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Isolation of Bacteria from Street Food

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Abstract: Three samples were collected from different areas of Chennai and plated in nutrient agar to obtain colonies of bacteria. Then they were subjected to biochemical tests to identify different types of bacteria in the sample. The bacteria which were isolated from these samples were found out to be from the family of Enterobacteriaceae.

Keywords: Isolation, gram staining, biochemical tests, Enterobacteriaceae.

I. INTRODUCTION

There are different types of bacteria present in different types of food. They can be both beneficial and pathogenic in nature. As street food materials are exposed a lot to the open environment they have a lot of chances getting contaminated and gives a suitable environment for the pathogenic bacteria to grow. The microbiological studies of food, aids in finding different pathogenic. Microbiological evaluation of raw materials also provides important information about the heat-processing parameters that would be necessary to meet the microbiological standards, guidelines, or specifications of a product. Microbiological evaluation of a food, food ingredient, and environment helps determine possible sources of a specific microbial type in a food and in the case of heated food, the source and nature of post heat treatment contamination.

II. MATERIALS AND METHODS

A. Sample Collection From Different Areas

Three samples were collected from three different areas as listed below:

Sample A: West Mambalam (shop near railway station)

Sample B: West Saidapet (collected from street vendor)

Sample C: Alwarpet (collected from street vendor)

B. Checking The pH Of The Samples

As the street vendors and the roadsides shops only have “Khatta pani” the pH was determined with the help of the pH strip as well as a pH meter. They readings ranged between 3.0-4.5 which showed that the samples were highly acidic. The samples were highly acidic due to the presence of tamarind, lemon extract and other acidic ingredients added to it.

The pH of the samples was checked to get a preliminary idea of the nature of bacteria present in the samples.

C. Laboratory Procedure

Procedures including sample collection, bacterial culture, microscopical examination and biochemical tests were used.

1) Colony Counting

2) Media preparation

The required amount of nutrient media was weighed and then mixed with double distilled water and was autoclaved with petri plates and the test tubes filled with 9 mL of double distilled water which will used for serial dilution. The agar was allowed to cool and was poured into the sterilized petri plates.

- Serial Dilution:** 1 mL of the samples were taken and added to 9 mL distilled water which was labelled stock. 1mL from this and was transferred to the test labelled 10^{-1} and this procedure was repeated to complete the serial dilution till 10^{-7} . this was done with all the three samples.
- Plating:** 0.1 mL was taking from tubes labelled 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} and plated in nutrient agar medium. The petri dishes were incubated overnight at 37°C in inverted positions. Pure cultures were obtained by picking the colonies from the plates which were incubated earlier and inoculating them in nutrient agar.
- Gram's Staining Procedure:** The pure cultures were subjected to grams staining procedure to identify the bacteria (whether it is gram positive or gram negative) present in different samples.
- Biochemical Tests:** The samples were subjected to different tests namely Oxidase, Catalase, Indole test, Methyl red test and Vogue Proskauer test to identify the species from the family Enterobacteriaceae.

III. RESULTS AND DISCUSSION

A. Results

1) *pH of the Sample Collected:* Table No 5.1.1: shows the pH of the collected samples which shows the liquid sample (khatta pani) was highly acidic in nature.

Samples	pH Strip	pH Meter
Sample A	4.0	4.25
Sample B	3.0	3.5
Sample C	4.0	4.5

2) *Total Viable Count (TVC):* As the table below shows the total viable colony count of the samples collected. A lot of contamination was seen in 10^{-1} , 10^{-2} , 10^{-3} and clear colonies were observed in 10^{-5} , 10^{-6} and 10^{-7} .

Table no 3.1.2: Number of colonies observed in nutrient agar

Dilution Factor	Sample A	Sample B	Sample C
10^{-1}	50	45	55
10^{-2}	43	40	42
10^{-3}	35	33	34
10^{-4}	23	20	26
10^{-5}	12	14	13
10^{-6}	5	6	5
10^{-7}	4	3	2

3) *Gram Staining:* When the three samples were subjected to gram's staining procedure, Gram negative bacteria was observed.

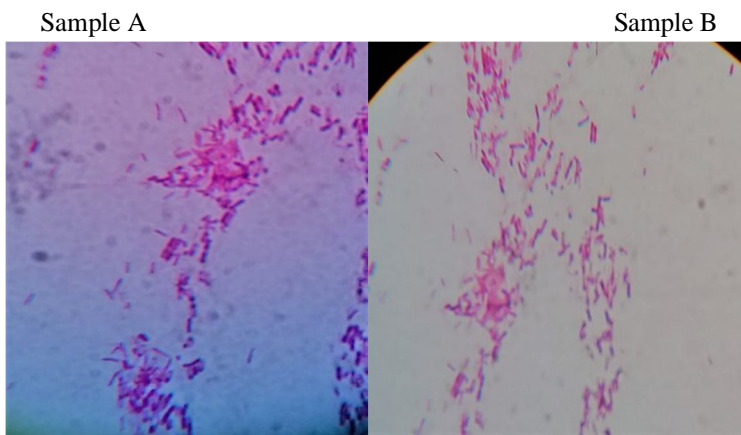


Figure 3.1.3.1- Grams staining picture of sample A (left) and sample B (right)

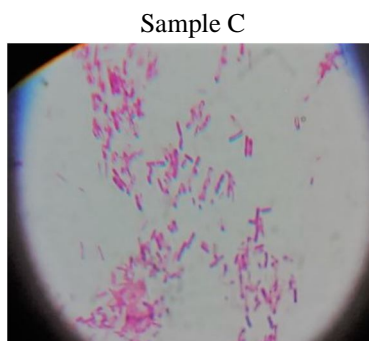


Figure 3.1.3.2- Gram staining picture of Sample C

4) *Biochemical Tests*: The table shows the results of all the three samples which were subjected to the biochemical tests.

Table 3.1.4: The results of the biochemical tests

Biochemical Tests	Sample A	Sample B	Sample C
Catalase	Positive	Positive	Positive
Oxidase	Positive	Positive	Positive
Indole	Positive	Positive	Positive
MR	Positive	Positive	Positive
VP	Positive	Positive	Positive

- a) *Catalase test*: Catalase is an enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants and animals). When a small amount of bacterial isolate is added to hydrogen peroxide, bubbles of oxygen are observed. The catalase test is done by placing a drop of hydrogen peroxide on a microscope slide.
- b) *Oxidase test*: The reagent is dark blue to maroon colour when oxidized, and colourless when reduced. Oxidase-positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron-containing haemoprotein) which reacts with the reagent and gives out purple colour.
- c) *Indole test*: This test done to check the ability of the microorganism to split amino acid tryptophan to form the compound indole. Tryptophan is hydrolysed by tryptophanase to produce three possible end products- one of which is indole. Kovac's or ehrlich's reagent which contains 4(p)-dimethyl amino benzaldehyde, this reacts with indole to produce a red coloured compound.
- d) *Methyl red test*: this test determines whether the microbe performs mixed acids fermentation when supplied glucose.
- e) *Vogues-Proskauer*: This test is used to determine if an organism produces acetymethyl carbinol from glucose fermentation.

IV. DISCUSSION

From the study the contamination was seen more in 10^{-1} with colony count of ranging from 50-55. Whereas the sample 10^{-7} was least contaminated than the others with colony count ranging 2-4. As the samples are acidic in nature, this favoured the growth of acidic bacteria. This is established from the results that in all the samples tested there were bacteria present.

This work revealed that all the food samples have different types of gram negative bacteria.

The tests revealed that the species which were present in the sample might be from the family Enterobacteriaceae etc. The presence of the species suggests the water used for the preparation was a result faecal contamination. The food samples obtained from the vendors were more contaminated as the samples were exposed to filthy, filled with flies and wastes. The vendors use the water whichever is available immediately for the preparation of food.

In general, this study demonstrated that the street vended foods which sold on the streets of Chennai are considerably contaminated. Lack of training on the proper handling and processing of food, poor personal hygiene of the vendors and unhygienic surroundings could be possible factors for observed problems in that locality. Therefore, educating the for the vendors on food safety and hygienic practices are essential to reduce contamination rate.

V. CONCLUSION

Most food borne pathogens are of soil or intestinal origin and are transmitted through poor food preparation, personal hygiene or public sanitation practices. Therefore, to ensure safety of the foods, producer and hawkers must maintain a high level of personal hygiene. Also, utensils used for the preparation should be properly cleaned.

The results suggest that there might be presence of Enteropathogenic bacteria. Such foods lead to hazardous effects to the consumers.

VI. FUTURE PERSPECTIVE

Further study should be conducted to isolate and characterize different bacterial and fungal species and know the quality of the sample collected by increasing the sample size as the current study was carried out only on small number of bacteria and small sample size.

Studies on plant sources which can be added to control the growth of a specified bacteria of interest.



VII. SUMMARY

Three samples from different places in Chennai were collected and were plated to obtain colonies. Higher contamination was observed in 10^{-1} with colony count ranging between 45-55. Gram negative bacteria were observed on grams staining. The biochemical tests catalase, oxidase, indole test, methyl red test and vogues-Proskauer test were positive. This suggested that the presence of Enteropathogenic bacteria.

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