge. Emerging Infectious Diseases 1997; 3(4):425-34.

Vaishnavi C, Singh S, Singh K. Bacterial profile of dairy products sold in Chandigarh. Tropical Gastroenterology. 2002 23(2):72-5.

WHO. Food safety and food borne illness. Fact Sheet. 2003;237:7.

Windrantz, P. and Arias, M. L. Evaluation of the bacteriological quality of ice cream sold at San Jose, Costa Rica. Archivos Latinoamericanos de Nutrición. 2000. 50:301-303