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Microbial Susceptibility In Raw Milk Due To Raw Water Adulteration In And Around Eluru, A.P.,

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Abstract
Concerns over diseases linked to milk consumption are growing. *Escherichia coli* and *Streptococci* is a major foodborne pathogen worldwide. The water used during handling and processing of milk products can be potential sources of microbial contamination with possible negative consequences on food safety. Especially, the water used in keeping the hygiene of milking and milk storage utensils is crucial to keep the quality and safety of the products. Some E. Coli species produce Shiga toxin, which can result in symptoms including diarrhoea and stomach pain and occasionally even fatal consequences like haemolytic-uremic syndrome. Even though pasteurization eliminates E. coli, milk products can still cause infections. Commonly *Streptococcus* it causes throat infection (pharyngitis), tonsil infection (tonsillitis), scarlet fever, skin sores (impetigo) and skin infection (cellulitis).
This study was conducted to assess the prevalence, risk factors, and microbial susceptibility of *E. coli, Coliforms, faecal streptococci* in raw milk and unhygienic practices in and around Eluru. Taking pasteurized milk as standard levels, the microbial susceptibility in raw milk is estimated. Microbial contamination of public drinking water supplies poses a serious threat to human health. Adulteration of raw water in milk for its quantity found to be microbial susceptibility other than the unhygienic practices in dairy farm. Some known contaminants of concern in drinking water parameter tests, Adulteration tests and Microbiological tests are used to detect the hygienic condition of a raw milk. The milk-handling practices are poor in the study areas. The same bacteria that are found in raw water samples are Escherichia coli, aerobic bacterial count and faecal streptococci —also found in higher concentrations in raw milk samples. Hygiene levels to be improved during milk collection, usage of clean milk transport containers, usage of filtered water, cleaning of surroundings and animal milk teats during milking.

Keywords: *Coliforms, streptococcus,* Raw milk, Raw water and Diseases

INTRODUCTION

Water used in the handling and processing of dairy products may be a source of microbiological contamination, which could have an adverse effect on the safety of food. The water used to maintain the cleanliness of the equipment used to milk and store milk is especially important for maintaining the products' quality and safety. Raw water is water found in the environment that has not been treated and does not have any of its minerals, ions, particles, bacteria, or parasites removed. Raw water with salmonella, E. Coli, and Giardia. These pathogens can cause stomach upset, diarrhoea, and vomiting. The oldest and youngest people are especially at risk for developing illnesses related to these contaminants because their immune system may not be strong enough to fight off the pathogens.
Acute neurological sickness first appeared in Eluru, a city in the southern Indian state of Andhra Pradesh, around the beginning of December 2020. On December 5, the first case was reported. Over the following week, hundreds more people became ill, and one person passed away. Therefore, the likelihood that Cyclone Nivar's intense rains flooded water channels, along with the potential for damaged drinking water pipes and Eluru's lack of a sewage treatment plant, could have all played a significant role in the town's drinking water supply becoming contaminated.

Pollutant Water is one of the most common adulterants in milk and is used in many types of milk adulteration. Milk loses some of its nutritional value, such as its protein and solid content when water is added, while its volume increases. Additionally, customers may be at risk for health problems if the water is tainted with pesticides or heavy metals. Using containers in sewage sites also exposes people to microbiological contamination from flies. Other pathogens that can be found in raw milk include: Salmonella, Streptococcus spp, Yersinia enterocolitica, Campylobacter jejuni, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis, Coxiella burnetti, and Mastitis-causing bacteria. Raw milk doesn't have high enough concentrations of antimicrobial compounds to kill pathogens. The antimicrobial components in milk can have either bactericidal, bacteriostatic, or no effect at all depending on the specific pathogenic species and strains involved.

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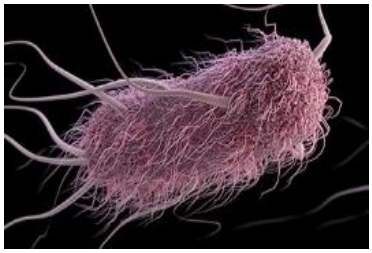
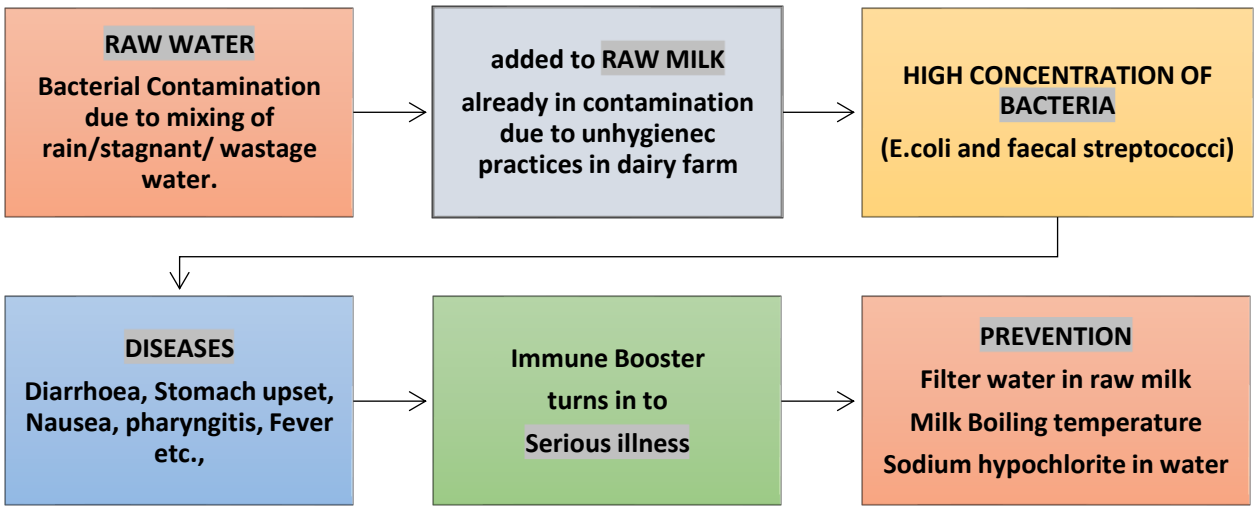


Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. *E.coli* is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or *E. coli*. The majority of persons with Shiga toxin-producing E. coli infections have vomiting, severe stomach cramps, and diarrhoea often bloody. Usually, three to four days after ingesting the germs, symptoms appear. Without medical attention, most patients recover in five to seven days. Reports of "E. coli present" in drinking water samples do not necessarily indicate the presence of this harmful strain; in fact, it is most likely absent. It does, however, suggest recent fecal contamination. All strains of E. Coli, including O157:H7, are eliminated by boiling or disinfecting tainted drinking water.

Streptococci are Gram-positive, spherical, non-motile, microaerophilic bacteria (cocci). They are facultative or stringent anaerobes that frequently appear in chains or pairs. Catalase testing yields a negative result for streptococci and a positive result for staphylococci. Streptococci can be found in a variety of natural settings, such as the mouth, intestinal tract, nasal passages, and throat mucosae of humans and other animals. When streptococci are found in drinking water, it means that there is faecal pollution. Commonly, *Streptococcus* it causes throat infection (pharyngitis), tonsil infection (tonsillitis), scarlet fever, skin sores (impetigo) and skin infection (cellulitis).

The most frequent symptoms of bacteria in raw milk include nausea, vomiting, diarrhea (often bloody), fever, headaches, and body pains. A number of serious or even fatal disorders, such as hemolytic uremic syndrome (which can cause kidney failure, stroke, and even death) and Guillain-Barré syndrome (which can cause paralysis), have been linked to raw milk consumption.



REVIEW OF LITERATURE

Milk can sustain a robust microbiota because of its high nutritional content. These microorganisms can enter milk through a variety of channels and once inside, they can perform a multitude of functions. For example, they can aid in dairy fermentations (such as those caused by Lactococcus, Lactobacillus, Streptococcus, Propionibacterium, and fungal populations), cause spoilage (such as those caused by Pseudomonas, Clostridium, Bacillus, and other spore-forming or thermoduric microorganisms), promote health (like those of lactobacilli and bifidobacteria), or even induce disease (like those caused by Listeria, Salmonella, Escherichia coli, Campylobacter, and mycotoxin-producing fungi) (Lisa Quigley 2013). Pregnant women run a serious risk of becoming ill from the germ *Listeria*, which is often found in raw milk and can cause miscarriage, or illness, or death of the newborn baby (U.S. FDA)

No coli forms are established in samples, taken directly from the pasteurizing equipment. The secondary infection with E. coli results from the use of vessels and equipment in the course of the technological process (I Gogov). No official control program for bovine virus diarrhoea virus (BVDV) has been implemented since BVDV was reported in 1980 in China (Li et al., 1983). Wastewater discharges in fresh waters and costal seawaters are the major source of faecal microorganisms, including pathogens (World Health Organization). Cholera, typhoid fever, bacillary dysentery, faecal indicator bacteria, coliforms, ammonia are major diseases due to polluted water.

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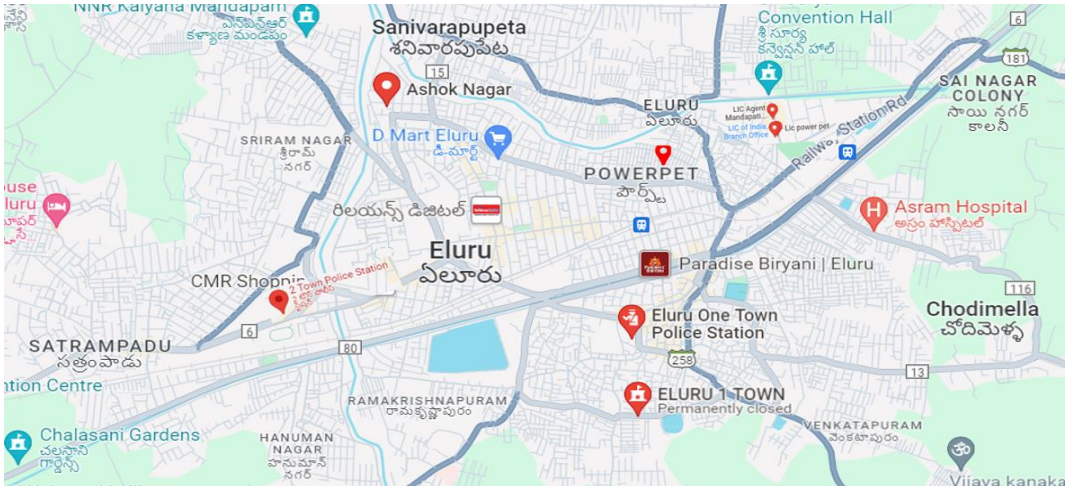
The longevity of certain bacteria of the typhoid group upon dairy utensils and in water; Typhoid bacilli are destroyed by drying, although they may survive for a considerable amount of time in areas with slight moisture content, such as clothes, soil, or faecal matter. While the majority of the organisms perished quickly, a small number of hardy individuals withstood drying for an extended period of time. Even when used only for rinsing, sodium hypochlorite was highly helpful in reducing the number of bacteria per bottle. Live steam was equally effective, but it was necessary to thoroughly wash the curd beforehand. otherwise, there may occasionally be a high count. referred to as "hot water". From a bacteriological standpoint, washing is ineffective and inadequate. (CARL R. FELLERS 1924). Add one tablespoon of bleach to a gallon of distilled water. That will clean and disinfect the surface without degrading the plastic. Sodium hypochlorite can also be used for point-of-use disinfection of drinking water, taking 0.2–2 mg of sodium hypochlorite per liter of water.

THE OBJECTIVES OF THE STUDY ARE AS FOLLOWS:

(1) To Analyse the Raw water physical and chemical parameters from the selected areas.
(2) To Identify the microbial susceptibility in both Raw water and Raw milk.
(3) To compare the risk factors of microbes and influence of raw water adulteration in raw milk.
(4) To study on controlling methods of microbes

STUDY AREA & SAMPLE COLLECTION



- Selected Eluru's predominant people colonies
- 25ml of Raw water and Raw Milk is collected in a plastic bottle from in and around five areas of Eluru
- The samples were kept in a cool box with ice packs and transported to the KRYSAL Microbiology Laboratory in Vijayawada for analysis.

Area 1	Eluru 2 town
Area 2	Eluru 1 town
Area 3	Ashok Nagar
Area 4	power peta
Area 5	LIC Road

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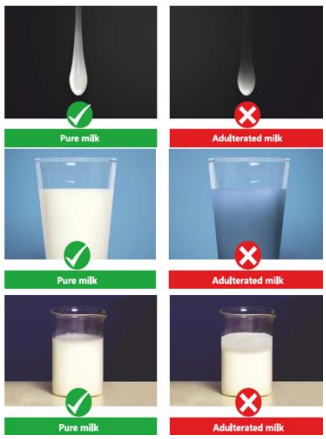
METHODOLOGY



1. ADULTERATION TEST

➢ **Detection of water in milk**

1. Put a drop of milk on a polished slanting surface
2. Pure milk either stays or flows slowly leaving a white trail behind
3. Milk adulterated with water will flow immediately without leaving a mark

➢ **Detection of detergent in milk**

1. Take 5-10ml of sample with an equal amount of water
2. Shake the contents thoroughly
3. If milk is adulterated with detergent, it forms dense lather
4. Pure milk will form very thin foam layer due to agitation

➢ **Detection of starch in milk**

1. Boil 2-3ml of sample with 5ml of water
2. Cool and add 2-3 drops of tincture of iodine
3. Formation of blue colour indicates the presence of starch

(in case of milk addition of water and boiling is not required)

2. MICROBIOLOGICAL TEST

The quality of microbes: The most popular method for assessing the presence of microorganisms in food is undoubtedly the standard plate count, which is also occasionally called the whole plate count. As the name suggests, the goal is to determine how many live microbe cells are present in a particular food sample.

✓ Various Inoculation Methods Used in Bacteriology, among those Pour plate method is used for present study:

Pour Plate Method

➢ The pour plate method is a laboratory technique for isolating and counting viable microorganisms like bacteria and fungi in a liquid sample that is added to a molten agar medium.

➢ In general, this technique counts the total number of CFUs (colony-forming units) on the surface of the solid medium.

➢ The procedure for standard CFU counting comprises

I. The substrate preparation,
II. The actual spreading,
III. Counting after incubation and
IV. Waste managements.

1. **Serial dilution** – If the sample is a liquid, it can be diluted serially with sterile broth or distilled water. If the sample is semisolid or solid, it must first be emulsified before being serially diluted to reduce the microbial load to the permissible limits.
2. In the pour plate method, the sample is either added to the Petri plate and then poured with the molten agar medium, or the sample is mixed with the molten agar medium before pouring.
3. **Agar preparation:** Autoclave 15 g agar in 800 ml water for 15 min. Add sterile concentrated minimal medium and carbon source. After medium has cooled to about 50°C, add supplements and antibiotics. Pouring 32 to 40 ml medium into each plate, expect about 25 to 30 plates per liter.
4. **Incubation:** Now the medium is allowed to solidify before being incubated at the appropriate temperature 25-30°C to grow the microbes present in the sample. The number of isolated colonies is counted after incubation.

Note: Check the plate by 24 hours of incubation, because if delayed over-growth can occur and colonies may fuse making the plate difficult to read. If no growth after 24 hours, incubate it for the next 24 – 48 hours (or more based on probable microorganism) before reporting no growth and discarding.

5. **Marking Colonies:**

Take the petri dish out of the incubator, label the bottom of the dish with a black marker wherever you see a colony forming unit (CFU), and count the number of bacterial colonies on the agar plate. Naturally, you might discover that the colonies are too tiny, too near to one another, or too many to count precisely.

6. **Bacterial Count Calculation:**

CFU/ml is equal to the total number of colonies multiplied by the dilution factor and this is divided by the volume of the culture plate.

$$CFU/ml = \frac{Total number of colonies * dilution factor}{volume of specimen used (aliquot)}$$

Bacteria/ml = 130 x 10^6 which would be equal to 1.3x10^8 which can also be written as 130,000,000

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SAMPLE ANALYSIS:

- **Microbiological quality of Raw milk from local vendors in and around Eluru Rural region:**

FOOD – Microbiological tests	Test method	Medium	Incubation: Temperature and Duration
Total plate count, cfu per ml	IS 5402 Part 1	Plate count agar	37degC for 72 hours
Escherichia coli, cfu per ml	IS 16067 Part 2	TBX agar	44degC for 24 hours
Faecal streptococci, cfu per ml	IS 5887 Part 2	Ethyl violet azide dextrose agar	37degC for 24 hours
Staphylococcus aureus, cfu per ml	IS 5887 Part 8.Sec1	Baird Parker agar	37degC for 24 hours
Salmonella , Detection per 25ml	IS 5887 Part 3.Sec1	(1) Buffered peptone water, (2) MKTTn broth, (3) RVS medium; (4) XLD agar; (5) BG agar	(1)37degC for 20 hours, (2)37degC for 24 hours, (3)41.5degC for 24 hrs, (4)37degC for 24 hours, (5)37degC for 24 hours,

- **Mapping of drinking water quality from different resources in the corporation area during pre monsoon period:**

WATER - Microbiological tests	Test method	Medium	Incubation: Temperature and Duration
Coliform bacteria, Per 100ml	IS 15185	Chromogenic coliform agar	37degC for 24 hours
Escherichia coli, per 100 ml	IS 15185	Chromogenic coliform agar & Tryptophan broth /Tryptone water	37degC for 24 hours & 44degC for 24 hours

3. WATER PARAMETER TESTS

- **https://law.resource.org/pub/in/bis/S02/is.3025.16.1984.pdf**

RESULT

Milk hygiene is the practice of important for ensuring cleanliness and safety of milk and preventing the spread of diseases. Milk hygiene is important for the health and welfare of cows and consumers.

To verify milk hygiene status, milk and domestic water samples were collected from local region and evaluated for various quality parameters. Raw milk samples were evaluated for adulteration and microbiological quality parameter – Escherichia coli. Domestic borewell and hand pump water samples were evaluated for potability quality parameters.

Data is compiled in Table 1 and 2 and conclusions drawn.

Table 1: PHYSICAL AND CHEMICAL PARAMETERS OF RAW WATER ALONG WITH IDENTIFICATION OF MICROBES

Sl. No.	Test parameters	Water sample 1	Water sample 2	Water sample 3	Water sample 4	Water sample 5	Acceptable limits, IS 10500:2012	Permissible limits in the absence of alternate source, IS 10500:2012
1	Visual appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear
2	pH at 25degC (IS 3025 Part 11)	7.31	7.16	7.24	7.30	7.16	6.5 to 8.5	No relaxation
3	Total dissolved solids (TDS), mg/L (IS 3025 Part 16)	1900	1150	2900	1950	3900	500, Max.	2000, Max.

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4	Colour (Hazen units) (IS 3025 Part 4)	<1	<1	<1	<1	<1	5, Max.	15, Max.
5	Odour (IS 3025 Part 5)	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable
6	Turbidity (NTU) (IS 3025 Part 10)	<1	<1	<1	<1	<1	1, Max.	5, Max.
7	Total alkalinity as CaCO3, mg/L (IS 3025 Part 23)	450	400	550	500	390	200, Max.	600, Max.
8	Total hardness as CaCO3, mg/L (IS 3025 Part 21)	882	529	500	735	294	200, Max.	600, Max.
9	Calcium as Ca, mg/L (IS 3025 Part 40)	200	192	200	180	168	75, Max.	200, Max.
10	Magnesium as Mg, mg/L (IS 3025 Part 46)	166	82	73	135	31	30, Max.	100, Max.
11	Chlorides as Cl, mg/L (IS 3025 Part 32)	265	235	230	550	245	250, Max.	1000, Max.
12	Fluoride as F, mg/L (IS 3025 Part 60)	<1.0	<1.0	<1.0	<1.0	<1.0	1.0, Max.	1.5, Max.
13	Iron as Fe, mg/L (IS 3025 Part 53)	<1.0	<1.0	<1.0	6.9	2.6	1.0, Max.	No relaxation
14	Sulphates as SO4, mg/L (IS 3025 Part 24.Sec1)	58	42	60	97	44	200, Max.	400, Max.
15	Coliform bacteria, per 100ml (IS 15185)	Present	Present	Present	Present	Present	Shall be absent	No relaxation
16	Escherichia coli, per 100ml (IS 15185)	Present	Present	Present	Present	Present	Shall be absent	No relaxation

- Raw Water sample 1, 3 and 4 is found to be at higher risk of contamination
- Raw Water sample 2 and 5 is found to be moderate levels of contamination

Note: cfu: colony forming unit; Adulteration tests were performed as per FSSAI DART manual for detection of adulteration with rapid tests (www.fssai.gov.in)

Microbiological tests were performed using test methods of Bureau of Indian Standards (www.bis.gov.in):
(1) Total plate count as per IS 5402 Part 1:2021;
(2) Escherichia coli as per IS 16067 Part 2:2023;
(3) Faecal streptococci as per IS 5887 Part 2:1976;
(4) Staphylococcus aureus as per IS 5887 Part 8.Sec1:2023;
(5) Salmonella as per IS 5887 Part 3.Sec1:2020.

Table 2: MICROBIAL TESTS IN RAW MILK

Sl. No.	**Test parameters**	**Raw milk 1**	**Raw milk 2**	**Raw milk 3**	**Raw milk 4**	**Raw milk 5**	**Pasteurized milk**	**Permissible limits, FSSAI**
	Adulteration tests							
1	Adulteration for Water in Milk	Present	Present	Present	Present	Present	Present	Shall be absent
2	Adulteration for Detergent in Milk	Absent	Absent	Absent	Absent	Absent	Absent	Shall be absent
3	Adulteration for Starch in Milk	Absent	Absent	Absent	Absent	Absent	Absent	Shall be absent

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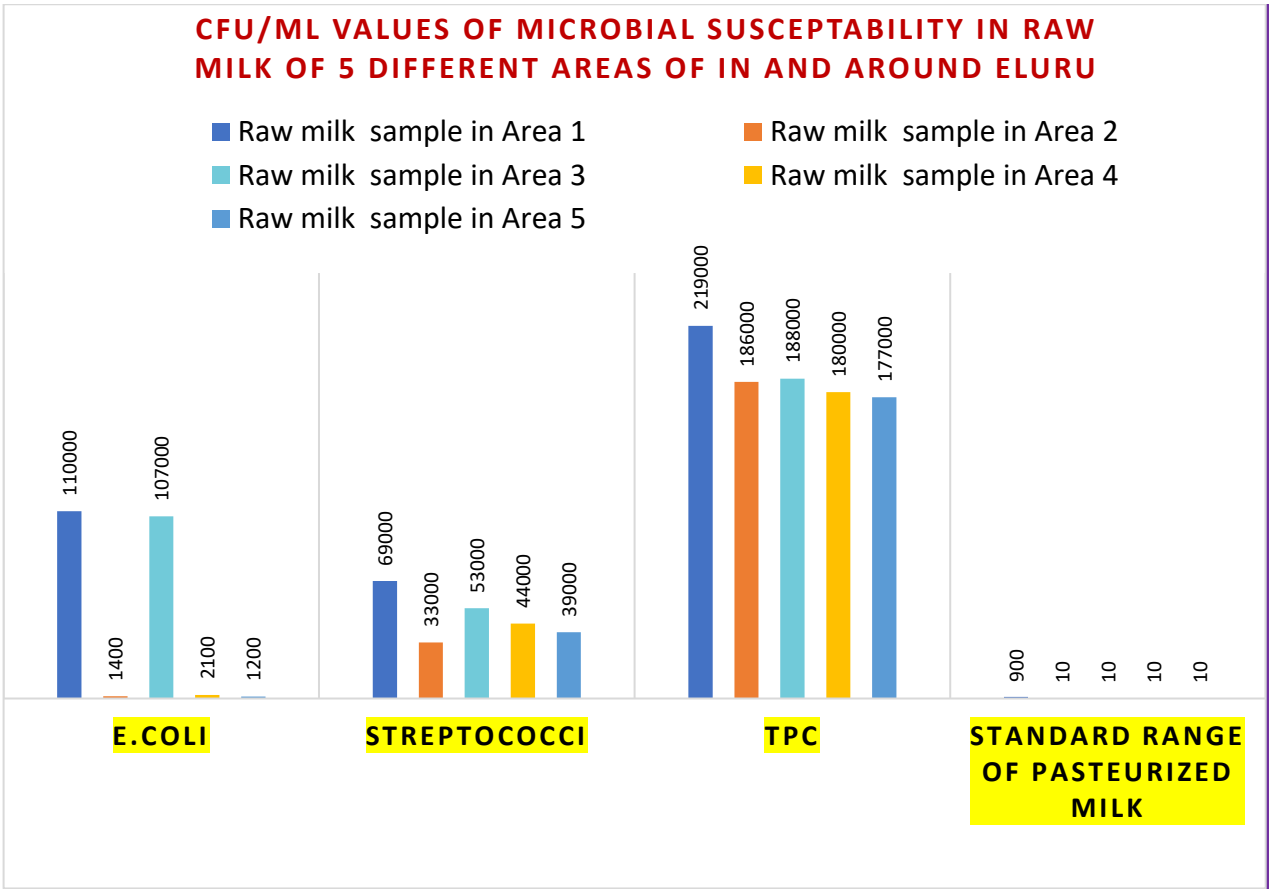
	Microbiological tests							
1	Total plate count, cfu per ml	219000	186000	188000	180000	177000	900	50000 for boiled milk
2	Escherichia coli, cfu per ml	110000	1400	107000	2100	1200	<10	<10/ml for milk products
3	Faecal streptococci, cfu per ml	69000	33000	53000	44000	39000	<10	Not specified
4	Staphylococcus aureus, cfu per ml	Absent	Absent	Absent	Absent	Absent	Absent	<50/ml for milk products
5	Salmonella , Detection per 25ml	Absent	Absent	Absent	Absent	Absent	Absent	Shall be absent

- **The result above demonstrates how raw water alters raw milk's quality.**

Note: cfu: colony forming unit; Adulteration tests were performed as per FSSAI DART manual for detection of adulteration with rapid tests (www.fssai.gov.in)
Microbiological tests were performed using test methods of Bureau of Indian Standards (www.bis.gov.in):

FIG:1 IN THE BAR GRAPH, THE RAW MILK QUALITY IS CLEARLY VISIBLE



- The bar graph above showed that streptococcus is the second most common bacteria after coliform bacteria.
- Areas 1 and 3 have high levels of streptococcus and E. Coli contamination.
- Areas 2, 4, and 5 have elevated levels of streptococcus contamination.
- The Second location has the lowest risk of microbiological susceptibility out of all of them.

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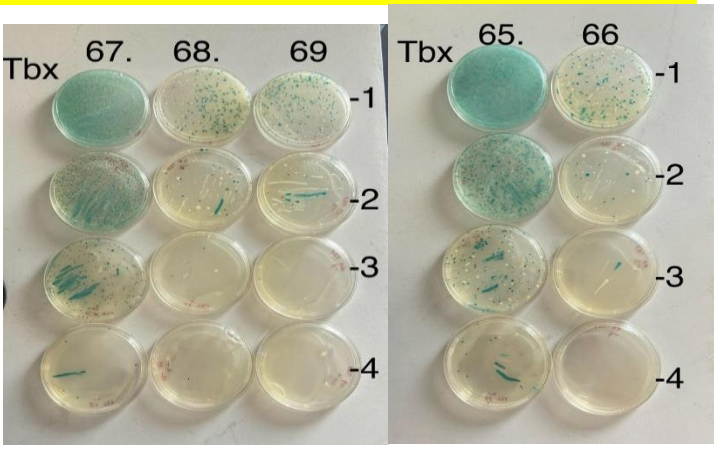
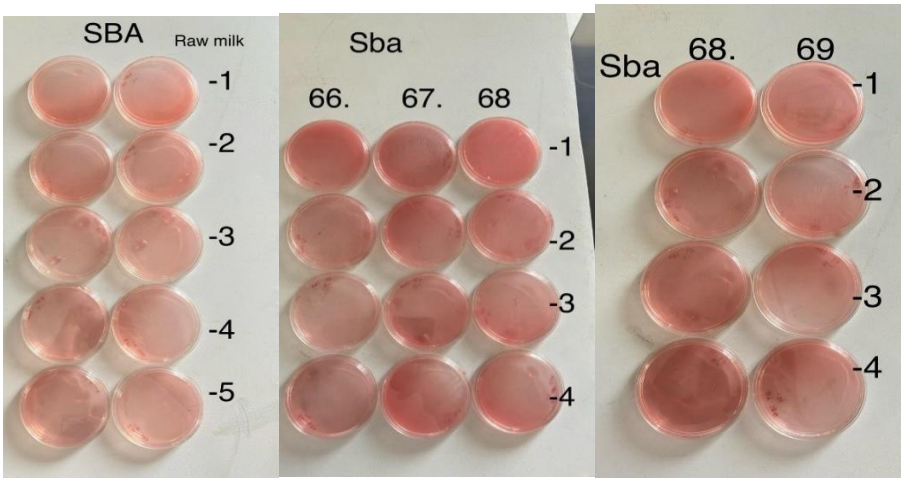
MICROBIAL SUSCEPTIBILITY TESTS IN KRYSL LAB VIJAYAWADA

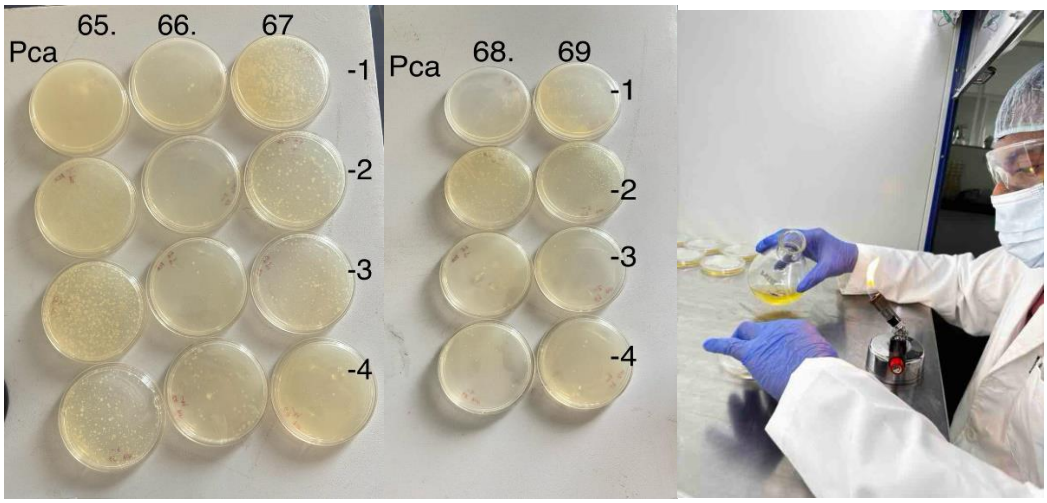
RAW MILK SUSCEPTIBILITY FOR *ESCHERICHIA COLI*



MILK SUSCEPTIBILITY FOR *STREPTOCOCCI*



RAW MILK SUSCEPTIBILITY FOR PLATE CELL COUNT



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DISCUSSIONS:

Adulteration of Raw Water in Raw Milk:

- Table 1 illustrates the moderate levels of raw water in Area Sample 2, and its impact on raw milk with low microbe levels in Figure 1.
- Both Raw water and Raw milk of Area 5 is at moderate levels of microbial suseptability
- Area 1's raw milk and raw water are both very susceptible to microbial contamination.

Raw Water Quality

After observing the table 1 we can conclude that -

- Raw water samples contain higher levels of dissolved solids and treatment measures are desired when such water is used for cleaning of milk containers.
- Raw water samples contain presence of Coliforms and Escherichia coli, bacteria which shall be absent when such water is used for cleaning of milk containers and hand washing.
- Chlorination of water sources is recommended.
- Alternatively, reverse osmosis treated water can be used for improving hygienic conditions.

Raw Milk Quality

After observing the table 2 and Fig 1 we can conclude that -

1. Pasteurized milk quality is acceptable and within acceptance limits.
2. Raw milk samples have presence of higher levels of Aerobic bacterial count, Faecal streptococci and Escherichia coli. These samples shall be processed (boiling) before use.
3. Hygiene levels to be improved during milk collection and in addition of water, usage of clean milk transport containers, cleaning of surroundings and animal milk teats during milking.

STUDY RESTRICTION

- Due to a lack of resources, the virulence and antibiotic resistance genes of the identified bacteria were not identified in this investigation.
- Raw milk without water adulteration tests is also not conducted.
- Survey for the illness is also not conducted from the area of sample collection.

CONCLUSIONS

This study revealed the presence of *Coliforms and Escherichia coli* in raw water as well as adulteration of unhygienic water practices in raw milk.

- ✓ Risk factors of health can be controlled by hygienic handling process of milk
- ✓ In the event that adding mineral water is not feasible, raw milk can still be treated with filterate or reverse osmosis water, which has the potential to reduce microbial contamination.
- ✓ Milk Boiled to 63°C (150°F) for at least 30 minutes

SIGNIFICANCE OF THE STUDY:

Milk Adulteration and Hygienic practices in dairy farm have been thoroughly studied and documented in earlier publications. But the quality of water adulteration in raw milk and microbial susceptibility study in raw water and raw milk in the Eluru district, however, have not yet been documented. Consequently, by this study, the baseline data may be established.

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