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ANTIBIOTIC RESISTANCE PATTERN AGAINST VARIOUS ISOLATES OF *STAPHYLOCOCCUS AUREUS* FROM MILK PRODUCTS KHOYA AND BURFI

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Abstract: A total of twenty eight samples of each khoya and burfi were taken from different shops in Multan. All the samples were found to be contaminated with *Staphylococcus aureus*. When these *S. aureus* isolates from burfi were subjected to various antibiotic, lincomycin, tetracycline, cephradine and co-trimaxazole sensitivity tests, were found to be 100% effective against *S. aureus*. The decreased order of susceptibility of other antibiotics (amoxil, amikin, augmentin, ampiclox, erythromycin, ampicillin and colistin) was 96.43%, 96.43%, 92.86%, 82.14%, 89.29%, 28.57% and 7.14%, respectively for *S. aureus* isolates from burfi. The increased order of susceptibility of *S. aureus* against various antibiotics collected from khoya samples were colistin (14.29%), ampicillin (17.86%), ampiclox (71.43%), tetracycline (75%), .erythromycin and lincomycin each (89.29%), amikin and co-trimaxazole each (92.86%), cephradine and augmentin each (96.43%). It can be concluded that hygiene of milk products is poor and resistant strains might have contaminated these products during the process of transportation or preparation.

Keywords: Antibiotic resistance, isolates, milk products (burfi and khoya) and *Staphylococcus aureus*.

INTRODUCTION

The milk products (Khoya and Burfi) are made of sugar by continuous boiling of milk and sugar. These sweets are widely used and sold under various unhygienic conditions in Pakistan. Food-poisoning outbreak due to burfi and pera (khoya) contaminated with *Staphylococcus aureus* was reported [Mondokhot and Chandiramani 1983]. Raw milk may also be a cause of food poisoning due to the presence of pathogenic organisms such as *Staphylococci, Salmonellae*, *Campylobacter*, etc. A variety of diseases may be potentially transmitted through milk. The source of pathogenic agents occurring in milk may be either a cow, or a human, and it may be transmitted by both [Seguin *et al.* 1999, Khan *et al.* 2000].

The emergence of antimicrobial-resistant bacterial pathogens has become a major public health concern. The use of antimicrobials in any venue, including disease treatment and growth promotion in domestic livestock, can potentially lead to widespread dissemination of antimicrobial-resistant bacteria [Tollefson *et al*. 1997, Gomez-Lus 1998, Witte 1998].

The resistance to antimicrobial agents in bacteria is mediated by several mechanisms, including (i) changes in bacterial cell-wall permeability, (ii) energy-dependent removal of antimicrobials via membrane-bound efflux pumps, (iii) modification of the site of drug action, and (iv) destruction or

inactivation of antimicrobials [Barbosa and Levy 2000, Schwarz and Chaslus-Dancla 2001]. The antibiotic resistance pattern of various antibiotics (methicillin, ampicillin, tetracycline, ampiclox, erythromycin, lincomycin, co-trimaxazole, penicillin, augmentin and orbenin) was reported and suggested that most of the isolates of bacteria were resistant to these antibiotics. The however erythromycin was found to be the most effective against these milk isolates [Farzana *et al*. 2004].
Therefore, the objective of this study was to determine the antimicrobial susceptibility of *S. aureus* strains isolated from retail milk products (sweets prepared from milk) in Multan, Pakistan.

MATERIALS AND METHODS

The study was conducted at Microbiology Laboratory, Department of Pharmacy, Bahauddin Zakariya University Multan. Twenty eight samples were collected from different areas in Multan during January 1994 to December 1994 for each of these two milk product khoya and burfi.

COLLECTION OF SAMPLES

These samples were aseptically collected in glass sterilized sample bottles and transported to the laboratory and processed within an hour of their collection.

DETERMINATION OF STAPHYLOCOCCAL COUNT

Staphylococcal count of each sample was carried out according to the procedures prescribed by FAO manual for microbiological food analysis [FAO 1979]. A ten fold serial dilutions of homogenized samples were made in Ringer's solution and the diluted samples were poured in sterilized Petri-dishes, of Baird-Parker agar (Oxide). This medium is used for isolation and identification of *Staphylococci* in these food stuffs. The media was prepared by dissolving 5.8 g per 100ml distilled water, autoclaved, cooled to 50 ºC and then mixed with 0.105 gm per 100ml tellurite emulsion (0.1 gm per litre Magnesium Chloride and 0.05% between 80) to enhance the cellular activities. Then this media was transferred to Petri-plates. When BP agar sets down, then inoculated the Petri-plates with 0.01 ml of appropriate dilution, and incubated at 37ºC for 24 hours (if the colonies do not appear then incubate up to 48 hours).
The numbers of colonies appeared on the plates were multiplied by the dilution factor to obtain the number of *Staphylococcus aureus* in these milk products (Khoya and Burfi).

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

Morphological and Biochemical Characterization of Bacterial strains were tested in Microbiology Laboratory. The presence of colonies were confirmed by the following tests.

Haemolysis	[Pelczar *et al*. 1999]
Coagulase	[Pelczar *et al*. 1999]
DNase	[Collins *et al*. 1995]
Catalase	[Pelczar *et al*. 1999)]

DISC DIFFUSION (BAUER-KIRBY) SUSCEPTIBILITY TEST

The disc diffusion test was done for each isolate on Mueller-Hinton agar (CM337-OXOID), 25 ml of medium was poured into 90 mm diameter sterile Petri-dishes to a depth of 4 mm on a level surface to make the depth of the medium uniform and left at 37^{o}C temperature overnight to check sterility [Bauer *et al*. 1966].

For inoculum preparation 5 ml tryptic soya broth (CM129-OXOID) was dispensed in screw capped tubes and sterilized by autoclaving at 121°C for 15 minutes. The tubes were cooled and kept in an incubator for 24 hours at 35°C to check sterility. The each isolate was inoculated in the sterilized 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 standards by the addition of sterilized plain broth [NCCLS 1993].

PREPARATION OF INOCULUM FOR DISC DIFFUSION TEST

Standardized bacterial suspensions were saturated with a sterile Dacron tip swab and excess culture was removed by turning the swab against the side of the tube. 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 to the entire surface. These plates were allowed to dry before applying discs, and after 15 minutes discs of given potency were applied on the inoculated plates with the help of forceps. These plates were then placed in an incubator at 35°C for 18 hours in an inverted position. After 18 hours of incubation, plates were examined and the zone of inhibition was measured.

RESULTS

In a total of thirty milk product samples, *S. aureus* were biochemically identified and 28 isolates were collected each in burfi and khoya. These twenty eight isolates each in burfi and khoya were subjected to antibiotic sensitivity tests (Tables 1 and 2). As all samples were found to contain large number of *S. aureus*.

ANTIMICROBIAL ACTIVITY OF GRAM-POSITIVE ORGANISMS

The antimicrobial activity was performed on Mueller-Hinton agar (CM337-OXOID) both for burfi and khoya. In Tables 1 and 2, the antibiotics sensitivity showed that *S. aureus* isolates were most susceptible to lincomycin, tetracycline, co-trimaxazole, cephradine, and augmentin in both milk products. In case of burfi lincomycin, tetracycline, cephradine

and co-trimaxazole were 100% sensitive to *S. aureus*, but augmentin and amikin, were slightly less sensitive. Whereas the isolates from burfi were moderately sensitive to ampicillin (53.57%), colistin (46.43%), ampiclox (17.86%), augmentin and erythromycin (7.14%), amoxil and amikin (3.57%). Whereas activity of erythromycin, ampiclox and colistin were in decending order against the isolates of *S. aureus* collected from burfi (Fig. 1).

Table 1: Antibiotic sensitivity of *staphylococcus aureus* from milk product *(*burfi).

Antibiotic used	Conc. of drugs in discs	Resistant	Moderately susceptible	Susceptible
Ampicillin (AM)	10 µg	5 (17.86%)	15 (53.57%)	8 (28.57%)
Lincomycin (MY)	15 ug	0	0	28 (100%)
Tetracycline (TE)	30 ug	0	0	28 (100%)
Ampiclox (AX)	30ug (Ampicillin 25 ug) (Cloxacillin 5 ug)	0	5 (17.86%)	23 (82.14%)
Amoxil (AML)	20 ug. Amoxicillin	0	1 (3.57%)	27 (96.43%)
Cephradine (V)	30 ug	0	0	28 (100%)
Co-trimaxazole (SXT)	25 ug	0	0	28 (100%)
Augmentin (AMC)	30 ug (Amoxycillin 20 ug) (Clavulanic acid 10 ug)	0	2 (7.14%)	26 (92.86%)
Amikin (AK)	30 ug	0	1 (3.57%)	27 (96.4%)
Colistin (CI)	10 ug	13 (46.43 %)	13 (46.43 %)	2 (7.14%)
Erythromycin (ER)	15 ug	1 (3.57%)	2 (7.14%)	25 (89.29%)

Table 2: Antibiotic sensitivity of *staphylococcus aureus* from milk product (khoya)

Antibiotic used	Percentage susceptibility of twenty eight isolates			
	Conc. of drugs in discs	Resistant	Moderately susceptible	Susceptible
Ampicillin (AM)	10 µg	10 (35.71%)	13 (46.43%)	5 (17.86%)
Lincomycin (MY)	15 ug	0	3 (10.71%)	25 (89.29%)
Tetracycline (TE)	30 ug	0	7 (25%)	21 (75%)
Ampiclox (AX)	30ug (Ampicillin 25 ug) (Cloxacillin 5 ug)	3 (10.71%)	5 (17.86%)	20 (71.43%)
Amoxil (AML)	20 ug. Amoxicillin	0	3 (10.71%)	25 (89.29%)
Cephradine (V)	30 ug	0	1 (3.57%)	27 (96.4%)
Co-trimaxazole (SXT)	25 ug	0	2 (7.14%)	26 (92.86%)
Augmentin (AMC)	30 ug (Amoxycillin 20 ug) (Clavulanic acid 10 ug)	0	1 (3.57%)	27 (96.4%)
Amikin (AK)	30 ug	0	2 (7.14%)	26 (92.86%)
Colistin (CI)	10 ug	15 (53.57%)	9 (32.14%)	4 (14.29%)
Erythromycin (ER)	15 ug	1 (3.57%)	2 (7.14%)	25 (89.29%)

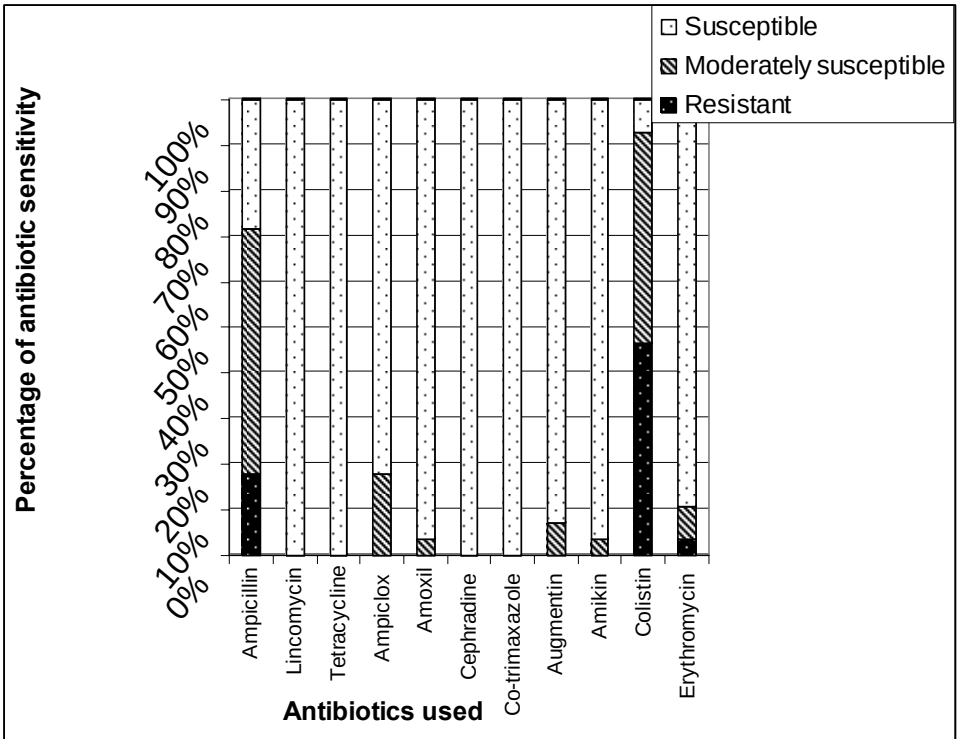

Fig. 1. Antibiotic sensitivity of *S. aureus* from milk product (Burfi)

In case of isolates from khoya amikin, co-trimaxazole, lincomycin, erythromycin, amoxil, tetracycline and ampiclox were in decending order of their effectiveness. The *S. aureus* isolates collected from khoya were found moderately sensitive to ampicillin (46.43%), colistin (32.14%), tetracycline (25%), ampiclox (17.86%), lincomycin and amoxil (10.71%), co-trimaxazole, amikin and erythromycin (7.14%), amoxil and augmentin (3.57%) given in Fig. 2.

DISCUSSION

The present study pointed out that milk products burfi and khoya taken from shops were contaminated with *S. aureus* and this contamination may be during processing and preparation of these sweets. These results show that the milk products may be processed in unhygienic conditions dusty environment which carry large number of bacteria. Moreover, contamination may be during selling of sweets to consumers at high temperature, also cause proliferation of bacteria.

The antibiotics were studied on the basis of their sensitivities against Gram-positive cocci (*S. aureus*) when cultured on Mueller-Hinton agar

(CM337-OXOID). The zones of inhibition of various antibiotics used were graded as sensitive, moderately sensitive and resistant.

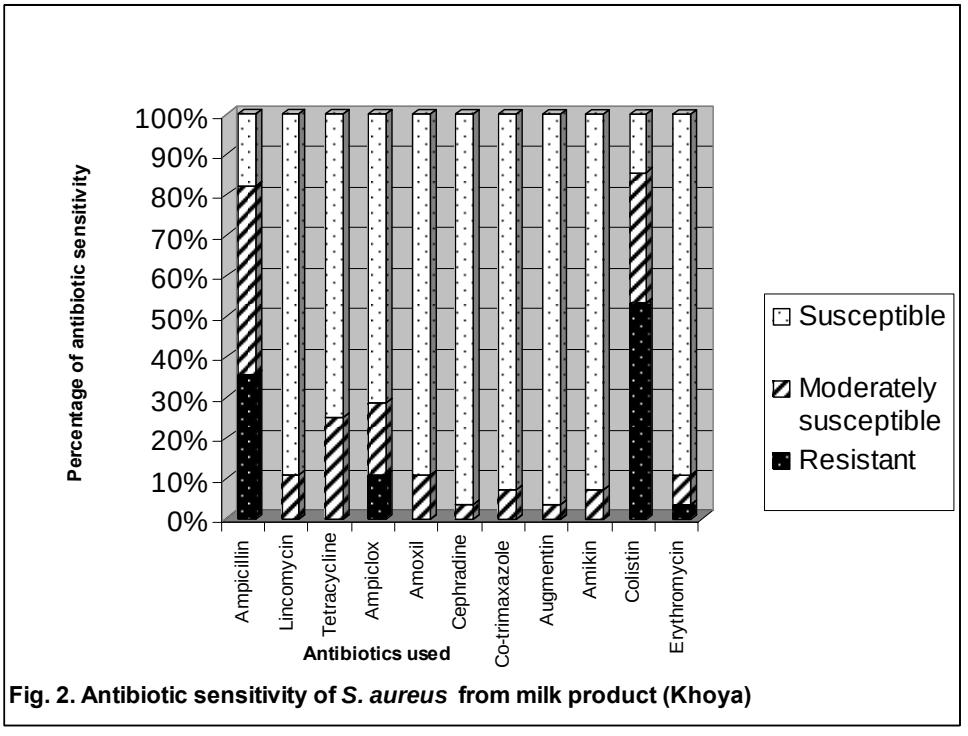

Fig. 2. Antibiotic sensitivity of *S. aureus* from milk product (Khoya)

In the study the *S. aureus* was found to be very sensitive to lincomycin, tetracycline, cephradine and co-trimaxazole, in tables 1 and 2 in burfi and khoya respectively. Whereas, in case of khoya activity of cephradine and augmentin was 96%, co-trimaxazole and amikin is 92%. The results of present study were similar to various antibiotics; as lincomycin, tetracycline, cephradine and co-trimaxazole found to be effective against *S. aureus* as represented by [Seligman 1973, Fong *et al.* 1976, Wiggins *et al.* 1978, Thomas 1988]. However, in present work some strains were slightly resisitant as for khoya [Martin *et al*. 1972].

Amoxil was effective against *S. aureus* 96% and 89% in burfi and khoya respectively. These results were comparable with the result of Blumberg [1974], who suggested that bacterial growth is retarded by inhibition of the formation of cell wall of bacteria. Moreover, peptidoglycan also inhibits the formation of certain enzymes like glucose-6-phosphate dehydrogenase and beta-lactamase which take part in synthesis of bacterial cell wall.

Ampiclox is sufficiently effective against more than 70% isolates of *S. aurues* both, collected from burfi or khoya. A few organisms were found to be moderately sensitive against Ampiclox. Acred and Sutherland [1967] noticed similar results that ampiclox was effective against *Staphylococci.* These results revealed that 3.57% *S. aureus* isolates were resistant to

erythromycin while ampicillin was found to be ineffective against 17.86% and 35.71% *S. aureus* isolates from burfi and khoya respectively as reported by Farzana *et al*. [2004]. In the study it was pointed out that ampicillin was found to be most effective against *S. aureus* either isolated from burfi or khoya. It was studied that very few *S. aureus* were found to be resistant, both isolated from burfi or khoya. It was also suggested that ampicillin was effective against the cell wall synthesis in bacteria by blocking certain enzymes [Blumberg 1974].

It has been reported that colistin seems to be effective against Gram-negative bacteria and not for Gram-positive bacteria. Our results also showed the same, that colistin to be less effective against *S. aureus* reported by Schwartz and co-workers [1960] and Taylor and Allison [1962].

The present work demonstrated that the resistant strains may have been transferred to milk products because of poor hygienic conditions, during preparation and subsequent handling. Therefore, thorough care during preparation should be taken in order to get a good quality product, safe for human consumption.

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ABBREVIATION USED

AM	: Ampicillin
AK	:Amikin
AML	: Amoxil
AMC	:Augmentin
BP agar	: Baird Parker Agar
Cl	:Colistin
ER	:Erythromycin
FAO	: Food Analysis Organisation
MY	: Lincomycin
NCCLS	:National Committee for Clinical Laboratory Standards
S. aureus	: *Staphylococcus aureus*
SXT	: Co-trimaxazole
TE	:Tetracycline
V	: Cephradine

References

Acred, P. and Sutherland, R. (**1967**) “Antibacterial activities of combination of ampicillin and cloxacillin”, *Antimicrob. Agents Chemother*, 6, 53.

Barbosa, T.M., and Levy, S.B. (**2000**) "The impact of antibiotic use on resistance development and resistance", *Drug Resist. Update*, 3, 303-311.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. (**1966**) "Antibiotic susceptibility testing by standardized single disk method", *Am. J. Clin. Pathol.*, 45, 493-496.

Blumberg, P.M. and Strominger, J.L. (**1974**) "Interaction of Penicillin with bacterial cell, penicillin-binding proteins and penicillin sensitive enzymes", *Bacteriol. Rev.*, 38, 291.

Collin, C.H., Lyne, P.M. and Grange, J.M. (**1995**) "Antimicrobial susceptibility tests", In: C.H. Collin, P.M. Lyne and J.M. Grange (Eds.) *Microbiological Methods*, 7th ed., Butter Worth-Heinemann, Linacre House, Oxford, U.K. pp. 179-205.

Farzana, K., Shah, S.N.H. and Jabeen, F. (**2004**) "Antibiotic resistance pattern against various isolates of *Staphylococcus aureus* from raw milk samples", *J. Res. Science, BZU. Pak*. 15, 145-151.

Food Analysis Organization (FAO), (**1979**) "Manuals of Food Quality Control" No. 4, Microbiological Analysis, Rome.

Fong, I.W., Engelking, E.R. and Kirby, W.M.M. (**1976**) "Relative inactivation by *Staphylococcus aureus* of eight cephalosporin antibiotics", *Antimicrob. Ag. Chemother.*, 9, 939.

Gomez-Lus, R. (**1998**) "Evolution of bacterial resistance to antibiotics during the last three decades", *Int. Microbiol*. 1, 279-284.

Khan, S.A., Nawaz, M.S., Khan, A.A. and Cerniglia, C.E. (**2000**) "Transfer of erythromycin resistance from poultry to human clinical strains of *Staphylococcus aureus*", *J. of Clin. Microbiol*. 38, 1832-1838.

Mandokhot, U., and Chandiramani, N.K. (**1983**) "Food born outbreak due to sweets", *Indian J. Med. Res*. 77, 190-193.

Martin, W.J., Gardner, M. and Washington, J.A. (**1972**) "In vitro antimicrobial susceptibility of anaerobic bacterial isolated from clinical specimen", *Antimicrob. Ag. Chemother*., 1, 148.

National Committee for Clinical Laboratory Standards (NCCS), (**1993**) "Methods for Determining Bactericidal Activity of Antimicrobial Agents. Tentative Guidelines", NCCLS Document M26-T, 2nd ed. Villanova, PA.

Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. (**1999**) "Host-Parasite Interaction; Nonspecific Host Resistance", In: *Microbiology Concepts and Applications*, 6th ed., McGraw-Hill Inc. New York, pp. 478-479.

Schwartz, B.S., Warren, M.R., Barkley, F.A. and Landis, L. (**1960**) "Microbiological and pharmacological studies of colistin sulphate and sodium colisin methane-sulfonate", *Antibiot. Annual*, 41.

Schwarz, S. and Chaslus-Dancla, E. (**2001**) "Use of antimicrobials in veterinary medicine and mechanisms of resistance", *Vet. Res.* 32, 201-225.

Seguin, J.C. and Walker, R.D., Caron, J.P., Kloos, W.E., George, C.G., Hollis, R.J., Jones, R.N. and Pfaller, M.A. (**1999**) "Methicillin-Resistant *Staphylococcus aureus* outbreak in a Veterinary Teaching Hospital: Potential human-to-animal transmission", *J. Clin. Microbiol*., 37, 1459-1463.

Seligman, S.J. (**1973**) "In vitro susceptibility of methicillin-resistant *Staphylococcus aureus* to sulfamethoxazole and trimethoprim", *J. Infect. Dis. (Suppl.)*, 128, 543.

Taylor, G. and Allison, H. (**1962**) "Colomycin-laboratory and clinical investigations", *Brit. Med. J*., 2, 161.

Thomas, C.G.A. (**1988**) "Medical Microbiology", 6th ed., Bailliere Tindall, pp. 205-229.

Tollefson, L., Altekruse, S.F. and Potter, M.E. **(1997**) "Therapeutic antibiotics in animals feeds and antibiotic resistance", *Rev. Sci. Tech.* 16, 709-715.

Wiggins, G.L., Albritton, W.L. and Feely, J.C. (**1978**) "Antibiotic susceptibility of clinical isolates of *Listeria monocytogenes*", *Antimicrob. Ag. Chemother.*, 13, 854.

Witte, W. (**1998**) "Medical consequences of antibiotic use in agriculture", *Science*, 279, 996-997.