Pak. J. Bot., 41(2): 935-943, 2009.

PREVALENCE AND ANTIBIOTIC RESISTANCE OF BACTERIA IN TWO ETHNIC MILK BASED PRODUCTS

KALSOOM FARZANA[1], SAEED AKHTAR[2*] AND F. JABEEN[3]

[1]*Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan*
[2]*University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan*
[3]*Quality Control Manager, Jeans Pharmaceuticals, Lahore, Pakistan.*

Abstract

Prevalence of food borne pathogens in milk products, *khoya* (a common ingredient in many traditional Indian sweets made by slowly evaporating milk under heat) and *burfi* (*khoya* cooked with sugar until it solidifies) and their sensitivity against different antibiotics was evaluated. *Coliform* indicated the lowest count ($7.5x10^3$ CFU/g) and the highest ($5.3x10^6$ CFU/g) in *burfi* whereas $6.5x10^3$ and $5.2x10^6$ CFU/g in *khoya* for 28 selected samples. Presence of *Staphylococcus aureus, Escherichia coli* and *Klebsiella* spp., was also confirmed in a large number in *khoya* and *burfi* samples. *S. aureus* represented the major part of bacterial flora in *burfi* and *khoya*. *Enterobacter* spp., and *E. coli* spp., constituted ~ 1.2%, in both *burfi* and *khoya*. The unidentified microflora comprised 12.56% and 8.41% in *burfi* and *khoya*, respectively. *E. coli* and *Enterobacter* spp., isolated from both *khoya* and *burfi* showed more susceptibility to Septran and Amikin. Ampiclox and Tetracycline exhibited higher degree of sensitivity against these isolates. However, *Klebsiella* spp., *Enterobacter* spp., and *E. coli* were found to be resistant to Urixin. Locally prepared milk products might be a potential source of bacterial contamination which poses a significant clinical threat to consumers through excessive use of various antibiotics against these micro-organisms.

Introduction

The origin of contamination by pathogenic bacteria varies with the type of product and the mode of production and processing. Contamination of milk and dairy products by pathogenic micro-organisms can be of endogenous origin, following excretion from the udder of an infected animal and /or exogenous origin, through direct contact with infected herds or through the environment (e.g., water, personnel). Treatment and processing of milk can inhibit or encourage the multiplication of micro-organisms (Brisabois *et al*., 1997). Food borne pathogens can survive and thrive in post-pasteurization processing environments, thus leading to recontamination of dairy products. These pathways pose a risk to the consumers from direct exposure to food borne pathogens present in unpasteurized dairy products as well as dairy products that become re-contaminated after pasteurization (Oliver *et al*., 2005).

Staphylococcus aureus by far is the most frequent pathogen associated with outbreaks (85.5% of the outbreaks), followed by *Salmonella* (10.1%) (De Buyser *et al.*, 2001). Cooked food products and raw milk were most commonly contaminated with food borne pathogens and many of them were resistant to different antibiotics. Milk products are often contaminated with enterotoxigenic strains of *S. aureus* (Chao *et al*., 2007). It is currently not possible to effectively and consistently exclude such multiantibiotic-resistant strains from the human food chain, which means that they continue to pose a significant clinical threat to consumers and concomitant economic threats to the food production and processing industry (Walsh *et al.*, 2005).

*Corresponding author E-mail: saeedbzu@yahoo.com

Presence of enterotoxigenic and antimicrobial resistant strains of *S. aureus* have become remarkably widespread in foods. This requires a better control of food contamination sources and distribution of antimicrobial-resistance organisms (Normanno *et al*., 2007).

Around 100 to 130 patients suffering from food poisoning and gastroenteritis were daily admitted to emergency wards of all major hospitals in Pakistan in 2007. A large number of children were also hospitalized for eating unhygienic food (Ali, 2007). Contamination of dairy foods with virulent pathogens render them to be a source of public health hazard. The possible contamination sources are either mastitis dairy cow or the milk itself (Carter, 1995). Growing concerns over food safety among the consumers call for the manufacturing and processing of foods under extremely hygienic conditions to avoid possible health challenges. Food safety conditions in Pakistan are not encouraging and milk products, specifically prepared by local manufactures, being unpasteurized, either exposed or improperly packed, are highly contaminated. The objective of the present study was to evaluate the level of prevalence of micoflora viz., *S. aureus, Enterobacter* spp., *E. coli* and *Klebsiella* spp., in frequently consumed dairy products and to asses their sensitivity against the most commonly used antibiotics.

Materials and Methods

Collection of samples: Thirty samples of *burfi* and *khoya* were collected in sterilized glass bottles from retail shops and were brought to the laboratory under low temperature for microbiological assay. The inocula were prepared by homogenizing 10 g of cooled and well-mixed samples in 100 ml chilled sterile normal saline solution containing 0.1 percent peptone.

Control strains: *E. coli* (ATCC 25922) and *S. aureus (*ATCC 25923) were used as control strains in this study.

Aerobic colony count (ACC): ACC was carried out by pour plate technique as reported previously (Case & Johnson, 1984). The homogenates were serially diluted in sterilized water, pour-plated in a thin layer of Nutrient Agar (Difco, BD Diagnostic Systems, Sparks, MD, USA) and were incubated at 37^{o}C for 24 h to determine CFU/g. The experiment was repeated twice and reported data represent mean values (CFU/g) of these measurements.

***Coliform* count:** *Klebsiella* spp., *E. coli* and *Enterobacter* spp., were enumerated in their selective media as *coliform* count. *Coliform* count was conducted by MPN technique, tubes containing gas in the inverted durham tubes were considered positive for the *coliform*s. To measure number of c*oliform*s present in the milk products (*khoya* and *burfi*), dilution was read from MPN table and results were computed by multiplying this number with the dilution factor (Cappuccino and Sherman, 1992).

Fecal *coliform* count: Fecal *coliform*s were obtained by MPN technique. 0.5 ml of *coliform* culture present in the tubes was incubated into 10 ml brilliant green bile broth tubes. The broth tubes were incubated at 44.5^{o}C for 48 hours and the results were recorded from MPN-table. Presences of fecal *coliform*s were confirmed by streaking

from positive brilliant green broth culture on eosin methylene blue agar (EMB) plates. Bacterial colonies developed were considered as fecal *coliform*s and were counted.

Identification, morphological and biochemical characterization of bacterial strains: The colonies isolated after purification were initially Gram stained and the isolates were biochemically characterized and identified up to species level by applying Baird parker agar, Manitol salt agar, Dnase test, Coagulase test, Oxidase catalase, Indole, methyl red, Voges-proskauer, simmons citrate, EMB as reported previously (Davidson & Henson, 1995; Holt, 1993; Pelczar *et al*., 1999)

Antibiotic sensitivity profile

Disc Diffusion Susceptibility Test: *Burfi* and *khoya* isolates; *Enterobacter* spp., *E. coli and Klebsiella* spp. were assessed for their sensitivity against different antibiotics viz., Urixin, Chloramphenicol, Ampicillin, Ampiclox, Nitrofurantoin, Tetracycline, Amikin, Amoxil, Augumentin and Septran as reported previously (Bauer *et al*., 1966).

The Disc Diffusion Susceptibility Test was used for each Gram-negative rod on Mueller-Hinton agar (CM337-OXOID) as growth medium. Medium was prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 15 min. These plates were stored at 2-8°C in sealed plastic bags for use within two weeks (Bauer *et al*., 1966). Tryptone soya broth (TSB) (CM129-OXOID) was dispensed in screw-capped test tubes and sterilized by autoclaving at 121°C for 15 min., for inoculum preparation. The test tubes were cooled and kept in an incubator for 24 h at 35°C to confirm sterility. Each isolated clinical strain was inoculated in the sterilized test tubes containing the medium and placed in an incubator overnight at 35°C. The presence of turbidity in broth cultures was adjusted according to 0.5 McFarland standard to obtain standardized suspension by adding sterile saline against a white background according to the methods outlined by National Committee for Clinical Laboratory Standards, NCCLS (Anon., 1993). Inoculum was spread evenly over the entire surface of the Mueller-Hinton agar plates by swabbing back and forth across the agar in three directions to give a uniform inoculum. Then the discs of given potency were applied on the inoculated plates with the help of forceps and incubated at 35ºC for 18 h in an inverted position. The results were recorded as zone of inhibition from the standard table.

Results and Discussion

The frequency distribution of *burfi* and *khoya* samples in relation to various microbial counts is given in Tables 1 and 2 respectively. The ACC, *coliform*s, fecal *coliform*s and *S. aureus* count examined by Standard Plate Count (SPC), indicated an excessive contamination in both types of dairy products. *Khoya* in general revealed more bacterial contamination as compared to *burfi* samples. The ACC was found to rang from 10^6 to 10^{11} CFU/g for both *khoya* and *burfi* and the highest number of samples i.e., 10 of 28, manifested a bacterial count of 10^7-10^8 CFU /g. *Coliform*s were found almost at the similar extent ranging from 10^3 to 10^7 CFU/g for both *khoya* and *burfi* samples however, a little variability in the number of samples of *khoya* and *burfi*, exhibiting extent of *coliform*s and fecal *coliform*s was observed (Tables 1 and 2).

Table 1. Microbiological profile (CFU/g) of milk products (*khoya*).

Test	No. of samples	Bacterial range (CFU/g)							
		10^3-10^4	10^4-10^5	10^5-10^6	10^6-10^7	10^7-10^8	10^8-10^9	10^9-10^{10}	10^{10}-10^{11}
ACC[a]	28	-	-	-	4	10	6	7	1
MPN-C[b]	26	4	8	9	5	-	-	-	-
MPN-FC[c]	26	4	8	9	5	-	-	-	-
S. aureus	24	-	-	12	8	4	-	-	-

ACC[a] = Aerobic colony count, C[b] = Coliform, FC[c] = Fecal Coliform.
The values are the mean of two experiments

Table 2. Microbiological profile (CFU/g) of milk products (*burfi*).

Test	No. of samples	Bacterial range (CFU/g)							
		10^3-10^4	10^4-10^5	10^5-10^6	10^6-10^7	10^7-10^8	10^8-10^9	10^9-10^{10}	10^{10}-10^{11}
ACC[a]	28	-	-	-	5	10	5	7	1
MPN-C[b]	26	3	8	10	5	-	-	-	-
MPN-FC[c]	26	3	8	10	5	-	-	-	-
S. aureus	24	-	-	10	8	6	-	-	-

ACC[a] = Aerobic colony count, C[b] = Coliform, FC[c] = Fecal Coliform.
The values are the mean of two experiments

S. aureus count in both *khoya* and *burfi* were found to be 10^5 to 10^8 CFU/g (Table 1 and 2) with 50% (12 of 24) samples of *khoya* indicating 10^5-10^6 CFU/g as compared to 42% (10 of 24) samples of *burfi* samples representing the similar extent of bacterial growth. With a little variability, level of contamination detected in both *khoya* and *burfi* for this pathogenic microorganism revealed a consistent and identical pattern (Tables 1 and 2). Microbiological assay of *khoya* and *burfi* clearly manifested a higher count of ACC (3 to 4 logs) as compared to other bacterial isolates i.e., *coliform*s and *S. aureus* (Tables 3 and 4). The degree of prevalence of the micro flora in these dairy products was found to be above acceptable limits and *coliform*s were found in 93% of the total samples examined. The highest CFU/g were $5.3x10^{6,}$ $5.2x10^6$ and the lowest were $7.5x10^3$ and $6.5x10^3$ in 86 % of the total samples tested for *coliform* and fecal *coliform* for *burfi* and *khoya* respectively (Tables 3 and 4). The average *coliform* load determined was $4.15x10^4$, $3.51x10^5$ in *burfi* and *khoya*, respectively (Tables 3 and 4).

Microbiological analysis of *khoya* and *burfi* samples revealed that *S. aureus* was the major part of bacterial flora in *burfi* (30.5%) and *khoya* (33.56%) (Fig. 1). The level of contamination with *coliform*s was found to be 28.49% and 30.95% in *burfi* and *khoya* respectively. The overall *coliform*s were 16.16% and 16.54%; and for *Enterobacter* spp., *E. coli* and, *Klebsiella* spp., the level of contamination with *Klebsiella* spp., was the highest i.e., ~ 11.17% and 7.6% in *burfi* and *khoya* samples respectively (Fig. 1). Prevalence of *Enterobacter* spp., and *E. coli* did not exceed 1.2%, in both *burfi* and *khoya*. The results of the present study clearly indicated that *S. aureus*, *coliforms* and fecal *coliforms* were the major contaminants of milk products (*burfi* and *khoya*).

This study also pointed out major count of *coliform*s (*Enterobacter* spp., *Klebsiella* spp., *E. coli*), fecal *coliform*s and *S. aureus. Coliform*s contamination was shown to be relatively less in both types of tested products as compared to fecal *coliform*s, *Staphylococcal* contamination was normally attributed to food handlers, since nasopharyngeal cavity of human beings is the reservoir of microflora from which these bacteria get localized on the skin, especially on hands (Kaplan 2005; Masud *et al*., 1988; Patel, 1985; Stone *et al*., 2001).

Table 3. Maximum, minimum and average values of various bacterial count (*burfi*) (CFU/g).

Nature of test	Samples (No.)	Max	Min	Avg
ACC[a]	28	2.0 x1 0^{11}	1.5 x 10^6	8.50 x 10^8
MPN-C[b]	26	5.3 x 10^6	7.5 x 10^3	4.15 x 10^4
MPN-FC[C]	26	5.3 x 10^6	7.5 x 10^3	4.15 x 10^4
S. aureus	24	7.0 x 10^7	2.2 x 10^4	1.45 x 10^5

ACC[a] = Aerobic colony count, C[b] = Coliform, FC[c] = Fecal Coliform.
The values are the mean of two experiments

Table 4. Maximum, minimum and average values of various bacterial count (*khoya*) (CFU/g).

Nature of test	Samples (No.)	Max	Min	Avg
ACC[a]	28	2.5x10^{11}	1.6x10^6	9.25x10^8
MPN-C[b]	26	5.2x10^6	6.5x10^3	3.51x10^5
MPN-FC[C]	26	5.2x10^6	6.5x10^3	3.51x10^5
S. aureus	24	7.2x10^7	2.4x10^4	1.56x10^5

ACC[a] = Aerobic colony count, C[b] = Coliform, FC[c] = Fecal Coliform.
The values are the mean of two experiments

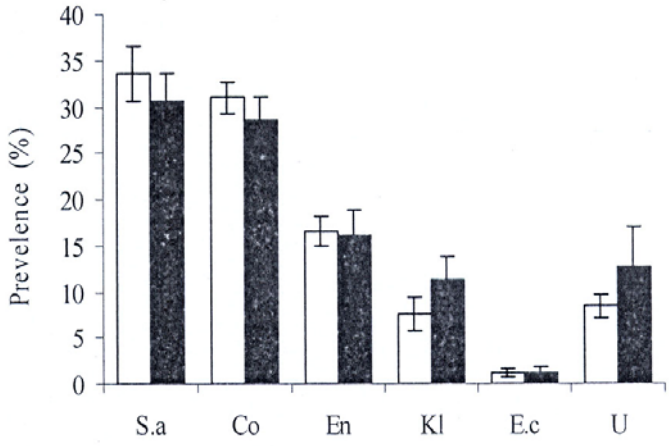

Fig. 1. Prevalence of bacteria in locally prepared dairy products *Khoya* (White bars) and *Burfi* (Black bars). Bacterial isolates *Staphylococcus aureus* (S.a), *coliform*s (Co), *Enterobacters* (En), *Escherichia coli* (E.c) and unidentified flora were determined as described in Materials and Methods, Data points shown are the mean of at least two repetitions, Bars represent ± SD.

The higher *Staphylococcal* count, as 1.7 x 10^6 for *khoya* and 1.9 x 10^3 for *burfi* was observed and the comparable range was present in milk products 10^5 to 10^8. (Gordon & Gibbon, 1999). Our study demonstrated 30.5% and 33.18% *S. aureus* in *khoya* and *burfi* and for *Enterobacter* spp. isolates, 56.92% and 55.8% were present in *burfi* and *khoya*, respectively.

Dust and skin of human beings were also known to contaminate food items with pathogens like *S. aureus* and *Klebsiella* spp., Manufacturers contaminate *khoya* and *burfi* during the process of sugar mixing and cutting of sweets into small pieces (Hobb's & Gilbert, 1978). *S. aureus* isolated from the milk products produce enterotoxins strains. *Coliform*s and fecal *coliform*s may enter the food through contamination with dust either directly or indirectly through utensils and equipments used in preparation of these milk

products. The food handlers and dust, constitute the major sources of microbial contamination of sweets. The food handlers also significantly contribute in contamination of *khoya* than in *burfi* (Masud *et al*., 1988). However, the presence of *S. aureus* and *Klebsiella* spp., will render these products unfit for human consumption, since sufficient number of these organisms will cause infection and intoxication. Multiplication and production of *S. aureus* would however, depend upon environmental factor like time, temperature, relative humidity and duration of storage and food factors, potential water activity (aw), moisture contents, nutrients present, additives used and associated microflora like *S. aureus* and *coliform*s and fecal *coliform*s (Garg & Mandokhot, 1984).

According to United States Environmental Protection Agency, (Anon., 2003), the presence of *E. coli* in the intestine and feces of warm-blooded animals is an indicator of fecal pollution. The grazing of cattle and land application of animal wastes may lead to the occurrence of enteric pathogens near the surface and ground waters. This potential contamination due to animal husbandry operations can be a serious threat to public health.

The present work reports high count of *S. aureus* in all samples of *khoya* and *burf* and that can be due to careless handling at various stages of processing. The presence of *coliform*s and fecal *coliform*s like *Enterobacter* spp., *Klebsiella* spp., and *E. coli* shows the unhygienic nature of these sweets prepared from milk (Hobb's & Gilbert, 1978).

Antibiotic sensitivity of gram-negative bacteria: Antibiotics resistance pattern of *E. coli*, *Enterobacter* spp., and *Klebsiella* spp., isolated from *burfi* and *khoya* samples has been shown in Figs. 2 and 3. *E. coli* isolated from *burfi* and *khoya* exhibited 100% resistance against Urixin, Chloramphenicol and Ampicillin. The level of resistance of *E. coli* against Ampiclox, Nitrofurantoin and Tetracycline declined almost with the same magnitude in both types of dairy products. The susceptibility of *E. coli* isolated from *burfi* and *khoya* when tested against Amikin, Amoxil, Septran and Augmentin was 100% (Figs. 2 and 3). *Enterobacter* spp., isolated from *khoya* demonstrated a greater degree of resistance against different antibiotics, particulary Ampicillin and Nitrofurantoin as compared to *Enterobacter* spp., isolated from *burfi* (Figs. 2 and 3). However, Amikin, Septran and Augmentin were still found to be as effective against *Enterobacter* spp., as *E. coli* from both *burfi* and *khoya* samples. One noticeable exception was observed with Amoxil, manifesting the similar efficacy i.e., ~ 29% resistance of *Enterobacter* spp., from both type of dairy products (Figs. 2 and 3).

Urixin, Chloramphenicol, Ampicillin, Ampiclox and Nitrofurantoin, remained ineffective against *Klebsiella* spp. In *khoya* and *burfi* isolates showing a greater variability (100, 64, 61, 36 and 39% resistance level of *Klebsiella* spp., respectively) in their effectiveness. Amikin and Amoxil demonstrated a simlar extent of susceptibility i.e., 14% resistance against *burfi* isolates while *khoya* isolates of *Klebsiella* spp., manifested relatively higher resistance (25 and 29% respectively) for these antibiotics. Similarly, *Klebsiella* spp., only showed some low resistance against Septran, among all the tested antibiotics (4%). Tetracycline and Augumentin indicated 100% efficacy against all *burfi* and *khoya* isolates examined in this study (Figs. 2 and 3). The activity of Septran, Tetracycline and Augmentin remained at the maximum for *Klebsiella* spp., *Enterobacter* spp., and *E. coli* isolates and the maximum resistance was observed towards Urixin against the *Klebsiella* spp., *Enterobacter* spp., and *E. coli* isolates of *khoya* and *burfi* (Figs. 2 and 3).

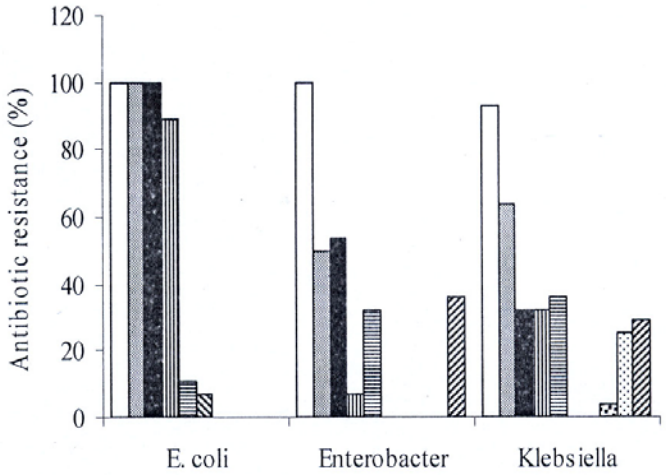

Fig. 2. Resistance of Gram- negative bacteria (%) against different antibiotics. Isolates from *burfi* were tested against Urixin (white bars), Chloramphenicol (grey bars), Ampicillin (black bars), Ampiclox (vertical lines), Nitrofurantoin (horizontal lines), Tetracycline (downward diagonal), Amikin (dotted lines), Amoxil (upward diagonal). Augumentin and Septran indicated 0 % resistance in all isolates and do not appear in the figure.

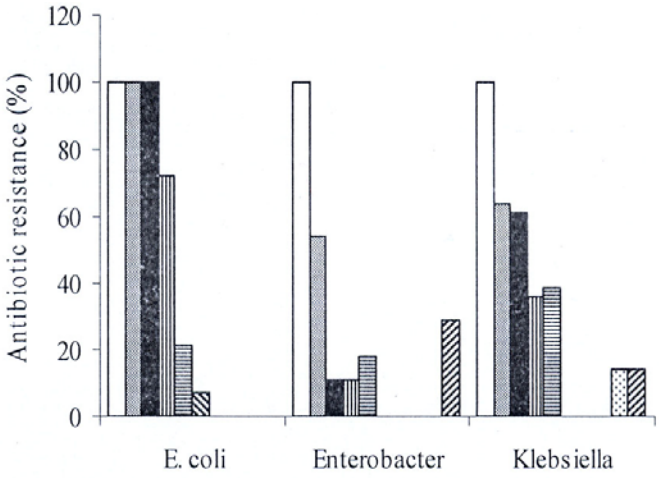

Fig. 3. Resistance of Gram- negative bacteria (%) against different antibiotics. Isolates from khoya were tested against Urixin (white bars), Chloramphenicol (grey bars), Ampicillin (black bars), Ampiclox (vertical lines), Nitrofurantoin (horizontal lines), Tetracycline (downward diagonal), Septran (large confetti) Amikin (dotted lines), Amoxil (upward diagonal). Augumentin indicated 0% resistance in all isolates and does not appear in the figure.

Dupont *et al*., (1978) confirmed the efficacy pattern of these antibiotics against *Enterobacter* spp., *E. coli* and *Klebsiella* spp. The researchers investigated different antibiotics for their resistances and found Amikin to be active against *Enterobacter* spp., *E. coli* and *Klebsiella* spp., which is consistent with the present results.

In the present study, *E. coli* was quite resistant to Ampiclox, but *Klebsiella* spp., and *Enterobacter* spp., were sensitive to Ampiclox. Blumberg & Strominger, 1974 investigated the mechanism of antibiotics efficacy substantiating Ampiclox to be effective against *Enterobacter* spp., *E. coli* and *Klebsiella* spp., by inhibiting the synthesis of cell wall mucopeptide. The resistance of this antibiotic against Gram-negative bacteria was caused by mutation of acquisition of R-plasmids (Chamberlain, 1976). It was further demonstrated that Amoxil's was highly effective against *Enterobacter* spp., *E. coli* and *Klebsiella* spp. (Stone *et al.,* 2001). Changes in micro-organisms lead to the constant evolution of new pathogens, development of antibiotic resistance and changes in virulence of known pathogens. In many countries, as people increasingly consume food prepared outside the home, growing numbers are potentially exposed to the risks of poor hygiene in commercial food service settings (Anon., 2007). Current evidence exists to suggest that not only are such antibiotic resistant strains more difficult to control in terms of human infection, they may also be more resistant to heat processes (Davidson & Henson, 1995). In our study, *Enterobacter* spp., and *Klebsiella* spp., were sensitive but resistance was also reported, when isolated from *burfi* and *khoya*. Roupas & Pitton (1974) studied the resistant strains of *Enterobacter* spp., *E. coli* and *Klebsiella* spp., by forming β-lactamase production. Chamberlain, (1976) suggested that this resistance might be due to the induction, mutation or by acquisition of R-plasmids.

It was noticed that *E. coli* and *Klebsiella* spp., were resistant to Chloramphenicol but *Enterobacter* spp., was less resistant to this antibiotic. Moreover, ribosome located bacterial resistance to Chloramphenicol is uncommon and resistance of Gram-negative bacteria is usually acquired by means of R-plasmids (Chamberlain, 1976). In our study, *E. coli, Enterobacter* spp., and *Klebsiella* spp. were found to be resistant to Urixin either isolated from *burfi* or *khoya* samples.

The current results indicated that locally prepared milk products might be a potential hazardous sources of pathogenic *S. aureus*, *Klebsiella* spp., *E. coli* and *Enterobacter* spp., Strict control measures must be applied to minimize and eliminate the contamination possibilities through milk and its products leading to minimized use of various antibiotics which are excessively used and becoming ineffective against such bacterial strains.

References

Ali, M. 2007. Food Poisoning cases on the rise. *Daily Times*, August 11, 2007, Pakistan.

Anonymous. 1993. *Methods for determining bactericidal activity of antimicrobial agents*. National Committee for Clinical Laboratory Standards, Tentative guidelines. Villanova, PA.

Anonymous. 2003. Concentrated animal feeding operations. *Federal Register*, 68: 71-76.

Anonymous. 2007. *Foodborne Disease Outbreaks*. Guidelines for Investigation and Control, Geneva, Switzerland.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turk. 1966. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 45:493-496.

Blumberg, P.M. and J.L. Strominger. 1974. Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. *Bacteriology Reviews*, 38: 291-335.

Brisabois, A., V. Lafarge, A. Brouillaud, M.L. de Buyser, C. Collette, B. Garin-Bastuji and M.F. Thorel. 1997. Pathogenic organisms in milk and milk products: the situation in France and in Europe. *Reviews Science. & Technology*, 16: 452-71.

Cappuccino, G.J. and N. Sherman. 1992. *Microbiology, A Laboratory Manual*. The Benjamin/Cummings Publishing Company, Inc., Redwood City, CA.

Carter, G.R. 1995. *Veterinary Microbiology*. Academic Press, Philadelphia.

Case, C.L. and T.R. Johnson. 1984. *Laboratory experiments in microbiology*. The Benjamin/ Cummings Publishing Company, Inc. CA.

Chamberlain, R.E. 1976. Chemotherapeutic properties of prominent nitrofurans. *Journal of Antimicrobial. Chemotherapy*, 2: 325-36.

Chao, G., X. Zhou, X. Jiao, X. Qian and L. Xu. 2007. Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China. *Foodborne Pathogens and Disease*, 4: 277-84.

Davidson, P.M. and T.M. Henson. 1995. Comparison of D55C - values of antibiotic resistant and antibiotic sensitive strains of *Salmonella*. *Journal of Food Protection*, 58: 40.

De Buyser, M.L., B. Dufour, M. Maire and V. Lafarge. 2001. Implication of milk and milk products in food-borne diseases in France and in different industrialized countries. *International Journal of Food Microbiology*, 67: 1-17.

Dupont, H.L., H. West, D.G. Evans, J. Olarte and D.J. Evans, Jr. 1978. Antimicrobial susceptibility of enterotoxigenic *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, 4: 100-2.

Garg, S.R. and V.U. Mandokhot. 1984. Studies on microbial and chemical profile of some Indian sweet meats and their significance. *Indian Journal of Dairy Science*, 37: 326-333.

Gordon, D.M. and F.F. Gibbon. 1999. The distribution of enteric bacteria from Australian mammals: host and geographical effects. *Microbiology*, 145: 2663-71.

Hobb's, B.C. and R.J. Gilbert. 1978.*Food Poisoning and Food Hygiene*. Edward Arnold.

Holt, J.G. 1993. *Bergey's Manual of Determinative Bacteriology*. Williams and Wilkins, Baltimore MD.

Kaplan, S.L. 2005. Implications of methicillin-resistant *Staphylococcus aureus* as a community-acquired pathogen in pediatric patients. *Infectious. Disease. Clinics of North America*, 19: 747-57.

Masud, T., A. Bari and A.A. Shah. 1988. Screening of milk and milk products for staphylococcal counts with special reference to public health. *Journal of Pakistan. Medical Association*, 38: 286-8.

Normanno, G., G. La Salandra, A. Dambrosio, N.C. Quaglia, M. Corrente, A. Parisi, G. Santagada, A. Firinu, E. Crisetti and G.V. Celano. 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology*, 115: 290-6.

Oliver, S.P., B.M. Jayarao and R.A. Almeida. 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathogens and disease*, 2: 115-29.

Patel, G.S. 1985. Bacteriological quality of pedha and burfi with special reference to certain bacteria of public health significance. *Journal of Food Science & Technology*, 22: 133-136.

Pelczar, M.J., E.C.S. Chan and N.R. Krieg. 1999. Host-Parasite Interaction; Nonspecific Host Resistance, In: *Microbiology concepts and applications*. McGraw-Hill., Inc. New York.

Roupas, A. and J.S. Pitton. 1974. R factor-mediated and chromosomal resistance to ampicillin in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, 5: 186-91.

Stone, S.P., L. Teare and B.D. Cookson. 2001. The evidence for hand-hygiene. *Lancet*, 357: 479-480.

Walsh, C., G. Duffy, J.J. Sheridan, S. Fanning, I.S. Blair and D.A. Mcdowell. 2005. Thermal resistance of antibiotic-resistant and antibiotic-sensitive *Salmonella* spp., on chicken meat. *Journal of Food Safety*, 25: 288-302.

(Received for publication 3 September 2008)