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Prevalence Of Antibiotic Resistant Bacteria And Analysis Of Microbial Quality Of Raw Milk Samples Collected From Different Regions Of Dehradun

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Abstract: Contaminated milk reduces the chances of high quality production of milk and milk- based products, and thereby, hit the economy badly. In the present study, the raw milk quality was analyzed from different regions of Dehradun city in Uttarakhand. The present study revealed the bacterial load in raw milk samples and their antibiotic sensitivity. Fifty raw milk samples were collected from different dairy owners in Dehradun city. The color of milk observed was white in appearance (90%) to yellow (10%). The pH range varies from 6.7 to 6.9. The microbial analysis of raw milk samples revealed dominant micro flora as *E.coli* > *Micrococcus* > *Lactobacillus sp.*> *Salmonella sp.*> *S. aureus* > *Klebsiella* species. Out of 50 samples, only 8% of raw milk was found in the category of good quality, 17% was in fair category and 25% was in the poor category with 41×10^7 CFU/ml bacterial count. Microbial characterization by Gram's staining technique, motility test and biochemical test revealed that 50 samples were containing *E.coli* and Micrococcus, 40 samples were containing lactobacillus, 35 samples were containing Salmonella and 30 samples were containing *S. aureus*, Klebsiella and other bacterial strains. In antibiotic sensitivity test of isolates, *S.aureus* was found to be resistant to penicillin and intermediate to erythromycin, Micrococcus was intermediate to Penicillin and erythromycin, *E.coli* was resistant to tetracycline and intermediate to chloramphenicol, Salmonella was resistant to penicillin and intermediate to streptomycin and tetracycline while Klebsiella was resistant to penicillin, chloramphenicol, erythromycin and intermediate to streptomycin and tetracycline. The bacteria found resistant are threat to mankind.
Keywords: raw milk samples, milk quality, antibiotic resistance.

Introduction

The National Dairy Development Board (NDDB) has revealed in an annual report for 2010-11 that India continues to be the largest milk producer. The estimated milk production for 2010-11 is 121 million tonnes. This means that the country has almost reached 17 percent of world milk production. As milk is consumed by people of all the age groups, the wholesomeness and quality of milk is of great importance. The health benefits of milk and dairy products are known to humanity since medieval times and may be attributed to the biological active components that are present in milk and also, due to their suitably modulated activities produced through the action of probiotics (1).

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (2). Milk is a compulsory part of daily diet for the expectant mothers as well as growing children (3). Milk being nutritious food for human beings also serves as a good medium for the growth of many microorganisms, especially *Lactobacillus, Streptococcus, Staphylococcus,* and *Micrococcus* species. Bacterial contamination of raw milk can originate from sources such as air, milking equipment, feed, soil, faeces and grass. Differences in feeding housing strategies of cows may also influence the microbial quality of milk (4). Indigenous sweet milk products are highly susceptible to variety of microorganism because of high nutritive

value and complex chemical composition (5). Microbial contamination can generally occur from three main, sources viz. within the udder, exterior of the udder and the surface of milk handling and storage equipment. Rinsing of milking machine and milking equipment with unclear water may also be one of the reasons for the presence of a higher number of microorganisms including pathogens in raw milk (6). Also, all cases of dairy illness continued to be of bacterial origin. Diseases associated with the consumption of milk include *Salmonella, Listeria monocytogenes, Staphylococcus aureus, Campylobacter,* Yersinia, pathogenic *Escherichia coli and Clostridium botulinum* (7). The presence of pathogenic bacteria in milk often emerge as a major public health concern, especially for those individuals who still drink raw milk (8). The presence of food-borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and excretion from the udder of an infected animal. Food borne pathogens are virtually inescapable reaching every aspects of life (9). Furthermore microbial contaminants are extremely difficult to pinpoint precision of their presence and role in food system (10). The pattern of acute poisoning may be different even within the region or a country (11). It is therefore essential to increase the number of studies carried out on the importance of food poisoning (12).

The most important microorganisms causing mastitis are *Staphylococci, Streptococci* and coliforms bacteria. The different diagnosis of clinical mastitis can be treated with various antibiotics (13). Antibiotics are used to treat diseases of cattle and as well as used as preservatives for milk (14). When low doses of antibiotics are used, they inhibit the growth of susceptible bacteria while resistance bacteria thrive and grow such as in the presence of tetracycline (15). Resistant bacteria occur in soil, water, plants and animals. The resistant bacteria present in environments are in contact with human beings and animals. It has been estimated that nearly equal tonnage of antimicrobial agents are used in man and in agriculture worldwide (16). The indiscriminate use of antibiotics has led to the development of multiple antibiotics resistances thereby rendering the antibiotic treatment ineffective (17). Incidence and prevalence of antibiotic resistance in bacteria present in milk and milk products is still common (18, 19). Antibiotic resistance in bacterial population during cold storage of raw milk is also reported (20).

Materials and Methods

Sampling

A total number of 50 raw milk samples (10 ml each) were collected in the morning from different dairy owners in diverse locations of Dehradun city. The samples were collected from nearby areas to be transported easily without any delay. The samples were collected in sterilized screw capped bottles (Borosil). All the possible precautions were taken to avoid external contamination at the time of collection of samples and during processing.

Physical appearance

All milk samples were observed for color and pH.

Total Bacterial Count / Standard Plate Count

Total viable count was carried out using tenfold dilution of samples up to 10^{-6} dilution. The overnight grown bacterial culture was spread onto the Nutrient Agar Medium (CDH) plates. The plates were incubated at 37°C for 24 hrs in inverted position in an incubator (8).

Calculation for the enumeration of the bacterial cells per milliliter was made with the formula:

$CFU/ml = \sum C / a \times d$

Where, $\sum C$ is the average of colony counted on all plates; a is the amount of plated samples of respective dilution and d is the dilution.

Isolation of bacteria from raw milk samples

The raw milk samples were spread on selective media (Eosin Methylene Blue agar, Mannitol Salt Agar, Salmonella Shigella agar, Lactobacillus agar) and incubated under optimal growth conditions. Each bacterial isolate were subcultured for further characterization. The experimental controls were also taken.

Characterization of isolates from raw milk samples

The obtained bacterial colonies were examined macroscopically for colony morphology (shape, color, texture) and microscopically by Gram's staining. Single isolated colony was picked for the preparation of smear and

stained for the examination of morphological characters of the isolates (21). Motility Test was done using wet slide method.

Bacterial isolates were further biochemically characterized by Indole, Methyl red, Voges Proskauer, Citrate utilization test (IMViC test) and Triple Sugar Iron Agar (TSI) test. Appropriate positive and negative controls were used to make a distinction between positive and "false-positive" reactions.

Antibiotic sensitivity of isolates

The bacterial isolates from raw milk samples were tested against five antibiotics: Penicillin G (P); Chloroamphenicol (C); Erythromycin (E); Streptomycin (S); Tetracycline (T) to know the resistance pattern of isolates using Kirby Bauer Method (22).

Table1. Physical appearance of milk

Location number	Location (Dehradun city)	Milk Color	Milk pH
1	Subash Nagar	White	6.7
2	Clement Town	White	6.7
3	Turner road	White	6.8
4	Defence colony	Yellowish white	6.8
5	Patel Nagar	White	6.7
6	Mazra	White	6.8
7	G.M.S road	White	6.7
8	Dharampur	White	6.9
9	Banjarawala	White	6.9
10	Kawali road	White	6.8
11	Balliwala	White	6.8
12	Saharanpur chowk	yellowish white	7.0
13	Moti bazaar	yellowish white	6.8
14	Neranjanpur	White	6.8
15	Khurbura	White	6.7
16	Kargi chowk	yellowish white	6.9
17	Saraswati vihar	White	6.9
18	Dharampur	White	6.9
19	Nehru colony	White	6.9
20	Bindal	White	6.6
21	Fish market	White	6.6
22	Yamuna colony	White	6.8
23	Krishna nagar chowk	White	6.9
24	Rajpur	light yellow white	7.0
25	Clock tower	White	6.9

Results

The color of milk samples observed was white in appearance (90%) to yellow (10%). The pH range varies from 6.7 to 6.9 (Table 1). Microbial count was determined by standard plate count as per BIS standards and compared with the standard chart of microbiological quality of raw milk (Table 2). Out of 50 samples, only 8% of raw milk was found in the category of good quality, 17% was in fair category and 25% was in the poor category (Table 3). Microbial characterization by Gram's staining technique, motility test and biochemical test revealed that 50 samples were containing *E.coli* and Micrococcus, 40 samples were containing lactobacillus, 35 samples were containing Salmonella and 30 samples were containing *S. aureus*, Klebsiella and other bacterial strains (Table 5). Antibiotic sensitivity was done against five antibiotics, i.e., Penicillin G (P), Chloroamphenicol (C), Erythromycin (E), Streptomycin (S) and Tetracycline (T) (Table 6). Isolated and characterized *Lactobacillus sp.* from milk sample was found to be sensitive to all five antibiotics (Penicillin, Chloramphenicol, Erythromycin, Streptomycin, Tetracycline). *S.aureus* was found sensitive to Chloramphenicol, Streptomycin, Tetracycline, intermediate to Erythromycin and resistant to Penicillin. *Micrococcus* was found sensitive to Chloramphenicol,

Streptomycin, Tetracycline and intermediate to Erythromycin and Penicillin. *E.coli* was found sensitive to Erythromycin, Penicillin and Streptomycin, intermediate to Chloramphenicol and resistant to Tetracycline. *Salmonella sp.* was found sensitive to Chloramphenicol, intermediate to Streptomycin, Tetracycline and resistant to Erythromycin, Penicillin. *Klebsiella sp*. was found intermediate to Streptomycin, Tetracycline and intermediate to Tetracycline and resistant to Erythromycin, Penicillin and Chloramphenicol.

Table 2. BIS Standard for raw milk plate count (SPC) (IS: 1479-1977, PART 111)

Count/ml	Quality/grade
<200,000	Better
2,000,001-1,000,000	Good
1,000,000-50,00,000	Fair
>5,000,000	Poor

Table 3. Analysis of raw milk quality

Total Milk samples	Mean Bacterial Count (CFU/ml)	No. of Samples	Quality Grade of Milk
50	15×10^4	8	Good
	20×10^5	17	Fair
	41×10^7	25	Poor

Table 4. Microbial examinations of raw milk samples

Bacterial Isolate	Total Positive samples
Lactobacillus sp.	40
Staphylococcus aureus	30
Micrococcus	50
Escherichia coli	50
Salmonella sp.	35
Klebsiella sp.	30
Other bacterial strains	30

Table 5. Biochemical tests for bacterial isolates

Tests → / Isolates ↓	Indole test	Methyl Red test	Voges-Proskaur test	Citrate Utilisation test	TSI test	Catalase test	Urease test
Lactobacillus sp.	-ve	-ve	+ve	-ve	A/A with no gas	-ve	-ve
Staphylococcus aureus	-ve	+ve	-ve	-ve	A/A with no gas	+ve	-ve
Micrococcus	-ve	-ve	-ve	+ve	-ve	-ve	+ve
Escherichia coli	+ve	+ve	-ve	-ve	A\A with gas	+ve	-ve
Salmonella sp.	+ve	-ve	+ve	+ve	Al/A with H2S	+ ve	- ve
Klebsiella sp.	-ve	-ve	+ve	+ve	A/A with gas	+ve	+ve

A=acid, Al=alkaline

Table 6. Antibiotic sensitivity of bacterial isolates

Antibiotics → Bacterial isolate ↓	**Penicillin**	**Chloroamphenicol**	**Erythromycin**	**Streptomycin**	**Tetracycline**
Lactobacillus sp.	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Staphylococcus aureus	Resistant	Sensitive	Intermediate	Sensitive	Sensitive
Micrococcus	Intermediate	Sensitive	Intermediate	Sensitive	Sensitive
Escherichia coli	Sensitive	Intermediate	Sensitive	Sensitive	Resistant
Salmonella sp.	Resistant	Sensitive	Resistant	Intermediate	Intermediate
Klebsiella sp.	Resistant	Resistant	Resistant	Intermediate	Intermediate

Discussion

Pathogenic bacteria in milk have been a major factor for public health concern since early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurized milk has been a major vehicle for transmission of pathogens. The health of dairy herd and milking conditions basically determine the milk quality. Another source of contamination by microorganisms is unclear teats. The use of unclear milking and transport equipments also contributed to the poor hygienic quality (23). A range of physical parameters and milk quality were studied of raw milk samples collected from different dairies in Dehradun. Out of 50 samples analyzed, 40 samples were white and 10 samples were yellow in color with pH range 6.7 to 6.9. These findings agreed with the reports of Judkins and Mack (24), who reported that normal milk has a yellowish color due to presence of fat, casein and the presence of small amount of coloring matter. These differences in color may be due to differences in nature of feed consumption or the breed of cow or the fat and solid contents of the milk (25). Microbial characterization of raw milk samples analyzed, showed the dominant micro flora in the order *E.coli* > Micrococcus > Lactobacillus sp.> Salmonella sp.> *S.aureus* > Klebsiella sp. among the isolates. Microbiological dominance in milk has been investigated by several workers (8). In the present study, out of 50 samples, only 8% of raw milk was found in the category of good quality, 17% was belong to fair category and 25% was lying in the poor category. Recently poor milk quality and antibiotic resistance is also observed at haridwar city in Uttarakhand by Singh and Chaudhary (26). In antibiotic sensitivity test of isolates, *S.aureus* was found to be resistant to penicillin, *E.coli* was resistant to tetracycline, Salmonella was resistant to penicillin while Klebsiella was resistant to penicillin, chloramphenicol and erythromycin. Antibiotic resistance in microbes isolated from milk sample is still a major problem and being observed by different workers in different region of India (27, 28). The microorganisms found resistant are threat to mankind.

Conclusion

On the basis of data obtained in the present study, conclusion may be drawn that microbial load and antibiotic resistance in milk distributed in Dehradun is increasing very fast. The main cause of microbial contamination of milk is due to milking from infected udders of the cows, unhygienic mechanical milking practices, unclean equipments or poor washing practices and improper storage conditions. Due to lack of awareness and negligence in this area, it is still observed.

Improving animal health, reducing antimicrobial use in animal husbandry, implementation of regulations to restrict the use of antibiotics in animals, application of modern technologies may improve the current situation and establish India as largest and best quality milk producer in the world. The evolving threat of antimicrobial resistance and options for action is being released by WHO (29) that will extend our knowledge to understand the cause and solution of the problem.

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