



SCREENING OF PATHOGENIC FLORA FROM RAW MILK AND MILK PRODUCTS IN RELATION TO PUBLIC HEALTH SOLD UNDER MARKET CONDITION AT NAGPUR

Sushma C. Meshram

30, Chandranagar near Hawrapeth Bridge, Post Bhagwan nagar, Nagpur – 440027.

ABSTRACT :

The purpose of studying this topic “ screening of pathogenic flora from raw milk and milk products in relation to the public health sold under market condition at Nagpur. “ present study reveals the presence of pathogenic flora in milk, cheese, butter, khoa, dahi, gulabjamun .It includes the study of isolates , identification of isolates, characterization of isolates and antibiotic susceptibility testing of isolates.

INTRODUCTION :

The significance of milk in human nutrition is now well established as it is considered as the best , ideal and complete food for all age groups. Among all microorganism E. coli is frequently contaminating organism and is reliable indicator of fecal pollution generally in insanitary condition of water, milk, food and other products.

Today the large amount of the milk produced in the world is converted into dehydrated milk products and food containing a large proportion of milk solids. In countries with the commercial dairying these processes are carried out in large capacity processing plants. In Africa milk is produced in most agricultural production systems. It is either sold fresh or consumed as the fermented milk and products such as butter , ghee and cheese. Sour milk is the most common products and milk is usually soured before any further processing is done. While there are several milk processing plants in Africa , much of the milk produced by rural small holders is processed on farm using traditional technology . It is important therefore to consider these processes look to possible technological inventions at this time when considering dairy development in the rural sector.

MATERIALS AND METHODS

All the Chemicals used in various tests are AR/GR grade.

All the Glasswares used are Borosil grade.

Media: (Hi Media)

- Nutrient agar.
- Nutrient broth.
- MacConkey agar.
- Eosine methylene blue agar.
- Mannitol salt agar.
- Salmonella shigella agar.
- Bismuth sulphite agar.
- Pseudomonas Isolation Agar.
- IMViC Media :-
- Trypton broth.
- Glucose peptone broth.
- Koser's citrate broth.
 - Grams Stains.
- Crystal violet
- Safranin

Samples: Raw milk, Dahi, Khoa, Gulabjamun, Kalakan, Malai pedha, Butter, Paneer, Cheese, Shrikhand.

Antibiotic disc:(Hi Media)

- 1) Chloramphenicol (30mcg)
- 2) Tetracycline (10mcg)
- 3) Amikacin (30mcg)
- 4) Kanamycin (30 mcg)
- 5) Bacitracin (10 units)
- 6) Novobiocin (5mcg)
- 7) Methicilin (10mcg)
- 8) Erythromycin (15mcg)
- 9) Amoxicilin (30mcg)
- 10) Gentamycin (10mcg)
- 11) Cephotaxime (30mcg)
- 12) Ceftriazone (30mcg)
- 13) Ampicillin (10 mcg)

RESULTS AND DISCUSSION

A) Microbial analysis of milk and milk products by standard plate count method

B) Microbiological analysis of isolates

This study includes 100 isolates isolated from different milk and milk products out of which 70 isolates were found to be organisms. Four to five suspected colonies from each bacterial plate were picked, cultured and they are identified by various morphological , biochemical and cultural characteristics.

B.1 Morphological characteristics of isolated bacteria

Isolated bacteria	Gram staining	Motility
A1	Gram negative short rods	Sluggishly motile
A2	Gram positive organism	Non motile
A3	Gram negative rods	Actively motile
A4	Gram negative short rods	Highly motile

On EMB media A1 isolate gave purple coloured colony with greenish meatallic sheen whereas on Mac Conkey agar pink coloured colonies were observed. During biochemical study, A1 was found to be Indole positive, MR positive, VP negative and Citrate negative. All sugars i.e., glucose , sucrose, lactose, mannitol were fermented with production of acid and gas. A1 isolate was found to be urease negative and catalase positive where as in TSI acidic slant and upliftment of butt was observed. All these characteristics resembles with E.coli. (table 12,13,14,15) (photo)

Rajiv Kumar and Amit Prasad in 2010 in the study of detection of E. coli and Staphylococcus in milk found that Dahi, Ice cream, Gulabjamun, Burfi, Khoa ,Butter contain E. coli. Maltin et al. in 1986, Hanjra et al 1989, Ahmed and Sallam, Sharma and Joshi 1992 also recorded also recorded the presence of E. coli in raw milk and milk products.

On MSA A2 isolate gave golden yellow coloured colonies where as on Mac conkey media pink coloured colonies were observed. During biochemical study, A2 was found to be Indole negative, MR positive, VP positive and Citrate negative. All sugars i.e., glucose, sucrose, lactose and mannitol were fermented with only production of acid and no gas. A2 isolate was found to be urease positive and catalase positive where as in TSI acidic slant and no upliftment of butt was observed. All these characteristics were similar to Staphylococcus aureus.(table 12,13,14,15)

M Maniruzzaman et al., in 2010 during the study of isolation and identification of bacterial flora from milk found that milk from apparently healthy buffalo-cows found that milk contain Stphylococcus aureus. Rajiv Kumar and Amit Prasad in 2010 in the study of detection of E.coli and Staphylococcus in milk found that Dahi, Ice cream, Gulabjamun, Burfi, Khoa ,Butter contain Staphylococcus aureus

On SSA A3 isolate showed presence of black coloured colony where as on Mac conkey agar colourless colonies were observed. The biochemical study showed that A3 isolate was found to be Indole negative, MR positive, VP negative and Citrate positive.A3 isolate fermented only two sugars glucose and mannitol with production of acid and gas. A3 isolate was found to be urease negative and catalase positive where as in TSI acidic slant with upliftment of butt as well as H₂S production was also observed. All the observed characteristics resembles to Salmonella typhi.(table 12,13,14,15)

On BSA A4 isolate gave black centered colourless colonies where as on Mac Conkey agar colourless colonies were observed. A4 isolate showed the biochemical chracterisation as the Indole negative, MR negative, VP negative, Citrate negative. A4 isolate fermented only two sugars i.e. glucose and sucrose with production of only acid but no gas. A4 isolate showed the Urease test positive, Catalase test positive, in TSI test acidic slant with upliftment of butt observed with the H₂S production. The above observed characteristics were similar to Proteus vulgaris.(table 12,13,14,15)

Graph for percentage of isolates obtained from different products

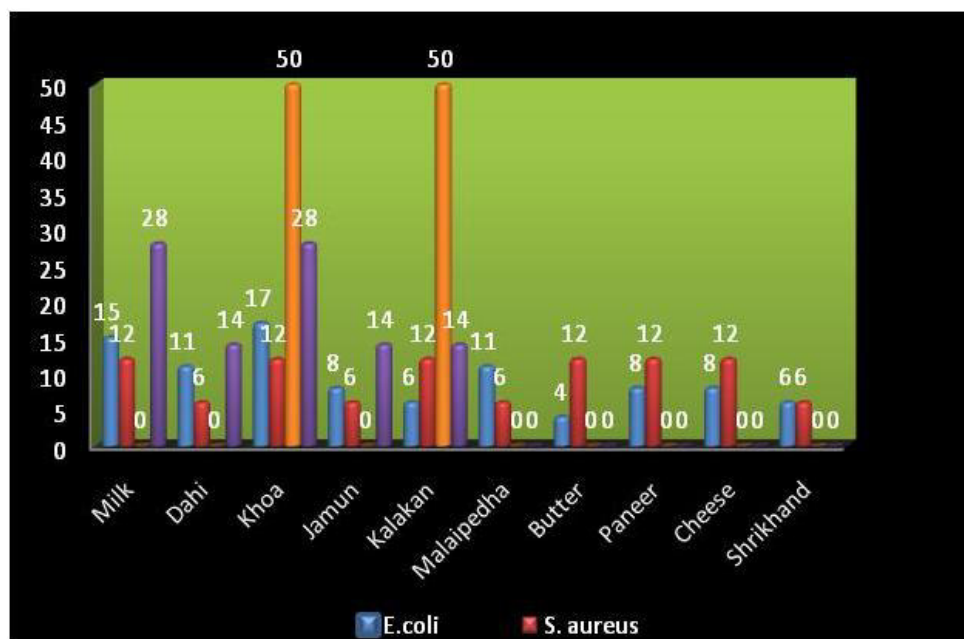


Table C] Percentage of isolates obtained from different products

Name of sample	E. coli n = 45	S. aureus n = 16	S. typhi n = 2	P. vulgaris n = 7
Milk	7 (15%)	2 (12%)	0	2 (28%)
Dahi	5 (11%)	1 (6%)	0	1 (14%)
Khoa	8 (17%)	2 (12%)	1 (50%)	2 (28%)
Gulabjamun	4 (8%)	1 (6%)	0	1 (14%)
Kalakan	3 (6%)	2 (12%)	1 (50%)	1 (14%)
Malai pedha	5 (11%)	1 (6%)	0	0
Butter	2 (4%)	2 (12%)	0	0
Paneer	4 (8%)	2 (12%)	0	0
Cheese	4 (8%)	2 (12%)	0	0
Shrikhand	3 (6%)	1 (6%)	0	0

Out of these 70 isolates 45 isolates were confirmed as *E. coli*, 16 isolates were confirmed as *S. aureus*, 2 isolates confirmed as *S. typhi*, 7 isolates were identified as *P. vulgaris*.

The highest contamination of *E. coli* was found in the khoa sample i.e., 17 % followed by the milk sample (15%) whereas least contamination of *E. coli* was found in the butter sample (4%)

The (12%) percent of *S. aureus* contamination was observed in milk, khoa, kalakan, butter, paneer, cheese samples whereas (6%) percent of *S. aureus* contamination was observed in dahi, gulabjamun, malai pedha, shrikhand.

The contamination of *S. typhi* was observed in only two samples i.e. khoa and kalakan The highest contamination of *Proteus vulgaris* was observed in milk and khoa sample followed by dahi gulabjamun and kalakan.

Naqvi in 1972, Martin et al. in 1986, Hanjra et al. in 1989 reported that 50 out of 100 milk samples were found to be contaminated with *E. coli*.

Rajiv Kumar and Amit Prasad in 2010 was found that out of all the milk samples the highest contamination was recorded in the milk collected from vendors (26%) followed by dairy farm (20%) and house milk (6.6%), this may be due to unhygienic handling of milk. In case of milk products, the highest contamination was recorded in Burfi (33.3%) followed by Dahi (20%), Gulabjamun (20%), Butter (20%), Khoa (13.3%), and Ice cream (6.6%).

M Maniruzzaman, M F R Khan in 2010 reported that among the bacteria isolated from milk of buffalo-cows 32.5% were *Staphylococcus* spp., 25% were *Bacillus* spp., 25% were *Lactobacillus* spp., 12.5% were *E. coli* and 5% were unclassified.] Antibiotic susceptibility testing of isolates

Diameter of **zone of inhibition measured in millimeter (mm)**

Table 17: Hi - Media Zone Size Interpretative Chart

Sr. No.	Antimicrobial agent	Symbole	Disc Content	Resistant	Intermediate	Sensitive
1	Amikacin	Ak	30 mcg	14	15-16	17
2	Amoxycillin When tested against <i>Staphylococcus</i> species	Ac	30 mcg	19	-	20
	When tested against Gram negative organisms			13	14-17	18
3	Ampicillin When tested against <i>Staphylococcus</i> spp and Gram	As	10 mcg	11	12-14	15

	negative organisms					
4	Bactracin	B	10 units	8	9-12	13
5	Cephotoxime	Ce	30 mcg	14	15-22	23
6	Ceftriazone	Ci	30 mcg	13	14-20	21
7	Chloramphenicol	C	30 mcg	12	13-17	18
8	Erythromycin	E	15 mcg	13	14-22	23
9	Gentamicin	G	10 mcg	12	13-14	15
10	Methicillin	M	5 mcg	9	10-13	14
11	Novobiocin	Nv	30 mcg	17	18-21	22
12	Tetracyclin	T	30 mcg	14	15-18	19
13	Kanamycin	K	30 mcg	13	14-17	18

Susceptibility of all the isolates were interpreted by comparing with the above standard chart.

Table .C.a For Gram positive bacteria

Isolated bacteria	B	NV	E	G	AMX	M	A	AK
S. aureus	16	13	11	29	23	14	22	28
Suceptibility	S	R	R	S	S	R	R	S

B = Bacitracin, NV = Novobiocin, E = Erythromycin, G = Gentamicin, AMX = Amoxycillin, M = Methicillin, A = Ampicillin, AK = Amikacin

(S = Sensitive, I = Intermediate, R = Resistant)

Table .C.b For Gram negative bacteria

Isolated bacteria	AK	C	K	TE	AMX	AX	CE	CI
E.coli	19	27	18	17	14	11	21	25
Suceptibility	S	S	S	I	I	R	I	S

S.typhi	22	20	17	20	17	13	24	27
Suceptibility	S	S	I	S	I	I	S	S
P. vulgaris	21	25	19	16	16	14	28	30
Suceptibility	S	S	S	I	I	I	S	S

AK = Amikacin, C = Chloramphenicol, K = Kanamycin, TE = Tetracyclin, AMX = Amoxycillin, AX = Ampicillin, CE = Cephotoxime, CI = Ceftriazone.

(S = Sensitive, I = Intermediate, R = Resistant)

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