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RESEARCH ARTICLE

# Isolation and Characterization of Bacteria Isolated from Ice Cream Samples in Hyderabad, India

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## Abstract

**Milk is a nutrient rich source of proteins. It also containing water, fat, and lactose. Ice-cream is milk based product providing nearly equal nutrients as compared with he whole milk. So, It can be serve as a good source for the growth of microorganisms as milk. In the present study, 10 ice-cream samples were collected and evaluated for the presence of various microorganisms. The collected samples were subjected or the isolation and identification of bacteria. The spread plate technique was used. About 25 bacterial species were isolated from the samples. These bacteria were characterized on the basis of morphological,cultural and biochemical tests. Those are *Escherichia coli* (8 species), *Enterobacter* (3 species), *Klebsiella* (7 species), *Proteus* (3) and *Staphylococcus* (4 species). Further, antibiotic sensitivity test was also performed these results showed that the most of the ice creams are in poor standard. As ice cream is a rich source of milk nutrient i.e, casein (milk protein), lactose (milk sugar), added sugars and cream rich in lipid, it acts as a good medium for growth and multiplication of microorganisms mainly bacteria and fungi. The organisms isolated in this study are mostly mesophilic, but they can also lead a latent life in psychrophilic.**

**Keywords:** Ice-cream, Bacterial isolates, *Escherichia coli*, Hyderabad.

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## INTRODUCTION

Milk is a nutritious basic food. It is rich in proteins, carbohydrates, fats, calcium, phosphorus, riboflavin and other B vitamins[1]. There are several kinds of milk products available and among them the most important is ice cream. The ice cream is frozen dairy product. The commercial ice cream production began in 1851 with the invention of the hand cranked freezer in 1846[2]. Water ice was definitely known in the year 1950. Eventually, the milk and cream were added into these mixtures making a product rich in taste, flavor and texture. Ice cream is a sweetened frozen food made from milk and cream which is typically sweetened with sugar[3-5]. This product resembled our present day ice cream. The popularity of ice cream is attributed to its refreshingly cool and sweet characteristics[5]. Though the origin of ice cream goes back to a few centuries, the future of ice cream seems endless. The percentage of the milk ice cream is 70% utilization for of total milk produced in the country. India produces annually 313 million liters of ice cream and related products. The growth rate of ice cream industries was estimated to be 25%-35%. The richness is nutritive constituents of ice cream. Although has been realized by all but the problem lies in the production and handling of this food is very complex and is associated with many problems. Since, ice cream is a milk-based product, it is rich in all milk nutrients and act a growth medium for various microorganisms. The growth and multiplication of containing microorganisms are possible during production, storage, handling, transportation and marketing of these products. The contaminating microorganisms in the food are traced in ingredients added and environmental factors such air, faults in storage tank, and crack in the plant and packaging materials.

Ojokoh[6] studied ice cream samples from the local market of Akure, Nigeria. He found that the samples contains *Staphylococcus* species, *Klebsiella* species and *Aspergillus* species (About 90% occurrence) and *Streptococcus* species (10% occurrence). He reported presence of three types of mould in the ice cream[6]. Similar studies were reported from various other regions[7-9]. Till now the reported bacterial species are *Alkaligenes, Bacillus cereus, Bacillus subtilis, Brucella abortus, B. melitensis, Cornybacterium diphteriae, Enterococcus faecium, E. faecalis, Enterobacter aerogenese, E. liquefaciens, Escherichia coli, Klebsiella, Micobacterium tuberculosis, Proteus, Pseudomonas, Salmonella, Shigella, Staphylococcus aureus, Streptococcus pyogenese* and *Vibrio*. Various mould (*Absidia, Alternaria, Aspergillus, Fusarium, Mucor, Neurospora, Penicillium* and *Rhizopus*) and yeasts (*Candida, Geotrichum, Rhodotorula, Saccharomyces*) were also reported in many studies[10-11].

With this background, in the present study, we have collected 10 samples from a local market of Hyderabad, India which then spread on a nutrient agar plate. The microorganism growth was observed. The isolated microorganisms were characterizes, identified and represented as colony forming units (CFU)/gm of sample.

## MATERIAL AND METHODS

In the present study, 10 different ice cream samples available in the markets were collected. They were evaluated of the presence of bacterial load.

### Collection of samples

The ice cream samples were collected from the local shops and kept in a frozen state till analysis.

### Preparation of sample

The collected sample (1ml) was aseptically transferred in to 9ml sterile distilled water. From this tube, 1ml of the sample were transfered to next dilution tube containing 9ml sterile distilled water. The diutions were processed till $10^{-6}$ dilution. From this dilution, 0.1ml sample was spread on nutrient agar plates to know total cfu/gm of the sample.

### Isolation of fungi

Serial diluted 0.1ml ice cream sample was inoculated on the potato dextrose agar (PDA) medium plates and incubated at room temperature for 48 to 72hrs. The intermitted observations were noted for the growth of fungi.

### Isolation of bacteria

### Isolation of coliforms

A loopful of ice cream sample was streaked on eosin methylene blue (EMB) agar plates for the isolation of coliforms. The plates were incubated at 30°C for 24hrs. The isolated coliforms were further confirmed by using lactose broth.

**Detection of *Staphylococcus aureus***

A loopful of ice cream sample was streaked on the Mannitol salt agar (MSA) media and incubated at 37°C for 24 hrs. The observed was done for the growth of *S.aureus*.

**Detection of *Salmonella***

Bismuth sulfite agar was used to isolate *Salmonella* spp. The ice cream samples (2.5g) were transferred into 22.5ml buffered peptone water and it was incubated at 37°C for 12hrs. Each pre-enrichment broth (5ml) was transferred to 10ml of tetrathionate broth and incubated at 42°C for 24hrs. Followed by transferring on sulfite agar and was incubated at 37°C for 24hrs. The plates were examined after 24hrs to 48hrs for typical colonies of *Salmonella* spp.

**Microscope observation**

The smears were examined under low power (10x) and then under oil immersion (100x) objectives for size, shape and arrangement of cells.

**Motility test**

The semisolid agar medium was stabbed with loopful of culture, incubated at 37°C for 24hrs the motility was confirmed by diffused growth of the organism around the stabbed area.

**Biochemical tests**

IMViC test was performed by using the test culture method.

**Indole test**

Indole broth was inoculated with the test culture (7ml) and incubated at 37°C for 24hr. The positive test gives a red ring following the addition of Kovac s reagent. The absence of red ring indicates a negative result.

**Methyl Red test**

MR-VP medium was used for methyl red test. The sterile glucose broth was inoculated with the test culture 7ml and incubated at 37°C for 24hr. Methyl red was added after incubation. Change in colour indicates a negative result.

**Voges Prokauer test**

The glucose broth was inoculated with the test culture (6ml) and incubated at 37°C for 24 hr. Barrit s reagent was added after incubation, change in colour of the broth from yellow to pink indicates positive results. Negative test gives no colouration.

**Citrate utilization test**

Simmons citrate agar was used for this test. The test organism was inoculated in Simmons citrate agar slants. The tubes were incubated at 37°C for 24hr. Positive results indicated by the growth of the colonies with blue colouration in the medium. While, negative results showed no growth and the medium was remain green.

**Catalase test**

A drop of 3% of hydrogen peroxide was placed on a glass slide along with a loopful of culture. The occurrence of brisk effervescence was observed for a positive test.

**Oxidase test**

A small amount of culture was streaked smoothly on an oxidase disc (Tetra methyl Para Phenylene Diamino Dihydrochloride) with a wire loop. A positive reaction was indicated by an intense deep purple colour appearing with in five to ten seconds.

**Rapid urease test broth**

Rapid Urease test Broth is used for rapid detection of urease production.

**Gelatin hydrolysis test**

There are several methods for determining gelatinase production, all of which make use of gelatin as the substrate. The standard and most commonly employed method is the nutrient gelatin stab method. The bacteria were streak on the gelatin stab and incubated at37°C for 24 hours. In positive test, the gelatin hydrolysis was observed in the culture.

**Hydrogen sulphide test**

This medium contains ferrous ammonium sulfate and sodium thiosulfate, which together serves as indicators for the production of hydrogen sulfide. Hydrogen sulfide production was detected when ferrous sulfide, a black precipitate, was produced as a result of ferrous ammonium sulfate reacting with $H_2S$ gas.

**Sugar fermentation test**

One drop of culture was inoculated into individual carbohydrate broth tubes and incubated at 37°C for 18-24 hours and observed for color change from blue to yellow due to acidity from fermentation.

**Identification of molds from ice cream sample**

Dilutions of the sample homogenate were prepared as in standard plated count method. (Saitou and Neil et al., 1987) about 0.1 ml of the diluted sample were plated on the previously solidified Sabouraud´s Dextrose agar medium and spread with asterile L-rod. Then, the plates were

incubated at 24°C for 3 days and colonies were observed.

**Effect of temperature, pH and salinity on growth of bacterial isolates**

All bacterial isolates were inoculated into nutrient broth and maintained at different pH range (pH 5, pH8 and pH9), temperature range (24°C, 40°C and 45°C) and different NaCl concentrations (2%, 5% and 7%). The bacterial growth was monitored after incubation for 24 hr.

**Antibiogram of bacterial isolates from ice cream**

The Kirby Buure test is a qualitative assay whereby disks of filter paper are impregnated with a single concentration of different antibiotics or any chemicals that will diffuse from the disk into the agar. The gentamycin, streptomycin, norfloxacin, ofloxacin and amoxicillin antibiotic were used in the present study. Antibiotics disks are placed on the surface of agar plate which has already been inoculated with test bacteria. The plates were again incubated for next 24 hr at 37°C. The area of zone of inhibition was measured from the growth culture.

## RESULTS

In the present study, the attempt has been made to isolate and characterized the bacterial cultures form the collected ice-ream samples.

**Collection of the ice-cream samples**

A total of 10 ice cream samples from different areas of Hyderabad was collected for isolation of bacteria on nutrient agar medium. A spread plate method was performed and the bacterial colonies were counted. The details about the samples were depicted in the supplementary data 1.

**Isolation of fungi from ice cream samples**

Only two samples out of 10 samples showed presence of fungi. These fungi are mucor and Penicillium spp.

**Isolation of bacteria from ice cream samples**

The colony count of each sample represented as CUF/gm of sample is depicted in Table 1.

**Microscopic examination of isolated cultures**

The isolated colonies were examined under the microscope and their characters were studied in detailed. Total 25 bacteria were isolated

**Table 1.** Colonies count of each collected sample

| S. No. | Name of ice cream | Count/gram |
|---|---|---|
| 1. | Cream bell | 17×10 |
| 2. | Kwality walls | 40×10 |
| 3. | Amul koolfi pista malai | 20×10 |
| 4. | Cream Ball | 40×10 |
| 5. | Amul | 2×10 |
| 6. | Dairy treat kulfi | 20×10 |
| 7. | Kwality walls feast choco bar mini | 10×10 |
| 8. | Kwality walls cornelto mini chocolate | 10×10 |
| 9. | Kwality walls paddle pop jiggly jelly | 50×10 |
| 10. | Dairy treat | 14×10 |

**Table 2.** Morphological characteristics of bacterial isolates from ice cream samples

| S. No. | Isolate | Shape | Gram staining | Motility |
|---|---|---|---|---|
| 1. | MKII 1 | Rods | Negative | Motile |
| 2. | MKII 2 | Rods | Negative | Motile |
| 3. | MKII 3 | Rods | Negative | Non-motile |
| 4. | MKII 4 | Rods | Negative | Non-motile |
| 5. | MKII 5 | Rods | Negative | Motile |
| 6. | MKII 6 | Rods | Negative | Motile |
| 7. | MKII 7 | Rods | Negative | Motile |
| 8. | MKII 8 | Rods | Negative | Motile |
| 9. | MKII 9 | Rods | Negative | Motile |
| 10. | MKII 10 | Rods | Negative | Motile |
| 11. | MKII 11 | Rods | Negative | Non-motile |
| 12. | MKII 12 | Rods | Negative | Motile |
| 13. | MKII 13 | Rods | Negative | Non-motile |
| 14. | MKII 14 | Cocci | Positive | Non-motile |
| 15. | MKII 15 | Rods | Negative | Motile |
| 16. | MKII 16 | Rods | Negative | Motile |
| 17. | MKII 17 | Rods | Negative | Non-motile |
| 18. | MKII 18 | Cocci | Positive | Non-motile |
| 19. | MKII 19 | Cocci | Positive | Non-motile |
| 20. | MKII 20 | Rods | Negative | Motile |
| 21. | MKII 21 | Rods | Negative | Non-motile |
| 22. | MKII 22 | Rods | Negative | Motile |
| 23. | MKII 23 | Rods | Negative | Motile |
| 24. | MKII 24 | Rods | Negative | Non-motile |
| 25. | MKII 25 | Rods | Negative | Non-motile |

**Table 3.** Biochemical test of the bacterial isolates from ice cream samples

| Isolate No | Indole production | Methyl red test | Vogues proskeur | Urease test | Catalase test | Oxidase test | H2S test | Gelatin hydrolysis test | Citrate Utilization test |
|---|---|---|---|---|---|---|---|---|---|
| MKII 1 | + | + | - | - | + | - | - | - | - |
| MKII 2 | - | - | + | - | + | - | - | - | + |
| MKII 3 | - | - | + | + | + | - | - | - | + |
| MKII 4 | - | - | + | + | + | - | - | - | + |
| MKII 5 | + | + | - | - | + | - | - | - | - |
| MKII 6 | + | + | - | - | + | - | - | - | - |
| MKII 7 | + | + | - | - | + | - | - | - | - |
| MKII 8 | + | + | - | + | + | - | + | + | - |
| MKII 9 | + | + | - | + | + | - | + | + | - |
| MKII 10 | - | - | + | - | + | - | - | - | + |
| MKII 11 | - | - | + | + | + | - | - | - | + |
| MKII 12 | - | - | - | - | + | + | - | + | + |
| MKII 13 | - | - | - | + | + | - | - | - | + |
| MKII 14 | - | + | - | - | + | - | - | + | - |
| MKII 15 | + | + | - | - | + | - | - | - | - |
| MKII 16 | + | + | - | - | + | - | - | - | - |
| MKII 17 | - | - | - | + | + | - | - | - | + |
| MKII 18 | - | + | + | - | + | - | - | + | - |
| MKII 19 | - | + | - | - | + | - | - | + | - |
| MKII 20 | + | + | - | + | + | - | + | + | - |
| MKII 21 | - | - | - | + | + | - | - | - | + |
| MKII 22 | + | + | - | - | + | - | - | - | - |
| MKII 23 | + | + | - | - | + | - | - | - | - |
| MKII 24 | - | - | + | - | + | - | - | - | + |
| MKII 25 | - | - | + | - | + | - | - | - | + |

**Table 4.** Sugar fermentation test of the bacteria isolates from ice cream samples

| Isolate | Glucose | Lactose | Manitol | Sucrose |
|---|---|---|---|---|
| Isolate | Glucose | Lactose | Manitol | Sucrose |
| MKII 1 | Acid and gas | Acid and gas | - | Acid |
| MKII 2 | Acid and gas | Acid and gas | - | - |
| MKII 3 | Acid and gas | Acid and gas | - | Acid and gas |
| MKII 4 | Acid and gas | Acid and gas | - | Acid and gas |
| MKII 5 | Acid and gas | Acid and gas | - | - |
| MKII 6 | Acid and gas | Acid and gas | - | Acid |
| MKII 7 | Acid and gas | Acid and gas | - | Acid |
| MKII 8 | Acid and gas | - | - | Acid and gas |
| MKII 9 | Acid and gas | - | - | Acid and gas |
| MKII 10 | Acid and gas | Acid and gas | - | - |
| MKII 11 | Acid and gas | Acid and gas | - | Acid and gas |
| MKII 12 | - | - | - | - |
| MKII 13 | Acid and gas | Acid and gas | - | Acid and gas |
| MKII 14 | Acid | Acid | Acid | Acid |
| MKII 15 | Acid and gas | Acid and gas | - | Acid |
| MKII 16 | Acid and gas | Acid and gas | - | Acid |
| MKII 17 | Acid and gas | Acid and gas | - | Acid and gas |
| MKII 18 | Acid | Acid | Acid | Acid |
| MKII 19 | Acid | Acid | Acid | Acid |
| MKII 20 | Acid and gas | - | - | Acid and gas |
| MKII 21 | Acid and gas | Acid and gas | - | Acid and gas |
| MKII 22 | Acid and gas | Acid and gas | - | - |
| MKII 23 | Acid and gas | Acid and gas | - | Acid |
| MKII 24 | Acid and gas | Acid and gas | - | - |
| MKII 25 | Acid and gas | Acid and gas | - | Acid and gas |

**Table 5.** Influence of temperature, pH and salt concentration of growth of bacterial isolates.

| S. No. | Bacterial Isolate | Effect of temperature | | | Effect of pH | | | Effect of NaCl | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 30°C | 40°C | 45°C | 5 | 8 | 9 | 5% | 8% | 9% |
| 1. | MKII 1 | + | + | - | + | + | - | - | - | - |
| 2. | MKII 2 | + | + | - | + | + | - | - | - | - |
| 3. | MKII 3 | + | + | - | + | + | - | - | - | - |
| 4. | MKII 4 | + | + | - | + | + | - | - | - | - |
| 5. | MKII 5 | + | + | - | + | + | - | - | - | - |
| 6. | MKII 6 | + | + | - | + | + | - | - | - | - |
| 7. | MKII 7 | + | + | - | + | + | - | - | - | - |
| 8. | MKII 8 | + | + | - | + | + | - | + | + | + |
| 9. | MKII 9 | + | + | - | + | + | - | - | - | - |
| 10. | MKII 10 | + | + | - | + | + | - | - | - | - |
| 11. | MKII 11 | + | + | - | + | + | - | - | - | - |
| 12. | MKII 12 | + | + | - | + | + | - | - | - | - |
| 13. | MKII 13 | + | + | - | + | + | - | - | - | - |
| 14. | MKII 14 | + | + | - | + | + | - | + | + | + |
| 15. | MKII 15 | + | + | - | + | + | - | - | - | - |
| 16. | MKII 16 | + | + | - | + | + | - | - | - | - |
| 17. | MKII 17 | + | + | - | + | + | - | - | - | - |
| 18. | MKII 18 | + | + | - | + | + | - | + | + | + |
| 19. | MKII 19 | + | + | - | + | + | - | + | + | + |
| 20. | MKII 20 | + | + | - | + | + | - | - | - | - |
| 21. | MKII 21 | + | + | - | + | + | - | - | - | - |
| 22. | MKII 22 | + | + | - | + | + | - | - | - | - |
| 23. | MKII 23 | + | + | - | + | + | - | - | - | - |
| 24. | MKII 24 | + | + | - | + | + | - | - | - | - |
| 25. | MKII 25 | + | + | - | + | + | - | - | - | - |

**Table 6.** Colony characteristic on cystine-lactose-electrolyte-deficient agar medium.

| Bacteria | Colony characteristic |
|---|---|
| *E.coli* | Opaque yellow colonies with a slightly deeper yellow center |
| *Proteus* | Translucent blue colonies |
| *Klebsiella* spp | Yellow to whitish-blue colonies, extremely mucoid |
| Enterobacter | Small yellow colonies |
| *Staphylococcus* spp | Pale yellow to deep yellow colonies |

from the collected ice-cream samples. They were named as MK II 1 to MKII 25. Shape of MK II 1 to MKII 25 bacterial isolates and their motility were observed. In gram staining, only three bacteria were gram positive while remaining 22 bacterial spp. were gram negative. The morphological characteristics are depicted in the Table 2.

**Biochemical tests**

The results of biochemical tests (indole production, methy red, vogues proskeur, urease, catalase, oxidase, $H_2S$ test, gelatin hydrolysis and citrate utilization test) are given in the Table 3. It was interesting that all the isolates showed catalase activity, *in vitro*.

**Sugar fermentation test**

All isolates except MKII 12 showed positive results in the glucose fermentation test. The results obtained from the various sugar fermentation test viz. glucose, lactose, manitol and sucrose is depicted in the Table 4.

**Table 7.** Biochemical identification of bacteria isolates

| S. No. | Isolate | Identification of the isolate |
|---|---|---|
| 1. | MKII 1 | *E.coli* |
| 2. | MKII 2 | *Enterobacter* |
| 3. | MKII 3 | *Klebsiella* |
| 4. | MKII 4 | *E.coli* |
| 5. | MKII 5 | *E.coli* |
| 6. | MKII 6 | *E.coli* |
| 7. | MKII 7 | *Proteus* |
| 8. | MKII 8 | *Proteus* |
| 9. | MKII 9 | *E.coli* |
| 10. | MKII 10 | *Enterobacter* |
| 11. | MKII 11 | *Klebsiella* |
| 12. | MKII 12 | *staphylococcus* |
| 13. | MKII 13 | *E.coli* |
| 14. | MKII 14 | *staphylococcus* |
| 15. | MKII 15 | *staphylococcus* |
| 16. | MKII 16 | *Proteus* |
| 17. | MKII 17 | *Klebsiella* |
| 18. | MKII 18 | *E.coli* |
| 19. | MKII 19 | *Klebsiella* |
| 20. | MKII 20 | *Klebsiella* |
| 21. | MKII 21 | *staphylococcus* |
| 22. | MKII 22 | *Klebsiella* |
| 23. | MKII 23 | *E.coli* |
| 24. | MKII 24 | *Enterobacter* |
| 25. | MKII 25 | *Klebsiella* |

**Table 8.** Antibiogram pattern of bacterial isolates

| S. No | Isolate | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 1. | MKII 1 | 1 | 0.8 | 0.1 | 0.3 | - |
| 2. | MKII 2 | 0.6 | 0.3 | 0.3 | 0.2 | - |
| 3. | MKII 3 | 0.6 | 0.3 | 0.3 | 0.2 | - |
| 4. | MKII 4 | 0.5 | 0.3 | 0.4 | 0.3 | - |
| 5. | MKII 5 | 0.9 | 0.7 | 0.2 | 0.3 | - |
| 6. | MKII 6 | 1 | 0.8 | 0.1 | 0.3 | - |
| 7. | MKII 7 | 1 | 0.8 | 0.3 | 0.2 | - |
| 8. | MKII 8 | 0.7 | 0.6 | 0.4 | 0.8 | - |
| 9. | MKII 9 | 0.7 | 0.6 | 0.4 | 0.8 | - |
| 10. | MKII 10 | 0.6 | 0.3 | 0.3 | 0.2 | - |
| 11. | MKII 11 | 0.7 | 0.2 | 0.5 | 0.1 | - |
| 12. | MKII 12 | 0.8 | 0.7 | 0.4 | 0.6 | - |
| 13. | MKII 13 | 0.5 | 0.4 | 0.4 | 0.3 | - |
| 14. | MKII 14 | 0.9 | 0.8 | 0.9 | 1 | 0.4 |
| 15. | MKII 15 | 1 | 0.8 | 0.1 | 0.3 | - |
| 16. | MKII 16 | 0.9 | 0.9 | 0.2 | 0.4 | - |
| 17. | MKII 17 | 0.6 | 0.3 | 0.4 | 0.2 | - |
| 18. | MKII 18 | 0.8 | 0.9 | 1 | 0.9 | 0.2 |
| 19. | MKII 19 | 1 | 0.7 | 0.9 | 0.8 | 0.4 |
| 20. | MKII 20 | 0.7 | 0.8 | 0.3 | 0.3 | - |
| 21. | MKII 21 | 0.6 | 0.3 | 0.3 | 0.2 | - |
| 22. | MKII 22 | 1 | 0.8 | 0.1 | 0.3 | - |
| 23. | MKII 23 | 0.9 | 0.9 | 0.2 | 0.3 | - |
| 24. | MKII 24 | 0.6 | 0.3 | 0.3 | 0.2 | - |
| 25. | MKII 25 | 0.6 | 0.3 | 0.3 | 0.2 | - |

1. Gentamycin; 2.Gentamycin; 3. Norfloxacin;
4. Ofloxacin; 5. Amoxicillin

**Effect of temperature, pH and salinity on growth of bacterial isolates**

All bacterial isolates can tolerate 40°C temperature and pH varies between 5 to 8. While, none of the isolated bacteria showed growth at 45°C temperature and 9 pH. The results of temperature, pH and salt variation are depicted in the Table 5.

The colony characteristics were summarized in the Table 6. Based on the above morphological and biochemical tests performed, it was noticed that of the twenty five isolates eight were Escherichia coli spp, three were Enterobacter species, seven were *Klebsiella* species, three were Proteus species and four were *Staphylococcus* spp (Table 7). Further, antibiotic sensitivity test was carried out.

**Antibiogram of bacterial isolates from ice cream**

All the isolated bacteria showed resistance towards gentamycin, streptomycin, norfloxacin, ofloxacin and amoxicillin antibiotic. However, there zone of inhibition were varies from 0.2 to 1cm. MKII 14, MKII 18 and MKII19 showed resistance to amoxicillin antibiotic. The antibiogram pattern of all bacterial isolates is depicted in Table 8.

## DISCUSSION

It was noticed that out of the twenty five isolates, eight are *Escherichia coli* species, three were *Enterobacter species*, seven were *Klebsiella species* and three were *Proteus* species and four were *Staphylococcus* species. Further, antibiotic sensitivity test was carried out. These results showed that the most of the ice creams are in poor standard. As ice cream is a rich source of milk nutrients i.e., casein (milk protein), lactose (milk sugar), added sugars and cream rich in lipid, it can act as good medium for growth and multiplication

of microorganisms mainly bacteria and fungi. The organisms isolated in this study are mostly mesophilic, but they can also lead a latent life in psychrophilic.

*Escherichia coli* and *Klebsiella* sp. were isolated from the samples and they are susceptible to pasteurization[7]. These bacterial apperence in the post pasteurization in ice cream may be due to defective heat process or to post pasteurization contamination by handlers with careless sanitary practices. Sanitary level of equipments, efficiency of pasteurization, health of workers & hygiene these factors contributes to microbiological aspects of ice- cream[12]. These bacteria reported to present in the food also which can described as index of food hygiene[13,14]. Other bacteria isolated were *Bacillus* sp. and *Streptococcus* sp. Their isolation specify favourable environment within the ice cream and potency of promoting growth of these organisms. Someof these bacteria can be very harmful to the health and may be capable of causing various ailments of humans which may be fatal. Some fungi (*Aspergillus* sp., *Rhizopus* sp., *Neurospora* sp.); yeasts and moulds were reported in ice cream[15].

For human consumption milk must be properly collected from a healthy, well fed female, & it should be free from colostrum. Utensils should be properly sterilized and special attention should be given towards the proper handling, cleanliness and hygiene of the workers. If ice-cream is not properly handled, not correctly made or stored it may cause food poisoning. Ice-cream is made from mixture of fat, sugar, milk solids, and emulsifying and flavoring agent which is mixed and freezing is done with simultaneous aeration of the liquid mixture. Pasteurizer is a container used to pasteurize the ice cream. These pasteurizing machines should be cleaned time to time. Utensils should be washed with warm water and sanitizing solution before use.

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