STUDENT STUDY PROJECT

ON

"ENUMERATION AND IDENTIFICATION OF MICROORGANISMS PRESENT IN VARIOUS RAW MILK SAMPLES"

Department of Microbiology

Dr. BRR Government Collage, Jadcherla
Mahabubnagar– 509301

Accredited by NAAC with "B++ "Grade// An ISO 9001-2015 Institution
Mahabubnagar (Dist.), Telangana State, India – 509301

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CERTIFICATE

This is to certify that the project work entitled **"Enumeration And Identification Of Microorganisms Present In Various Raw Milk Samples" Jadcherla, Mahabubnagar District, and Telangana. "**is a bonafide work done by the students of **III MZC (EM) Mr. B. Sai Vamshi, Miss. K.Vineetha Bai, Miss. M. Umadevi, Miss. P. Mounika, Mr. T. Pedda Chennaiah** under my supervision for the award of Project Work in Microbiology, Department of Microbiology, Dr. BRR Government College, Jadcherla and the work hasn't been submitted to any other College/University either in part nor in full, for the award of any degree.

Signature of Head of the Department

SumeraTabasuum
Lecturer of Microbiology

26/5/2023
Signature of external examiner

Signature of Internal Examiner

Signature of Principal
PRINCIPAL
Dr. B.R.R. Goverment Degree College
JADCHERLA

DECLARATION

We are hereby declaring that the project work entitled with **"Enumeration and Identification of microorganisms present in various raw milk samples.Jadcherla, Mahabubnagar District and Telangana. "**Is a genuine work done by us under the supervision of **Sumera Tabassum,** for the Department of Microbiology, Dr.BRR. Government College and it has not been under the submission to any other Institute/University either in part or in full, for the award of any degree.

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ABSTRACT

Lactobacillus species play a major role in raw milk and also contribute to the therapeutic aspects of human health. Microorganisms present in raw milk, including milk from cows, sheep, goats and humans. Milk, due to its high nutritional content, can support a rich micro biota. These microorganisms enter milk from a variety of sources and, once in milk, can play a number of roles, such as facilitating dairy fermentations. Raw milk sample was used in this study to isolate and identify the lactobacillus and to find out the incidence of Lactobacillus in raw milk. Biochemical tests were used for identification of isolates of Lactobacillus from raw milk up to the genus level. A total 16 samples of local dairy products including cow milk, buffalo milk, sheep, and goat were collected from different areas of Jadcherla. Enumeration serial dilution revealed from $10^{-1}$to 10^{-4} based on serial dilution and characterization of bacteria from raw milk samples that showed Lactobacillus, Escherichia coli, Streptococcus. The present investigation indicated that unhygienic and poor sanitary practices or practiced by the village formers for the production of handling of raw milk

Keywords: Lactobacillus, Biochemical, Antimicrobial

INTRODUCTION

INTRODUCTION:

Milk is a highly nutritious food that can be obtained from a variety of animal sources such as cows, goats, sheep and buffalo, as well as humans, for human consumption. However, the high nutrient content of these milks, which includes proteins, fats, carbohydrates, vitamins, minerals and essential amino acids all at a near neutral pH and at a high-water activity, provides an ideal environment for the growth of many microorganisms. It is generally accepted that the lactic acid bacteria (LAB), a group of bacteria that ferment lactose to lactate, are a dominant population in bovine, goat, sheep and buffalo milk, prior to pasteurisation. In India milk is produced in non standardized and is usually supplied to the consumers of the urban and rural areas by milkmen for fulfilling consumers demand production of quality milk is necessary. Milk provides a favourable environment for the growth of microorganisms. Yeasts, moulds and a broad spectrum of bacteria can grow in milk, particularly at temperatures above 16°C. Microbescan enter milk via the cow, air, feedstuffs, milk handling equipment and the milkmaid. Once microorganisms get into the milk their numbers increase rapidly.

Escherichia coli are the predominant facultative anaerobe of the human colonic flora. The organism typically colonizes the infant gastrointestinal tract within hours of life. E.coli is differentiated on the basis of pathogenic features, emphasis will be placed on the mechanisms of diseases and the development of diagnostic techniques based on the virulence factors.Lactobacilli can be found in rich carbohydrate – containing niches, including those associated with animal's raw milk. Streptococcus is a harmful bacterium, can cause different infections. These infections range from minor illness and deadly diseases. These usually reach the milk, via the milker, from equipment either contaminated by humans or not adequately disinfected. In the past, infections by streptococcus have been spread by raw milk, but this risk has been eliminated by pasteurization.

OBJECTIVES

- Isolation and identification of Enterobacteriaceae form different Raw Milk samples.
- Bacterial Isolation Characterization from Raw Milk.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

- Particularly for LAB, the physiological and molecular mechanisms in response to growth temperature have been studied due to the impacts on food processing (De Angelis and Gobbetti, 2004).
- According to Konig and Berkelmann-Löhnertz (2017) and Sanchez et al. (2019), ingeneral the optimal growth temperature of lactobacilli is between 30 to 40°C; however, there are strains that can grow at temperatures ranging from 2 at 53°C as was observed for L. plantarum strains that were able to grow at low temperatures (4°C to 16°C) (Dalcanton et al., 2018). Likewise, other authors have found that some Lactobacillus spp.
- The growth pattern of probiotics can be modified by changing their nutritional factors and their physiological stage. Meanwhile, high intensity ultrasound (HIUS) can be employed to increase probiotics' biomass. The one-factor-at-a-time (OFAT) approach was employed to investigate the influence of the growth medium (MRS broth, whole milk, and skim milk), culture age (1 day and 7 days old) and ultrasound parameters (time and amplitude) on the kinetic parameters of L. acidophilus. The oldest culture (7 days) had a greater lag phase and time to reach the end of the sigmoidal curve (Tmax) ($p < 0.05$) as well as a lower rate (maximum growth potential max) compared to the youngest culture (1 day). Regarding the growth medium, skim milk presented the greatest L. acidophilus counts ($p < 0.05$).
- The presence of E. coli organisms in milk and milk products is an indication of unsanitary production and\or improper handling of either milk or milk utensils (El-zubeir and Ahmed, 2007&Olfa et al., 2013.
- Milking udder with sub-clinical mastitis and wet environment lead to contamination of bulk tank milk and hence raw milk reaches the consumers with elevated Coliform count (FAO, 2008; Zadoks et al., 2007).
- The result of present study is in line with Endale et al. (2013) at different critical points in Mekelle (44.4%);11.1% at farm level, 11.1% at milk vending shops, 22.2% at cafteria and Vahedi et al. (2013) in 42 (42%) from raw cow milk; but it is higher than that reported by Olfa et al.
- (2013) 13 out of 50 milk samples (32.5%) were contaminated with E. coli in Sfax, Tunisia from raw cow's milk from different localities.

STUDY AREA

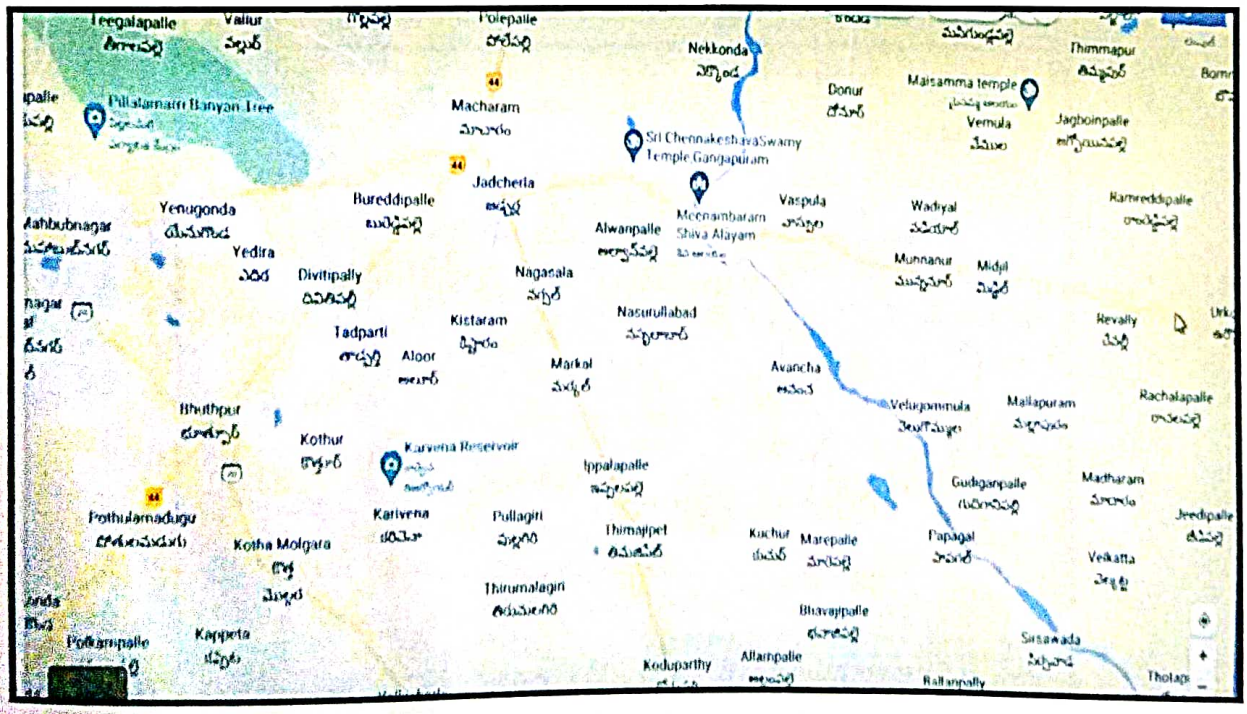
STUDY AREA:

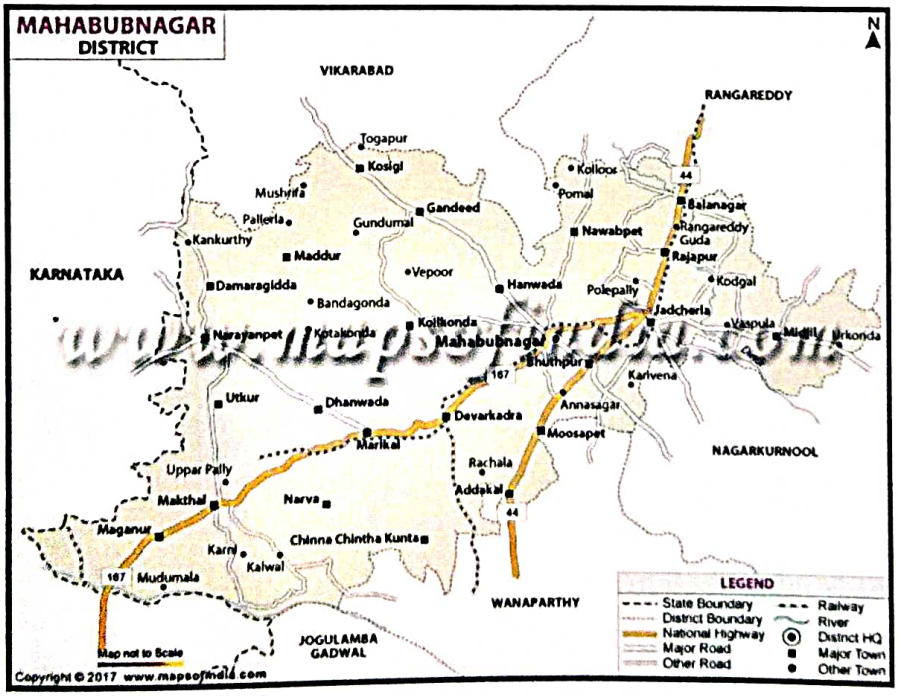
Jadcherla is a town in Mahbubnagar district in the state of Telangana. It is located in Jadcherla mandal in Mahbubnagar revenue division.Jadcherla is located at 16.7738°N 78.1367°E and at an altitude. Jadcherla is located 86 km from Hyderabad 130 km from Kurnool and 21 km from Mahabubnagar.

We collected the different raw milk samples from different areas and villages in Jadcherla Mandal.

Places where we collected the raw milk samples are named below:

- Divitipally
- Thimmajipet
- Alwanpalle
- Gollapalli
- Kaverammapata
- Kothapally
- Marchal
- Midjil

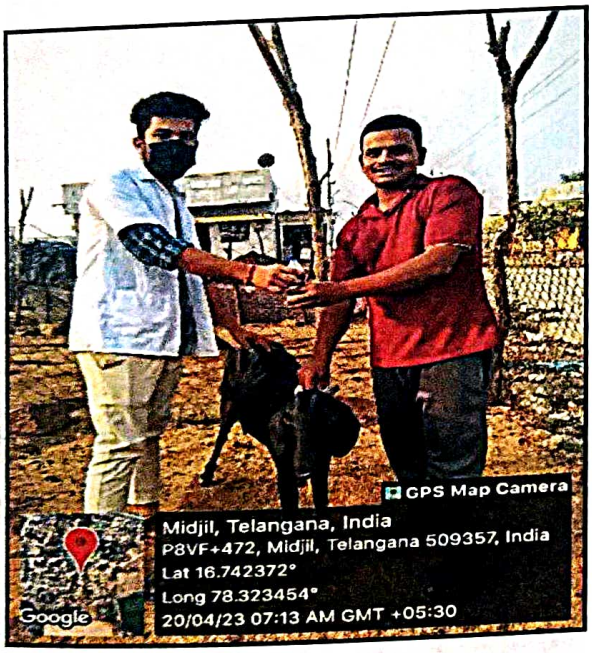


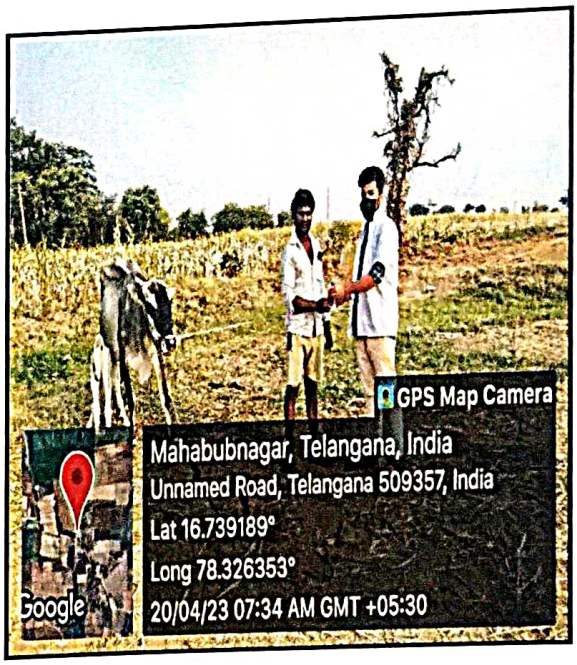


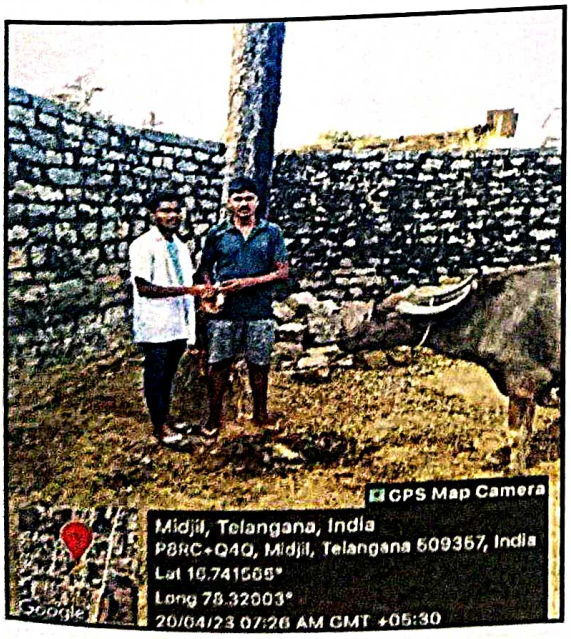
MATERIALS AND METHODS

1. Sample collection:

- We collected the different milk samples on early morning from different villages and areas locality of Jadcherla, Mahabubnagar dist.
- Total 16 milk samples in a sterilized screw cap tubes without any contamination from 4 different dairy animals (cow, buffalo, sheep and goat) from each village
- We took some photos from Gps camera while collecting the samples in villages.
- The collected samples were kept in the refrigerator and transported to the laboratory. For further examinations all the possible precautions were taken to avoid contamination.

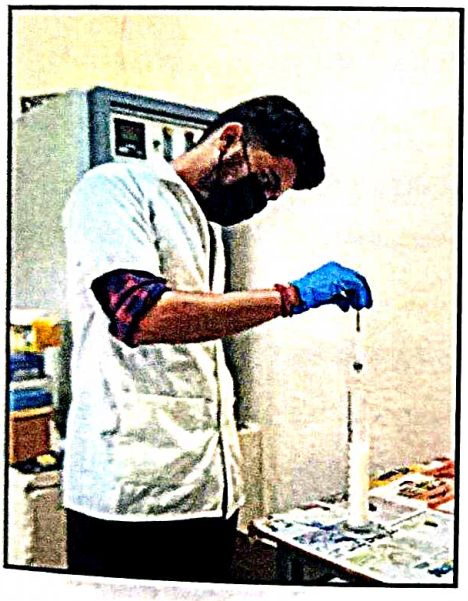


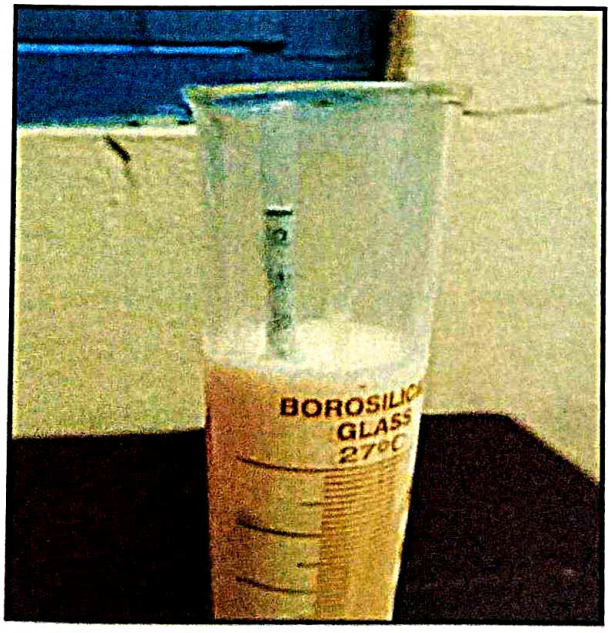




2. Analysis of milk density:

All milk samples were tested for pH specific gravity and by visually nasally and lingual to determine colour, flavour and texture by taking 16 different individuals and mean values were taken by lactometer. For milk testing, we taken a measuring cylinder and poured the pure milk in it and lactometer is dipped in milk. In lactometer, the pointup to which it sinks in the pure milk is marked after that adds water in that milk and marked at the point up to which it sinks in water. It sinks less in milk than water because as we know milk is denser than water.





3. Serial Dilution:

- Set up the sterilized glass test tubes in a rack. Label each tube clearly to indicate the dilution of its contents after the fold serial dilution of its contents after the fold serial dilution has been carried out 10-1 to 10-5.
- Use a measuring cylinder to dispense 10 ml of distilled water to the first sterile labeled test tube and then add 9 ml of distilled water to remaining sterile test tubes.
- Use a micro pipette to transfer 1 ml of the raw milk sample to the first tube and mix the sample properly with the diluents by vortex shaker this is the first ten- fold sample.
- Use micropipette equipped with a new sterile tip for carrying out a second 10-fold diluting.
- Continue the series of ten-fold diluting until you reach the final test tube.
- Repeat the same process for all milk samples with new sterile test tubes.

4. Preparation of media:

- The isolation of bacteria the raw milk samples was carried out using selective Medias such as,
- Eosin Methylene Blue Agar (EMB).
- De Man, Rogosa& Sharpe agar (MRS).
- Xylose lysine Deoxycholate agar (XLD).
- First, we weighed 13.4 gm of MRS Agar on weighing balance for 200ml of water and this mixture was poured in 250ml of conical flask and sealed with cotton plug.
- Then we weighed 7.0 gm of EMB Agar for 200ml of water and this mixture was poured in 250ml of conical flask and sealed with cotton plug.
- For XLD Agar we taken 11 gm for 200ml of water in 250ml of conical flask and sealed with cotton plug.
- All these materials are placed in autoclave.
- An Autoclave is a machine that provides a physical method of sterilization by killing bacteria, spores present in the material put inside of the vessel using steam under pressure.

5. Pouring and Streaking Method:

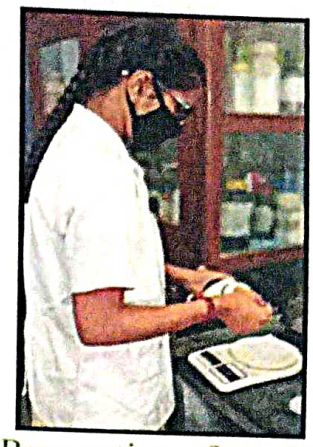
- The **Pour Plate Method** is a plating technique that is commonly used for obligate and anaerobic bacteria.
- This technique is used to isolate microbial colonies by serial dilution and then counting the colonies.
- After Autoclave, we taken the Medias out from the autoclave and transported to laboratory.
- For Pour Plate Technique we taken the sterile Petri plates and poured the different liquid medias in each Petri plate and leaved it for solidification.
- After solidification of media, we done streaking method.
- For, **Streak Plate Method** Sterilize all the instruments, which are required for the streaking procedure.
- Set up the Bunsen burner and we sanitized our hands before handling the process.
- We label the Petri dish such as name of the media used and the culture being inoculated.
- To pick a sample we used inoculation loop which is sterilized by heating it in burner.
- A loopful of sample is streaked on the first quadrant in a back-and-forth motion on the agar plate.
- Sterilize the inoculation loop by heating it in the Bunsen burner.
- Repeat the same streaking process by using other samples on different agar Medias.

6. Incubation:

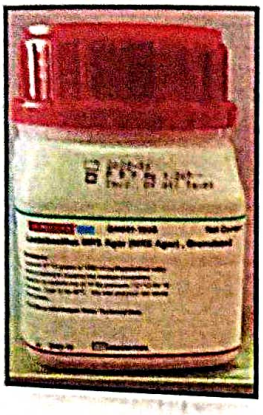
- An incubator is a device used to grow and maintain microbiological cultures or cell cultures.
- Incubate the all-Petri plates for 24 to 48 hours to grow the cultures.
- The incubator maintains optimal temperature, humidity and other conditions such as the CO_2 and oxygen content of the atmosphere inside.

Serial Dilution of milk samples

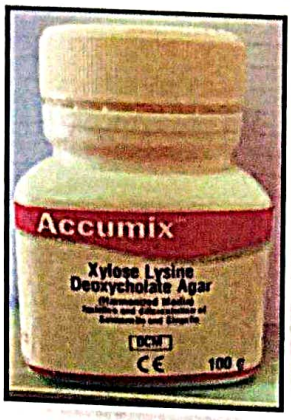
Serial Diluted milk samples



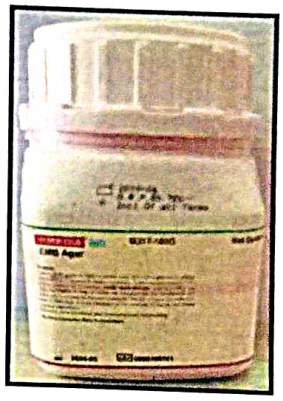
Preparation of media



MRS – Media



XLD - Media



EMB- Media

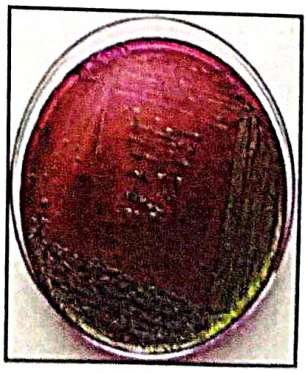
Autoclaving of Media

Pouring and inoculation of milk samples

Incubation of sample inoculated plates

Colonies on MRS media

Colonies on XLD- Media



Colonies on EMB- Media

7. Gram Staining:

Principle:

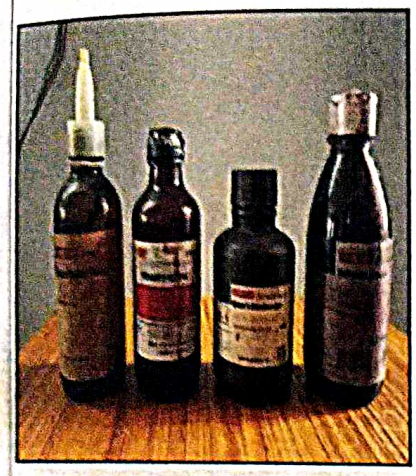
The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Gram-positive microorganisms have higher peptidoglycan content, whereas gram-negative organisms have higher lipid content. A Gram stain helps diagnose harmful bacteria. Under a Gram stain, different kinds of bacteria change one of two sets of colours (pink to red or purple to blue) under a special series of stains and are categorized as "gram-negative" or "gram-positive," accordingly.

Reagents Used in Gram Staining:

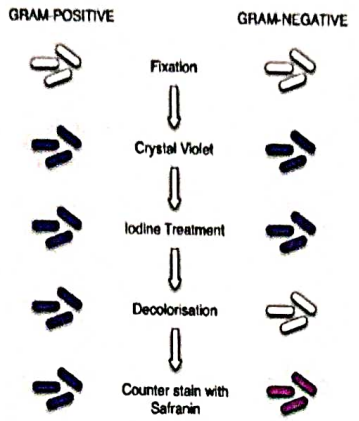
- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counter stain

Procedure:

- Take a clean, grease free slide.
- Prepare the smear of suspension on the clean slide with a loopful of sample.
- Air dry and heat fix.
- Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
- Flood the gram's iodine for 1 minute and wash with water.
- Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
- Add safranin for about 1 minute and wash with water.
- Air dry, blot dry and Observe under Microscope.

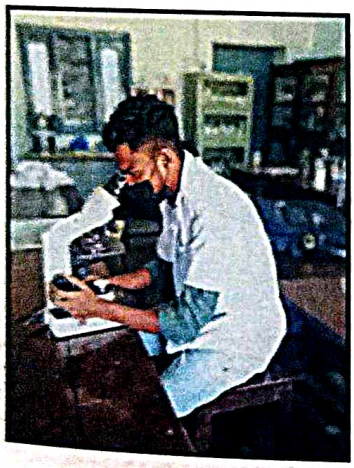


Gram Staining Reagents

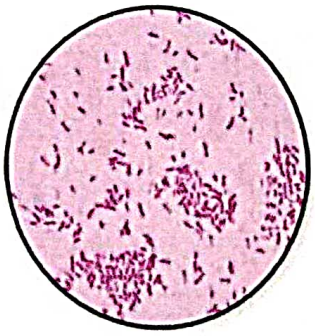


Gram staining Procedure

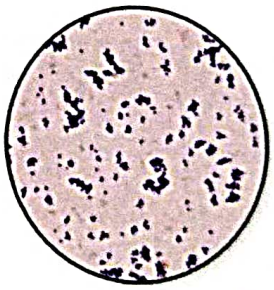
Doing of Gram Staining



Observation Under Microscope



Gram Negative Bacteria



Gram Positive Bacteria

BIOCHEMICAL TESTS

8.Biochemical Test:

I. Indole Test

II. Methyl Red Test

III. Citrate Test

IV. Catalase Test

V. Urease Test

I. Indole test

Principle:

Indole tests are a biochemical process, which is used to identify the indole producing organism from trypton. Trypton is an important amino acid which is found in most bacteria cell protein.

Reagents:

- Indole Kovac's reagents
- P-Dimethylaminocinnamaldehyde [DMACA] - 10.0gm
- Hydrochloric Acid,37% -100.0ml
- Amyl alcohol - 750.0ml

Procedure:

Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours. To test for indole production, add 5 drops of Kovác'sreagent directly to the tube. A positive indole test is indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within secondsof adding the reagent. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy. Indole positive bacteria: E. coli, Vibrio cholera Indole negative bacteria: Klebsiella, Salmonella, Shigella sp.

II. Methyl Red Test & Voges Proskauer test

Principle:

Some bacteria have the ability to utilize glucose and convert it to stable acid like lactic acid, acetic acid or formic acid as the end product. These bacteria initially metabolize glucose to pyruvic acid, which is further metabolized the '" mixed acid pathway to produce the stable acid. The type of acid produced differs from species to species depends on the specific enzymatic pathways present in the bacteria. The acid so produced decreases the PH to 4.5 or below, which is indicated by a change in the colour of methyl red from yellow to red.

In the methyl red test, the test bacteria are grown in a broth medium containing glucose. If the bacteria has the ability to utilize glucose with production of stable acid, the colour of methyl red changes from yellow to red, when added in to the broth culture.

The mixed acid pathway gives 4 mol of acidic products (mainly lactic acid and acetic acid), 1 mol of neutral fermentation product, 1 mol of CO2, and 1 mol of H2 per mol of glucose fermented. The large quantity of acids produced causes a significant decrease in the PH of culture medium.

Reagents:

- MRVP broth (pH 6.9)
- Ingredients per litre of deionizer water
- Buffered peptone = 7.0 gm
- Glucose= 5.0 gm
- Dipotassium phosphate = 5.0 gm
- Methyl red solution = 0.02%
- Dissolve 0.1 gm of methyl red in 300 ml of ethyl alcohol, 95%
- Add sufficient distilled after to make 500 ml
- Store at 4 to 8 degree C in a brown bottle. Solution is stable for 1 year.

Procedure:

- Prior to inoculation, allow medium to equilibrium to room temperature.

- Using organisms taken from an 18-24 hours pure culture, lightly inoculate the medium.
- Incubate aerobically at 37 degrees C for 24 hours.
- Following 24 hrs. of incubation, aliquot 1ml of the broth to a clean test tube.
- Re incubate the remaining broth for an additional 24 hrs.
- Add 2 to 3 drops of methyl red indicator to aliquot.
- Observe for red color immediately.

III. Citrate Utilization Test

Principle:

Sodium citrate is considered as both a carbon source and an energy source. Nitrogen source is NH_4^+. The presence of enzymes such as citrate permease [citrase] facilitates the citrate into bacterium. Bromothymol blue is considered as a PH indicator for the citrate utilization test oxygenises required and the process is done on the slants. The citrate is oxidised from bacteria and is extracted with the release of CO_2 from the medium. Sodium from sodium citrate combines with CO_2 and water, to form an alkaline product [sodium carbonate]. The change in the PH of the solution gives details about the presence and absence of the test. Colour change of the solution to blue indicates the absence of the citrate test. If there is no colour shift, that means there is no growth in the medium which indicates the absence of the citrate test.

Reagents:

- Simmon's citrate agar [PH 6.8]
- Bromothymol blue indicator solution
- Koser'sliquid citrate medium [PH .6.8]

Procedure:

- Prepare Simmon citrate agar in test tubes, taking 5 ml medium by autoclaving for 30 minutes tilt the test tube containing melted citrate medium to prepare distinct slant and butt.
- Inoculate the given sample of organism were on the slant of the media using sterile inoculation loop and label the tubes
- Incubate the tubes at 37°C for 24 -48 hours.
- Observe the color change in the medium

IV. Catalase test

Principle:

The enzyme Catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of Catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

$$2H_2O_2 \rightarrow\rightarrow\rightarrow\rightarrow\rightarrow\rightarrow 2H_2O+O_2 \text{ (gas bubbles)}$$

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Reagents:

3% hydrogen peroxide

Procedure:

Place a microscope slide inside a Petri dish. Keep the Petri dish cover available. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H2O2 onto the organism on the microscope slide and observe for immediate bubble formation (O2 + water = bubbles). Observing for the formation of bubbles against a dark background enhances readability.

V. Urease test

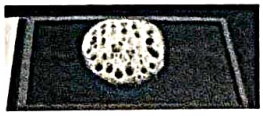
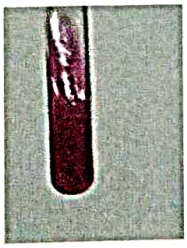
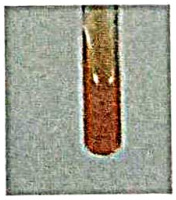
Principle:

Urease is the product of decarboxylation of amino acids. Hydrolysis of urea produces ammonia and CO2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the colour change of phenol red from light orange at pH 6.8 to magenta (pink) at pH 8.1Rapid urease-positive organisms turn the entire medium pink within 24 hours. Weakly positive organisms may take several days, and negative organisms produce no colour change or yellow as a result of acids production.

$$\underset{\text{Urea}}{(NH_2)_2CO} + 2\,H_2O \xrightarrow{\text{Urease}} \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O} + \underset{\text{Ammonia}}{2\,NH_3}$$

Procedure:

Christensen's Urea Agar (4, 5) Use a heavy inoculum from an 18 to 24 hours pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a colour control. Incubate tubes with loosened caps at 35^{0}C.Obserbe the slant for a colour change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt. Note that any degree of pink is considered a positive in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea should be used. Rapidly urease-positive Protease (Proteus spp., Organelle morganii, and some Providencia stuartii strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., Klebsiella or Enterobacter) will typically produce a weak positive reacti0on on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish colour if the organism is urease negative.

Test	Positive Result	Negative Result
Indole test		
Methyl Red Test		
Citrate Utilization Test		
Catalase test		
Urease test		

DRUG SUSCEPTIBILITY OR RESISTANCE OF MICROORGANISMS

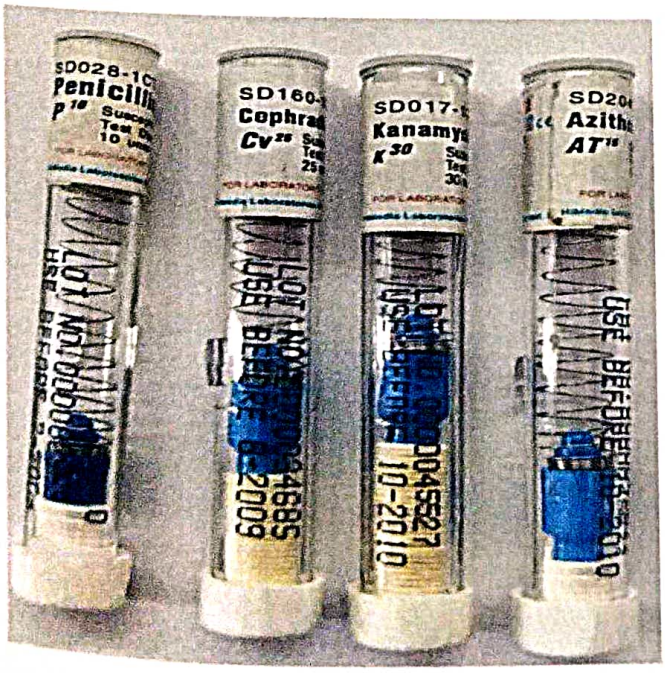
Determination of Antimicrobial Activity

The Antimicrobial activities exhibited by lactobacillus species which indicates the cell free solution of isolated lactobacillus species were able to inhibit the growth of all the test microorganisms. This experiment clearly indicates that the inhibitory metabolites produced by isolated lactobacillus species.

Penicillin-G, Azithromycin, Kanamycin, Cephradine

Procedure:

- The purpose of antimicrobial test is lactobacillus have ability to resist the pathogenic bacteria.
- Prepare a nutrient agar medium by placing it in autoclave to sterile the media
- Pour the media in petri palates and leave it for solidify.
- After solidification of media, take the bacteria culture with the help of loop and spread the culture.
- And place the discs (Penicillin-G, Azithromycin, Kanamycin, Cephradine) on culture media.
- Incubate the petri plates for 24 to 48 hours.

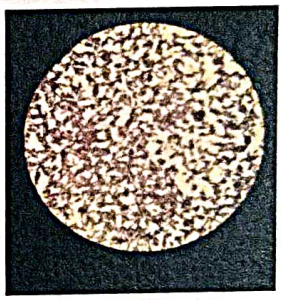
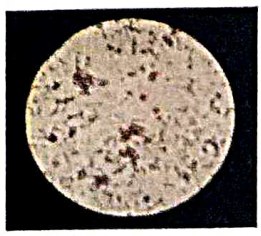


RESULTS

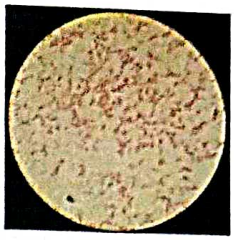
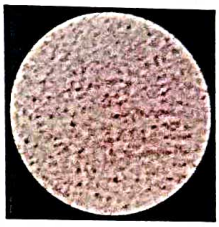
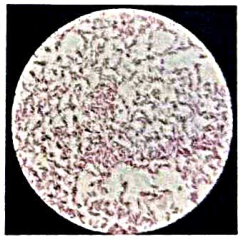
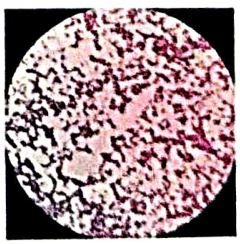
PHYSICAL EXAMINATION OF RAW MILK SAMPLES

S.NO	VILLAGE NAME	SAMPLE NAME	COLOR	FLAVOUR	TEXTURE
1	MIDJIL	COW 1	CREAMY WHITE	SWEET AROMA	CREAMY
2	DIVITIPALLY	COW 2	WHITE	NORMAL	CREAMY
3	MARIKAL	COW 3	OPAQUE WHITE	NORMAL	CREAMY
4	ALWANPALLY	COW 4	WHITE	FLAT	NORMAL
5	GOLLAPALLY	BUFFALO 1	WHITE	NORMAL	CREAMY
6	KAVERAMMAPETA	BUFFALO 2	WHITE	NORMAL	CREAMY
7	KOTHAPALLI	BUFFALO 3	CREAMY WHITE	SWEET AROMA	THIN
8	MARIKAL	BUFFALO 4	WHITE	NORMAL	CREAMY
9	MIDJIL	GOAT 1	OFF WHITE	FLAT	CREAMY
10	DIVITIPALLY	GOAT 2	WHITE	NORMAL	CREAMY
11	MARIKAL	GOAT 3	WHITE	NORMAL	CREAMY
12	ALWANPALLY	GOAT 4	WHITE	NORMAL	WATERY
13	GOLLAPALLY	SHEEP 1	WHITE	NORMAL	CREAMY
14	KAVERAMMAPETA	SHEEP 2	WHITE	SWEET AROMA	CREAMY
15	KOTHAPALLI	SHEEP 3	OFF WHITE	NORMAL	CREAMY
16	MARIKAL	SHEEP 4	WHITE	NORMAL	CREAMY

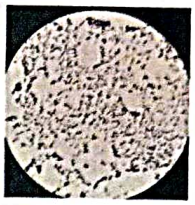
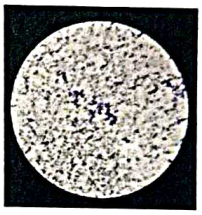
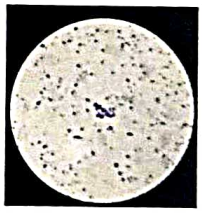
GRAM STAINING OF MRS

S.NO	MILK SAMPLE	MEDIA USED	COLONY MORPHOLOGY	GRAM STAINING	MICROSCOPIC VEIW
1.	Cow	MRS	Long, slender rods to shots	Negative	
2.	Goat	MRS	Long, slender rods to shots	Negative	
3.	Sheep	MRS	Long, slender rods to shots	Negative	
4.	Buffalo	MRS	Long, slender rods to shots	Negative	

GRAM STAINING OF EMB

S.NO	MILK SAMPLE	MEDIA USED	COLONY MORPHOLOGY	GRAM STAINING	MICROSCOPIC VEIW
1.	Cow	EMB	Rod Shaped	Negative	
2.	Goat	EMB	Rod Shaped	Negative	
3.	Sheep	EMB	Rod Shaped	Negative	
4.	Buffalo	EMB	Rod Shaped	Negative	

GRAM STAINING OF EMB

S.NO	MILK SAMPLE	MEDIA USED	COLONY MORPHOLOGY	GRAM STAINING	MICROSCOPIC VEIW
1.	Cow	XLD	Mucoid or smooth	Positive	
2.	Goat	XLD	Mucoid or smooth	Positive	
3.	Sheep	XLD	Mucoid or smooth	Positive	
4.	Buffalo	XLD	Mucoid or smooth	Positive	

BIOCHEMICAL TEST RESULTS

Lactobacillus Bacteria

S.NO	MILK SAMPLES	INDOLE TEST	METHYL RED TEST	CITRATE TEST	CATALASE TEST	UREASE TEST
1.	COW	--	--	--	--	--
2.	GOAT	--	--	--	--	--
3.	SHEEP	--	--	--	--	--
4.	BUFFALO	--	--	--	--	--

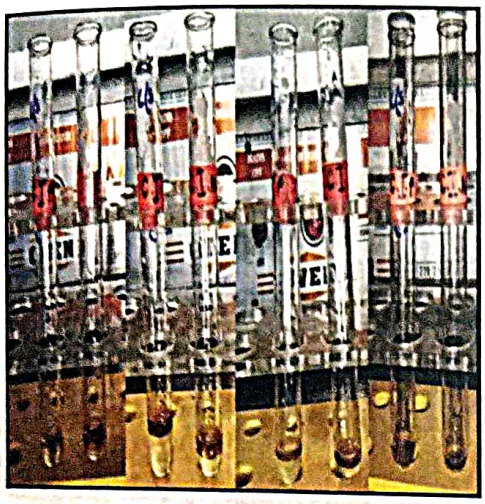
Escherichia Coli Bacteria

S.NO	MILK SAMPLES	INDOLE TEST	METHYL RED TEST	CITRATE TEST	CATALAS TEST	UREASE TEST
1.	COW	+	+	+	+	–
2.	GOAT	+	+	+	+	–
3.	SHEEP	+	+	+	+	–
4.	BUFFALO	+	+	+	+	–

Streptococcus Bacteria

S.NO	MILK SAMPLES	INDOLE TEST	METHYL RED TEST	CITRATE TEST	CATALASE TEST	UREASE TEST
1.	COW	+	+	–	+	–
2.	GOAT	+	+	–	+	–
3.	SHEEP	+	+	–	+	–
4.	BUFFALO	+	+	–	+	–

BIOCHEMICAL TEST IMAGES

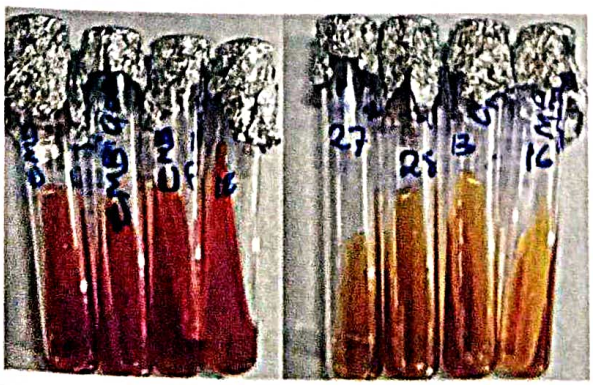


INDOLE TEST

MR – VP TEST

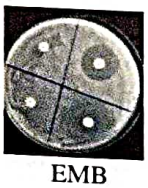
CITRATE TEST

CATALASE TEST



UREASE TEST

DRUG SUSCEPTIBILITY OR RESISTANCE OF MICROORGANISMS

S.NO	MICROORGANISM		PENICILLIN-G	AZITHROMYCIN	KANAMYCIN	CEPHRADINE
1	LACTOBACILLUS	 MRS	-	+	+	-
2	ESCHERICHIA COLI	 EMB	-	+	+	-
3	STREPTOCOCCUS	 XLD	-	+	+	-

RESULTS

In the field of Dairy microbiology lots of works regarding Milk and its products safety and security issues have been addressed; like the specific endogenous oxidative stress (identification strategy of microbial contaminants in the milk and milk products is still in its infancy. Nevertheless, a few earlier researches reported that microbial contamination in milk and milk products could take place from three principal sources: inside the udder; the exterior of the udder and the surface of milk handling; and storage equipment. Our project investigation also showed a microbial contamination in most of the samples. All samples were found to harbour the total viable bacteria and, were biochemically identified. The organisms like E. coli, Streptococci spp and lactobacillus spp. were found in all sample. In this study, some isolates showed drug resistance .All the three Isolates were found to be highly resistant against Pencilline – G and Cephradine antibiotics used. The overall study showed that all the pathogenic isolates exhibited the partial drug resistance. According to the current study results, the presence of microorganisms in the studied samples is sufficiently indicative of health risk upon consumption of the milk tested unless appropriate microbiological measures are not taken.

CONCLUSION

Present study revealed the presence of a range of pathogenic bacteria which were of public health significance. Maintenance of proper hygiene during handling and processing of milk as well as proper application of sterilization procedure such as pasteurization and UHT could ensure milk quality and most importantly consumers' safety.

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