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Antibacterial Activities: An Analysis on Isolated Bacterias from Daily use Dairy Products

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ABSTRACT

A study was conducted to analyze the status of important daily used dairy products whether they are safe to use. For the purpose, the Isolates from daily used dairy products were identified as E. coli, Pseudomonas, Lactobacillus, Staphylococcus, based on morphological and biochemical tests; the isolates were further treated for antibacterial activities test. The disc diffusion method of Kirby- Bauer was used for antibacterial test. Susceptibility of all isolates namely E.coli, Pseudomonas, Lactobacillus, Staphylococcus against antimicrobial was tested using NCCLS, USA (National Committee for Clinical Laboratory Standards, USA) guidelines. Antimicrobial agents used were gentamycin, tetracycline, erythromycin and streptomycin. The results showed the resistance of isolates against the antimicrobes used.

Key words: *Antibacterial agents, Bacterias, Standard tests, Morphological and Biochemical.*

INTRODUCTION

Food Standards Agency of the United Kingdom defines *dairy* as "foodstuffs made from mammalian milk. Known as high energy-yielding food products, production plant for the processing of milk is called a dairy or a dairy factory. The source of these products primarily are the milk of cows, goats, sheep, yaks, camels, and other mammals for the human consumption apart from breastfed infants.

Milk, known as balanced diet, is a major part of human food and an aqueous colloidal suspension of proteins, fats and carbohydrates that contains numerous vitamins and minerals. Moreover, with complex biochemical composition and high water activity, milk exhibit an excellent culture media for growth of microorganism besides some of microorganisms produce undesirable effects to consumers.

Number of milk/dairy products including cheese, paneer, curd, yogurt, kefir and clabbered milk, are available in the market since the time immemorial, and have been inseparable part of the human diet and include all forms of cultured, soured or fermented milk. Further, fermented milk products are not consumed directly and form starting materials for other dairy products [1, 2].

In India, as milk form the basis of different fermented products, these products are processed primarily to conserve the nutritional values of milk [3]. The recognition of dairy products with probiotics bacteria as functional foods that provide health benefits beyond basic nutrition and emerging clinical evidence to their potential in preventing some diseases have notable enlarged their consumption and stimulated innovation and new product development [4, 5].

The perusal of earlier studies projected it as potentially beneficial but unfortunately, its harmful act in providing suitable medium for transmission of pathogenic organisms, as observed that fresh, non pasteurized milk generally contains varying numbers of microorganisms.

Incidentally, in India subcontinent, such products, called Indigenous milk products are major source of calcium, magnesium and phosphorous, also carry toxic metabolites of organisms and ingestion of these metabolites cause food borne infections. It has been observed, invariably that soft cheeses (e.g., brie, cheese, cottage cheese) having more water content prepare a suitable environment for bacteria, viruses or molds to multiply quickly [6, 7].

With a view to the above literature, a study has been planned (a) to analysis of variety of daily use dairy products (b) To identify and characterize the isolated strains (c) to study the antibiotic susceptibility level of the strains (*E. coli, Pseudomonas, Lactobacillus* and *Staphylococcus aureus*).

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MATERIALS AND METHODS

Collection of samples:

The present study work was carried out in the Department of Microbiology of Dolphin College of Life Sciences, Chunni Kalan. A total of 10 dairy samples were collected from different sites/ locations (Table 1). Each sample was labelled to show serial number and source. The samples were collected in sterilized screw capped containers and transported to lab. All samples were kept at 4^0C until processed. The type of dairy sample, source and sample number is given in table 1:

Table No. 1*:* Showing type of sample, sample number and source

S.NO	SAMPLE	SAMPLE NO.	SOURCE
1.	Paneer	1.	Dairy
2.	Khoya	2.	Dairy
3.	Curd	3.	Surya
4.	Butter	4.	Vimal
5.	Milk	5.	Verka
6.	Mayonnaise	6.	Mr. Bactor
7.	Lassi	7.	Verka
8.	Cream	8.	Amul
9.	Icecream	9.	Basant
10.	Flavoured milk	10.	Nestle

Processing of samples:

Isolation of various micro-organisms from different samples.

Serial dilution:

1 ml of the sample was mixed in 9 ml sterile normal saline (0.85% Nacl) test tube. 9 test tubes were taken for each sample and were labelled as 1 to 9. Uniform suspension was made by vigorous shaking on magnetic shaker for few minutes. The resultant suspension was called master sample. Master sample is further serially diluted 10 folds from 10^{-1} to 10-9 concentration. 15 ml of selective media was poured in each petridish and allowed to

solidify. Upon solidification of media, 0.1 ml of inoculum was spreaded into petriplates with the help of spreader under aseptic conditions. Procedure was repeated for all other samples and labelled. All plates were incubated at their respective temperatures for 24- 48 h in an incubator in inverted form and were observed for appearance of distinct individual colonies.

E.Coli was isolated on Eosin Methylene Blue (Emb) Agar and Macconkey Agar Plates Whereas *Lactobacillus* was isolated using De Man Ragossa Sharpe (Mrs) Medium; Kings Medium was used for isolation of *Pseudomonas* while *Staphylococcus* was isolated by using Baird Parker Agar and Blood Agar.

The isolated colonies were mixed with many forms of life therefore; the sample obtained was inoculated on petriplates in order to obtain pure culture. The isolates so obtained were identified using colony morphology, gram's staining methods and Biochemical tests.

The morphological and biochemical characterizations were carried out using gram staining by standard methods [7] and for biochemical identification of bacterias, different standarderized test have been used including Indole production test, Methyl-Red and Voges-Proskauer (MRVP) test, Catalase Test and Citrate Utilization Test.

Further, Slants of nutrient agar media were made and kept for solidification. After solidification pure colonies of isolates were streaked on these slants and incubated at 37° C for 2-3 days. After growth these slants were stored at 4° C for further use.

Antibiotic Susceptibility Test:

For antibiotic susceptibility, the disc diffusion method of Kirby- Bauer were used. Susceptibility of isolates (*E.coli, Pseudomonas, Lactobacillus, Staphylococcus*) against antimicrobial was tested using NCCLS, USA (National Committee for Clinical Laboratory Standards, USA) guidelines. Antimicrobial agents tested were gentamycin, tetracycline, erythromycin and streptomycin (Table 2). The agar used was Muller Hinton agar that is rigorously tested for composition and pH. Further, the depth of agar in the plate is a factor to be considered in the disc. Suspension of isolates (*Escherichia coli, Pseudomonas, Lactobacillus* and *Staphylococcus*) were streaked on Muller Hinton agar plates with the help of sterile swabs. The surface was allowed to dry for 5 minutes. Antibiotic disks were placed on the surface of the agar using flame sterile forceps. Plates were inverted and incubated for 24 h at 37^0 C. All plates were examined for the presence and size of inhibitory zone. Antibiotic-susceptible disks (Oxoid) were stored in sealed containers and were maintained at 4^0C the day of the trials.

Table No. 2: **Antibiotics with concentration used to test susceptibility against *S. aureus, Lactobacillus, E. coli, Pseudomonas.***

S.NO	Antibiotics	Antibiotics concentration (µg)
1.	Gentamycin	30
2.	Tetracycline	30
3.	Erythromycin	10
4.	Streptomycin	10

RESULTS AND DISCUSSION

In the present study, *E. coli, Pseudomonas*, *Lactobacillus, Staphylococcus* was isolated from different dairy products (Figure 1). Isolates was confirmed by morphological and biochemical procedures. Five biochemical tests widely employed in the classification of *E. coli, Pseudomonas, Lactobacillus* and *Staphylococcus* were done to confirm their presence. Further, all the isolates were tested for susceptibility test against antimicrobials Gentamycin (G), Streptomycin (S), Teramycin (T) and Erythromycin (E) using Disc diffusion method.

On morphological examination (Figure 1), four strains namely *E. coli, Pseudomonas, Staphylococcus* and *Lactobacillus,* based on staining (table 3), have been identified [8].

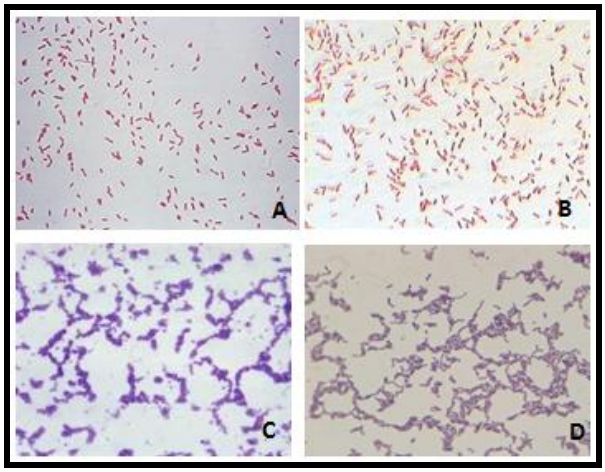

Fig. 1: (A-D) Showing Gram's straining reactions; A, *Pseudomonas; B, E.coli; C, Stahylococcus; D, Lactobacillus*

Table No. 3: **Showing morphological characteristics of isolates Gram's Staining were performed and bacteria appeared as**

S.NO	Strains	Characters
1.	*Escherichia coli*	Gram- negative, rod shaped
2.	*Pseudomonas*	Gram-negative, rod-shaped
3.	*Lactobacillus*	Gram-positive, rod shaped
4.	*Staphylococcus*	Gram- positive, spherical

Analysis of Dairy Products:

Bacteriological examination of dairy samples was done to check the contamination in samples and results have been depicted in table 4. For this purpose, serial dilution was done and then samples were streaked on different culture medias (Figures. 2 A-F).

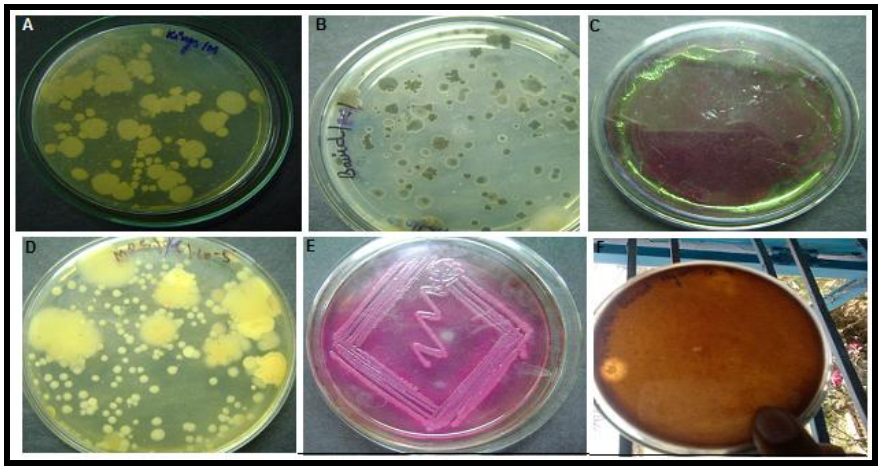

Fig. 2: (A-F): Showing isolates from various dairy samples: A. *Pseudomonas;* B. *Staphylococcus; C. Escherichia coli; D. Lactobacillus; E. Escherichia coli* on MacConkey agar; F. *Staphylococcus* on Blood agar.

Table No. 4: Bacteriological analysis of dairy samples from different sources

S.NO	SAMPLE	*E. coli*	*Pseudomonas*	*Lactobacillus*	*Staphylococcus*
1.	Paneer	_	+	+	+
2.	Khoya	_	+	+	+
3.	Curd	+	+	+	+
4.	Butter	_	+	+	+
5.	Milk	_	+	+	+
6.	Mayonnaise	+	+	+	+
7.	Lassi	_	+	+	_
8.	Cream	+	+	+	+
9.	Icecream	_	_	_	_
10.	Flavoured milk	_	_	_	_

Biochemical Characterization of Microorganisms: Different biochemical tests have been performed to confirm the presence of microorganisms already confirmed on morphological basis (Table 5).

The results are as follows:

Escherichia coli* -** indole, MR and catalase positive whereas VP, citrate were negative confirming the presence of *E. coli*, ***Pseudomonas* -** MR, and catalase positive whereas indole and VP negative confirming the presence of *pseudomonas*, ***Lactobacillus- Catalase negative confirming lactobacillus, ***Staphylococcus* –**MR and catalase positive whereas indole and citrate were negative and VP may or may not be positive (Figure 3).

Table No. 5: Summary of Biochemical Characterization of microorganisms (*E.coli, Pseudomonas, Staphylococcus, Lactobacillus*) isolated from samples (Dairy products)

S.No	Indole test	MethylRed (MR)	Voges Proskaur VP)	Citrate test	Catalase test
Observations	**Red coloured ring on top of test tube**	**Red colouration throughout the tube**	**No crimson red colour**	**No change in colour**	**Production of bubbles**
1.	+	+	_	_	+
2.	+	+	_	_	+
3.	+	+	_	_	+
4.	+	+	_	_	+
5.	+	+	_	_	+
6.	+	+	_	_	+
7.	+	+	_	_	+
8.	+	+	_	_	+
9.	_	_	_	_	_
10.	_	_	_	_	_

Fig. 3 (A-E): Showing biochemical tests of isolates (*E. coli, Pseudomonas, Staphylococcus, Lactobacillus): A.* Indole tests- positive confirmed by presence of cheery red colour at the top; *B.* Methyl red tests – positive confirmed by presence of red colour; *C.* Voges proskaur test – negative confirmed by no formation of crimson red colour; *D.* Catalase tests - positive confirmed by formation of bubble; *E.* Simmon citrate test – negative confirmed by no change in colouration.

Out of 10 samples, 8 samples showed positive results (Table 5). Two samples showed negative results as nothing was isolated from them. The samples which showed negative results were found to form yellow ring near the surface of medium in case of indole test.

In MR test, yellow colouration in test tubes which is the indicative of negative results whereas in VP test, pink colouration

occurred in test tubes within 2-5 minutes. which is the indicative of negative results.

In citrate utilization test, growth with intense blue colour occurred. Blue colour was due to the alkaline pH which results from utilization of citrate.

In catalase test no bubble formation occurred in case of negative test,

The results of antibiotics used against the microbes isolated have been illustrated in Table 6.

Table No. 6: Summary of Antibiotic sensitivity test of microorganisms (*E.coli, Pseudomonas, Staphylococcus, Lactobacillus*) isolated from samples (Dairy products)
Zone Diameter Interpretation Chart (In Centimetres)

Samples No.	Tetracycline	Streptomycin	Erythromycin	Gentamycin
1.	2(Resistant)	3 (Resistant)	2.9 (Resistant)	2.4(Resistant)
2.	1.4(Resistant)	1.8(Resistant)	1 (Resistant)	1.9 (Resistant)
3.	0.8 (Resistant)	1.2 (Resistant)	1.5 (Resistant)	2.4 (Resistant)
4.	2.5 (Resistant)	1 (Resistant)	_(Susceptible)	0.5 (Resistant)
5.	2 (Resistant)	2.3 (Resistant)	1.5 (Resistant)	2.9 (Resistant)
6.	0.5(Resistant)	1 (Resistant)	_(Susceptible)	2.5 (Resistant)
7.	1.9 (Resistant)	2.9 (Resistant)	3.1 (Resistant)	2.9 (Resistant)
8.	2.4 (Resistant)	2.2 (Resistant)	2.4 (Resistant)	2.5 (Resistant)
9.	_	_	_	_
10.	_	_	_	_

The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food born disease, is not significant if *E. coli* is normally a ubiquitous organism [9] yet the pathogenic strains if present could be harmful to consumers. *S. aureus* on the other hand releases a toxic chemical, enterotoxin. As little as 1.0 µg of the toxin in contaminated food produces symptoms of illness.

Milk is one of the most important nutrients and protein dense food, because it is an excellent source of nine essential nutrients and casein, a major milk protein. Dairy products like curd and paneer, khoya made from milk and their consumption plays a significant role in the supply of important nutrients and protein required for good health. These milk products are very essential in the Indian diet so their contamination can cause varied health hazards.

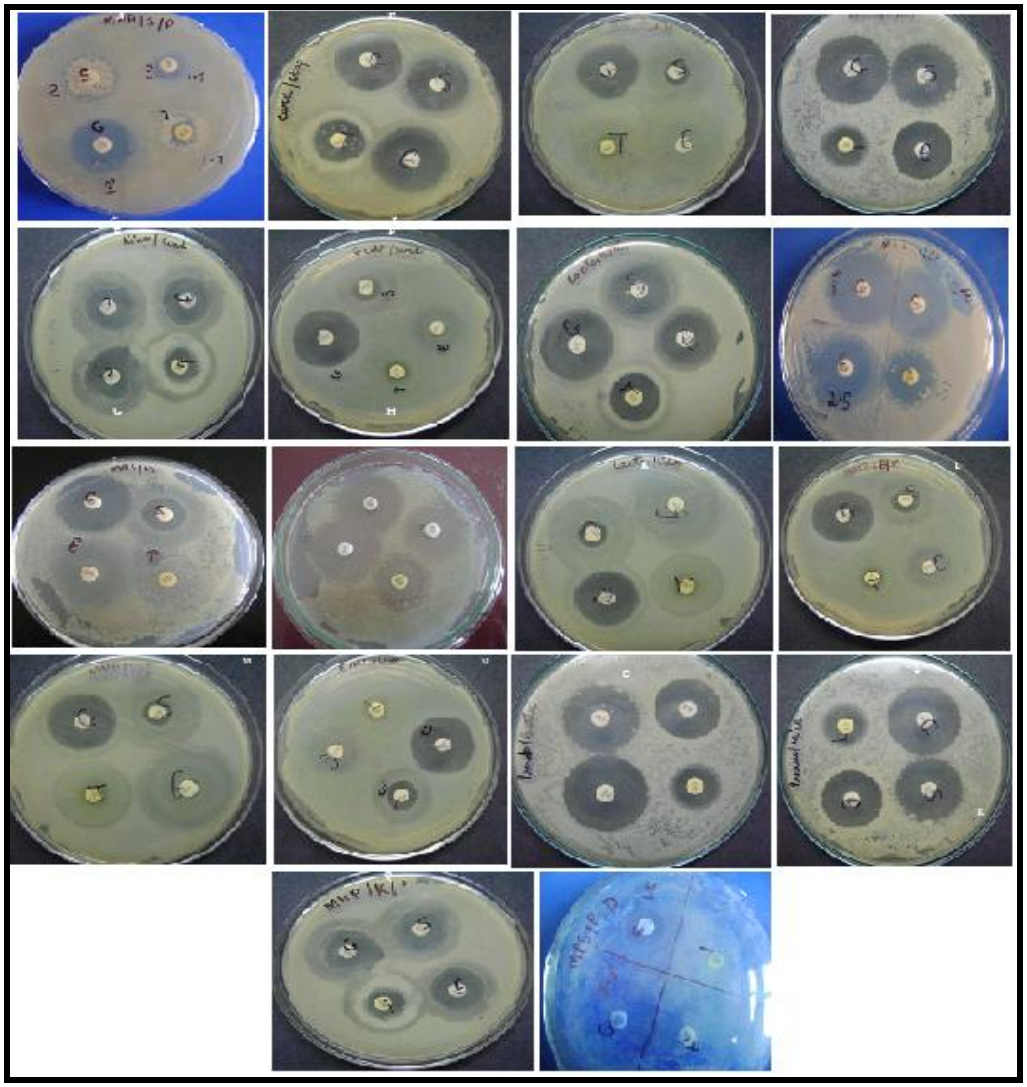

Fig. 4 (A-R): Susceptibility of isolates (*E.coli, Pseudomonas, Lactobacillus, Staphylococcus*) against antimicrobial Gentamycin (G), Streptomycin (S), Teramycin(T) and Erythromycin (E) using Disc diffusion method.

WHO's (2007) was reported that, it is important to handle food in such a way that the microorganisms present do not have a chance to multiply and to prevent food from becoming contaminated with other microorganisms by:

1. Wash and dry hands before preparing any food and after handling foods (milk, curd, etc).
2. Ensure that food preparation areas and equipment are clean.
3. Protect kitchen areas or restaurants and food from insects, pests and other animals.
4. People with gastrointestinal illness, such as vomiting or diarrhoea, should not handle food intended for consumption by others.

The results of the present study indicate that strict preventive measures should be adopted to ensure contamination free milk products for the good health of all consumers. For this, consciousness and care is required from the point of generation to the point of consumption of these widely consumed milk products. *Lactobacilli* are usually sensitive to antibiotic that inhibit cell wall synthesis, such as penicillin and ampicillin [10, 11], our *lactobacilli, Pseudomonas, Staphylococcus* isolates were resistant to Gentamycin followed by Streptomycin. Isolates from *Escherichia coli* showed variability in their pattern of resistance to the antibiotics used in this study. This variability could be due to inherent differences among the isolates. Antibiotic-resistant probiotic strains may benefit patients whose normal intestinal microbiota has become unbalanced.

REFERNCES

1. Anjum MS, Lodhi K, Raza AA. Dairy industry issues and policy alternative special report series No- 14. In: Pakistan Eco. Anal. network proj. Islamabad, **1989**; 2: 1131- 1135.
2. Thapa T. Small-scale milk processing technologies: Other milk products. Report of the FAO E-mail Conference on Small-Scale Milk Collection and Processing in Developing Countries, May 29-July 28, **2000**; FAO, Rome, Italy.
3. Harper N, Richard H.L. Survival and growth of food borne microorganisms in processed and individually wrapped cheese slices . J. Environ. Health., **2008**; 70: 10-14.
4. Boylstona TD, Vinderolab CG, Ghoddusic HB, Reinheimer JA. Incorporation of *Bifidobacteria* into cheeses: challenges and reward. Inter. Dairy Jour., **2004**; 14: 375-387.
5. Ong L, and Shah NP. Probiotic Cheddar cheese: Influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. Food Sci. Technol., **2009**; 42: 1260-1268.
6. Ono K and Yamamoto K. Contamination of meat with Campylobacter Jejuni in Saitama. Japan Inter. Food Microb. Jour., **1999**; 47: 211-219.
7. Bichai F, Payment P, Barbeau B. Protection of water borne pathogens by higher organisms in drinking water. Canadian Microb. Jour., **2008**; 54: 509-524.
8. Aneja KR. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International Publishers. **2005**; New Delhi, India.
9. Bergey DH, Holt JG, Krieg NR, Sneath PHA. Bergey's Manual of Determinative Bacteriology, 9th ed. (eds. Breed RS, Murray EGD, Smith NR) Williams and Wilkims, **1994**; Baltimore.
10. Hahn, G. Pathogenic bacteria in raw milk situation and significance. In: Bacteriological quality of raw milk. Int. Dairy Federation, Brissels (Belgium), **1996**; pp: 67-83.
11. Danielsen M, and Wind A. Susceptability of Lactobacillus species to antimicrobial agents. Int. J. Food Microbiol., **2003**; 82: 1-11.

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