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Screening and isolation of microbial contaminants from carbonated and non-carbonated soft drinks of Delhi

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Abstract
Foodborne pathogens are a global public health problem, their abundance in the various range of foods is posing a great danger of their spread among communities. Soft drinks are gaining popularity day by day because of various flavours retailed all over the world. This investigation was carried out so as to analyse the microbial load in carbonated and non-carbonated drinks available in the local market of Delhi. Total plate count method was performed with the different samples, among them only Banta sample showed a bacterial load of 1.7 x 10^3 Cfu/ml. Cold drinks were further analysed for the presence of enteric bacteria, Staphylococcus aureus. Presence of food associated microbes is either air-borne or water-borne which may invade into the drinks. Variety of microbial contaminants can be eliminated from the drinks by maintaining proper plant sanitation processing and storage.
Keywords: *Staphylococcus aureus, Klebsiella pneumoniae, soft drinks, coagulase test and IMViC test.*

Introduction

The soft drinks industries are rapidly growing from last few years. A variety of soft drinks are available such as still, carbonated with a different flavour and soda water. Soft drinks acquire their characteristic flavours from cola, citrus or other herbal extracts. These beverages are acidulated with edible acids [1,2].

In recent years, consumers increasing awareness of microbial safe food and drinks influenced industries to develop food with the minimal microbial load. These thrust quenchers should be free from undesirable micro-organisms. Presence of pathogenic micro-organisms may lead to food poising outbreaks among communities.

In India, transmissions of diseases through soft-drinks are due to improper sanitisation, adulteration in raw material and water. Soft drinks are made up of potable water incorporated with carbon dioxide under high pressure, sugars, permitted flavouring agents, colouring agents, preservatives, emulsifying agents, stabilising agents, acids. Due to the presence of acids, pH of the drink is low and carbonation inhibits the growth of microbes, although Escherichia coli 0157 and Salmonella species can persist for weeks in chilled, fruit juices [3,4].

Spoilage occurs in non-carbonated drinks because fungus tends to grow easily on sugar-rich drink. Some of the fungal species has been reported which even tolerate high amount of carbon dioxide. Microbial contamination leads to the deterioration of the product into an undesirable form. Deterioration may cause excessive gas production, turbidity or taints in the soft-drinks [5].

Different soft drinks including pepsi, gatorade, limca, sprite and banta (local drink) were investigated microbiologically. Banta is a traditional lemon flavoured carbonated drink widely consumed in all parts of Delhi. Banta is a soda water packed in codd bottle which enclosed with marble and rubber washer in the neck and

sells by local vendors. Empty codd bottles are filled upside down and pressure of the gas in the bottle forces the marble against the washer, sealing in the carbonation. In this study, the presence of different isolates was investigated using specific media and confirmatory tests including IMViC test, coagulase test.

Materials and Methods

Collection of sample and storage

Soft drinks were collected from local market of Delhi. Four soft drinks (pepsi, limca, sprite and banta) were carbonated, one was non-carbonated orange flavoured energy drink (gatorade) purchased in their PET bottles. All soft drink samples were stored at 4˚C until used.

Microbial analysis

Plate count method was performed to investigate the total microbial load of soft drinks. The sample was diluted in maximum recovery diluents up to 10^{-3}. The diluted samples were spread on to plate count agar and incubated at 30±1℃ for 72 hours.

To investigate the presence of coliform, soft drinks were diluted in maximum recovery diluents and was spread on violet bile red agar spreader and incubated at 37˚C for 24 hours. To calculate the number of micro-organism/ml following formula was used-

$$N = \sum C / (\boldsymbol{n_1} + \mathbf{0.1}\, \boldsymbol{n_2}) \times d$$

Where a ∑C= sum of colonies counted on all the dishes retained, $\boldsymbol{n_1}$=number of dishes retained in the first dilution, $\boldsymbol{n_2}$=number of dishes retained in the second dilution and d= dilution factor corresponding to two significant figures.

To detect *Staphylococcus aureus*, the sample was transferred into cooked meat medium and incubated for 24 hours at 37˚C. After 24 hours, samples were streaked on to Baird Parker Agar and incubate at 37˚C. To confirm the presence, gram staining was done followed by coagulase test using rabbit plasma.

To detect *E.coli*, the sample was transferred in macConkey broth inserted with Durham's tube in order to trap the gas produced by microbes and incubated at 37˚C. After 24 hours, the sample was streaked on to macConkey agar and eosin methylene blue agar. To confirm the strain, a bio-chemical test was done (IMViC).

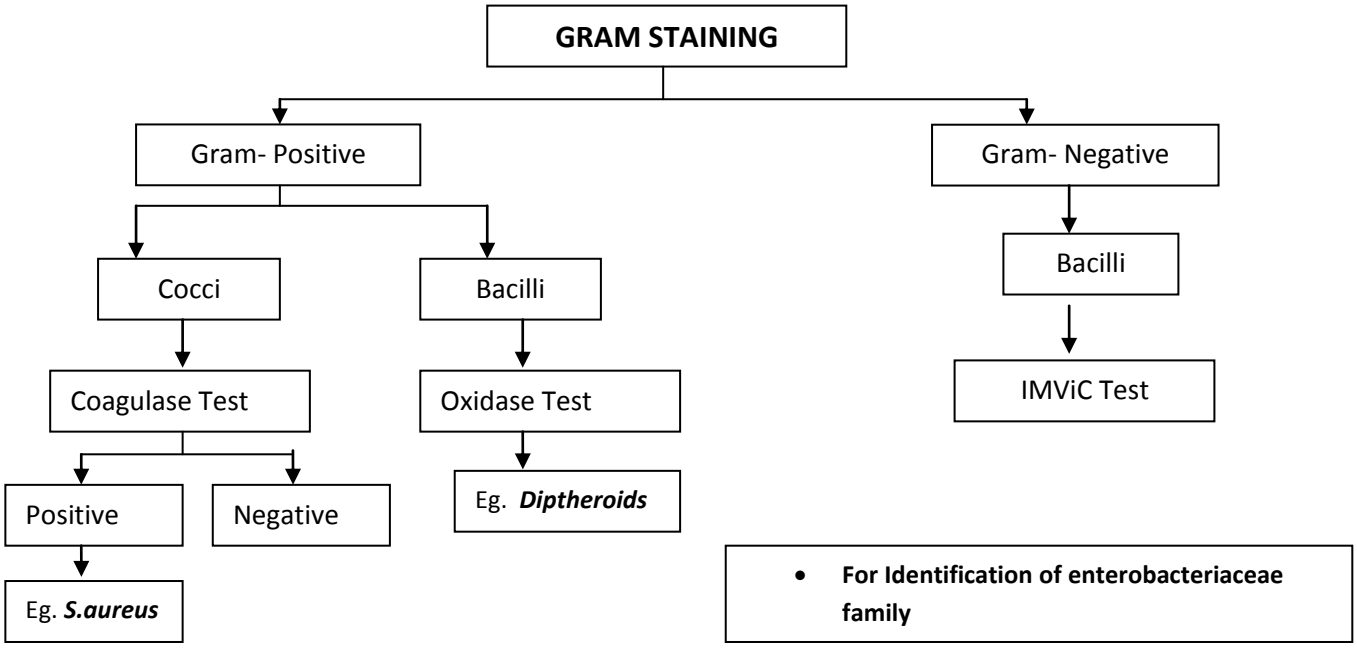

Fig. 1 Scheme for identification of the organism isolated from soft drinks

Results

In total plate count method, among five samples four samples (pepsi, gatorade, limca and sprite) shows no growth in plate count agar whereas local drink has numerous colonies. Initially, the direct sample was spread and colonies appeared which was Too Numerous to Count (TNTC) as shown in Figure 2. In subsequent dilution 10^{-1}, 10^{-2} and 10^{-3} countable colonies were appeared (Table 1). The number of micro-organisms per millilitre was 1709.09cfu/ml.

Table 1 Number of colonies were counted in duplicate plates and CFU/ml was determined

Dilutions	Plate 1	Plate 2
Direct	TNTC	TNTC
10^{-1}	144	157
10^{-2}	40	35

Coliform was not found in any of the samples. *Staphylococcus aureus* was absent in pepsi, gatorade, limca and sprite but local drink was contaminated with the specific species. Characteristic black coloured colonies appeared on baird parker agar (Figure 3a). These microbial contaminations may occur due to unhygienic handling, bottling or poor sanitation. The colonies were morphologically analysed under the microscope which appeared to be gram positive, cocci. These colonies were further tested by coagulase test. Coagulase enzyme was found on the surface of *Staphylococcus* which enables the conversion of fibrinogen into fibrin and may lead to blood clot within the human if present as a food contaminant. Colonies expressed positive coagulase result (Figure 3b).

Among lactose fermenting Enterobacter, *Escherichia coli* was not found in pepsi, gatorade, limca, sprite but few colonies were visualized in the local lemon drink (Banta). Colonies appeared pink on eosin methylene and macconkey agar plates. These were analysed through standard gram staining protocol. Under the microscope, the organism was gram negative with short rods. Further colonies were investigated biochemically through IMViC test. Organism showed positive voges proskauer and citrate test which means organism have a citritase enzyme which breakdown sole carbon source citrate into oxaloacetate lead to increase in pH due to the synthesis of sodium bicarbonate which will turn bromothymol blue into green in colour. Among Enterobacteriaceae family, *Klebsiella pneumoniae* showed positive voges proskauer and citrate test (Figure 4).

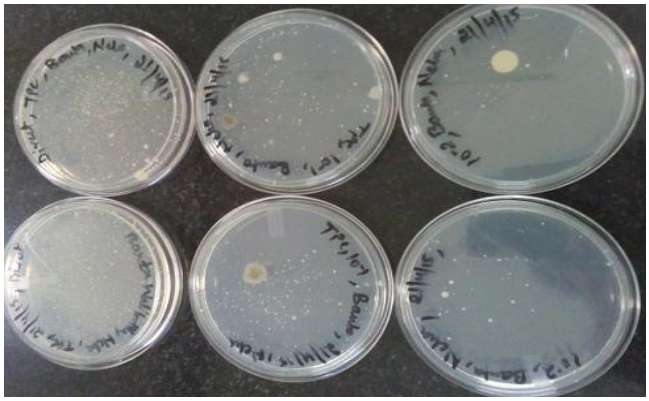

Fig. 2 Gradual decrease was reported from dilution 10^{-1} to 10^{-2} in the banta sample which confirmed positive total plate count test.

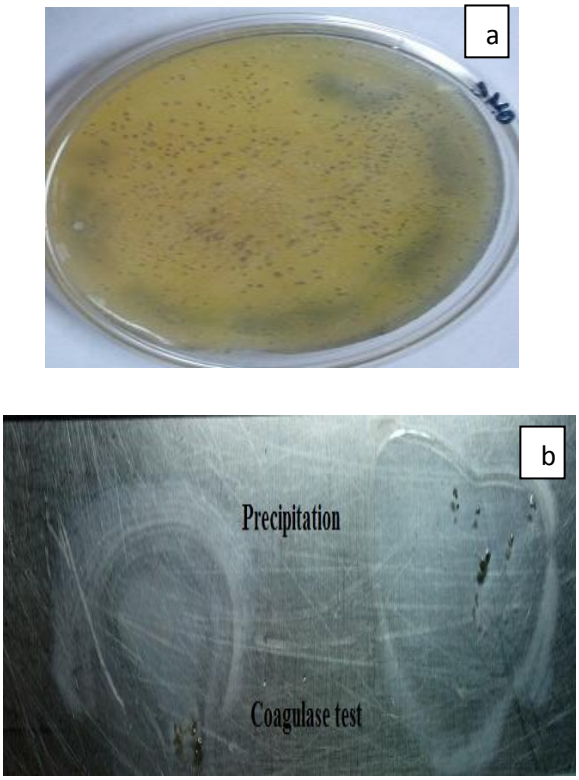

Fig. 3 Identification and confirmatory test for *Staphylococcus aureus* . a Black coloured colonies

on Baird Parker Agar in Banta sample, b Precipitation of fibrinogen indicate the presence of coagulase enzyme in *Staphylococcus aureus* species.

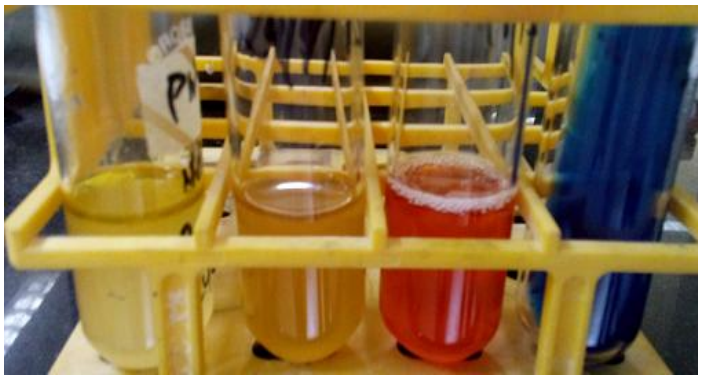

Fig. 4 Banta sample confirmed positive IMViC test for *K . pneumoniae*

Discussion

Soft-drinks are spoiled by varieties of microbes including bacteria and fungi. Microbial contamination of the beverage can occur either during processing, handling, storage or adulterated raw material[6]. Therefore proper hygiene, handling and sanitation lead to the good quality of product. It has been documented that micro-organism is becoming resistant to preservation techniques. The low pH and carbonation considerably limit the growth of number and type of micro-organisms but some are acidophilic those have a tendency to survive under acidic conditions [7].

The use of unsafe or non-potable water for the preparation of soft drinks may lead to spoiled product and undesirable product for human consumption. In this investigation, pepsi, limca and sprite was carbonated beverage which inhibits the growth of micro-organisms due to the presence acids, preservative and pressurised carbon dioxide whereas gatorade was a non-carbonated sports drink, it also did not show any growth because of proper hygiene and plant sanitization.

It was distinct that microbial flora was only found in a local beverage (Banta) collected from the local market. The major microbial contaminants were *Staphylococcus aureus* and *Klebsiella pneumoniae*. Presence of Klebsiella spc indicates that it could come from water. The presence of coagulase positive *Staphylococcus aureus* in the local drink can pose a significant hazard to human health. The coagulase enzyme was found on the cell wall of Staphylococcus aureus and this enzyme inhibits the functioning of protease and lead to coagulation of blood. Staphylococcus spc were a possible contaminant from handlers and utensils used especially during processing.

It is therefore suggested that local drink should be processed and stored properly in order to avoid contaminants. Municipal treated or non- potable water should be avoided during processing otherwise water-borne microbes may incorporate into the drink. And the processing environment should be sterilized and packing material (codd bottle) should be well sterilized. It would seem essential for public health prospective to set up control measures for all the ingredients used in the soft drinks.

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