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"Microbiological quality of fresh fruit juices and their antibiotic profile in Beed city"

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ABSTRACT

Due to the lack of knowledge regarding the relationship between the vendor's sanitary status and the level of contamination, a descriptive study was conducted in order to add to the body of comparable studies and assist the government in developing the best possible policy. Fruit juices are widely consumed in many tropical nations and are known for their high nutritional value, mineral content, and vitamin content. Methodology: The most probable number [MPN] test was used to quantify the coliform population in both water and juice samples after a sample of various fruit juices was gathered from five different public hotspot places in Beed. The research was then expanded to include the isolation of coliforms using a selective solid media and the detection of several Enterobacteria species utilising quick biochemical tests. Five fruit juice samples were gathered from five different vendors, grown on agar, subjected to pertinent biochemical analyses, and isolated. To get the average CFU for the vendor, the CFU of isolates purchased from one vendor were averaged. The sellers were questioned, and the investigator also recorded his observations of the vendors. Both were used to create an inventive hygiene score that was utilised to determine the vendor hygiene score. The scatter plot and spearman's rho were computed after statistical analysis of the vendors' average CFU.

KEYWORDS: Fresh Fruits juice, Gram staining, Antibiotic, Bacillus, staphylococcus aureus.

INTRODUCTION

Every year, India produces roughly 9 million tonnes of fruit. Fruit juices that are both packaged and freshly produced have a market potential Malik et al (2020). Due to its nutritious benefits and fresh flavour, fruit juice is thought to be the most consumed non-alcoholic beverage across all age groups Nayik et al (2013). Juices are fat-free and contain naturally occurring phytonutrients that promote better health. Juice's main components are fruit pulp, sugar, and water. To make them, the liquid pulp of mature fruits is extracted, and then it is combined with sugar, water, and ice. The body receives a lot of growth components from these juices in the form of vitamins and minerals, which has led to an increase in their consumption in recent years. The finished product is unpasteurized and unfermented for human consumption Amin et al (2018). According to Shakir et al. (2009), there is a wealth of research demonstrating the nutritional and health benefits of consuming fresh fruit juices. Fresh fruit juices are crucial parts of the human diet.Fruit juices are widely consumed in many tropical nations and are known for their high nutritional value, mineral content, and vitamin content. Juice contamination is largely caused by contaminated raw materials and equipment, extra processing conditions, inappropriate handling, and the prevalence of unsanitary circumstances (Oliveira et al., 2006). Furthermore, according to a few investigations (Chumber et al., 2007), fruit juice consumption may be linked to some food-borne disorders. Foodborne diseases are conditions brought on by consuming tainted foods or drinks. Fruit juice contains a variety of microorganisms that are similar to those found in the corresponding fruits. Mould, yeast, and bacteria are frequently present in fruits (Hariyadi, 2013). The gastrointestinal system is the principal organ affected by these food-borne illnesses. Fruits that haven't been properly washed introduce microorganisms to extracts, contaminating them. Additionally, using polluted water, living in unsanitary conditions—often with swarms of houseflies—exposing oneself to airborne dust, and the environment itself can all be causes of contamination. According to studies by Buchmann et al. (1999), Sandeep et al. (2002), and Barro et al. (2006), these juices have the potential to be sources of a variety of bacterial pathogens, including Bacillus and S. aureus. Therefore, the current study's objective is to compare the microbiological contamination of fruit juices in local Beed city markets.

MATERIAL AND METHOD

Laboratory Procedure Laboratory procedures such as sample collection, sample processing, bacterial culture, microscopical examination and biochemical tests were used to determine colony count, isolation and identification of indicator organisms and selected pathogens. The fresh unpasteurized fruit juices prepared in different street vended shops were obtained from Beed city. Collection of the Sample A total of five (5) Samples of mosambi, pineapple, orange, Grapes and pomegranate locally prepared unpasteurized fruit juices were collected randomly from different street vended shops of Beed city. All the samples were collected on a voluntary basis from participating shops and cafes in a sterilized (50) ml container aseptically, labelled and brought immediately to the laboratory after processed it immediately.

Serial Dilution a 1mL of the juice sample was added into 9mL of sterile distilled water to prepare stock solution. Then the test tubes were labelled as (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} ,10^{-5}, 10^{-6},10^{-7},10^{-8},and10^{-9}). After that, 1mL from the mixture sample was transferred into the first test tube10^{-1}shacked well in order to get equal distribution of microorganisms. And then, 1mL from the first test tube was transferred into the next test tube and again shacked. Finally, the procedure was repeated to complete the serial dilution up to 10^{-9}. Gram Stain Procedure The slide was placed with heat fixed smear on staining tray. Drop of crystal violate(primary stain) was added and allowed for 60 seconds and then washed with distilled water and also one drop of Iodine (mordent) was added and then allowed for 60 seconds after this washed by distilled water. Then 95% ethyl alcohol (decolarilzation) was added and allowed for 30 seconds then washed by distilled water. After that safranin (Counter stain) was added and allowed for 60 seconds and then washed with distilled water. Finally, the slide was put under microscope and then the purple and pink colour from a single colony of the slide under microscope was observed. Then, the purple colour from a single colony of the slide under microscope indicated that the bacteria were gram positive whereas the pink colour under microscope indicated that the bacteria were gram negative. Microscopic investigation for Gram reaction and morphological features of suspected colony was determined using standard method of Gram„s staining. Biochemical test Several biochemical tests were carried out in order to have a presumptive identification of the potential bacteria chosen before. Most of the methods were done according to the microbiology laboratory manual. The biochemical tests performed were catalase, coagulase, oxidase, IMViC test [Iodole production test, Methyl red test, Voges -Proskaur test, Citrate utilization test], and Urease test. Catalase test: In this test, 1-2 drops of hydrogen peroxide solution were poured on slide. Isolated colony of bacteria was taken with the help of a sterile inoculating loop and immersed in the hydrogen peroxide solution. The slide was left for bubbling. Coagulase Test: In this test, one drops of normal saline solution was taken on a clean slide and a single isolated colony was mixed in saline solution and left for few minutes for clotting.

IMViC test: IMViC reaction are a set of four useful reaction that are commonly employed in the identification of microbes. IMViC test consist of four different tests each of the letters in “IMVIC” stand for one of these tests I- Indole, M- Methyl red, V- Voges- proskuer, C- Citrate. Indole test: Some bacteria can produce indole from amino acid tryptophan using the enzyme tryptophanase. Production of indole is detected using Ehrlich‟s reagent or Kovac‟s reagent. Indole reacts with the aldehyde in the reagent to give a red colour. An alcoholic layer concentrates the red colour as a ring at the top. Methyl red (MR) test: This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in colour at a pH of 4.4 or less. Voges Proskauer (VP) Test: While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol Acetyl-methyl carbinol (acetone) is an intermediate in the production of butylene glycol. In these test two reagents, 40% KOH and alphanaphthol were added to test broth after incubation and exposed to atmospheric oxygen. If acetone is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha- naphthol to produce red colour. Role of alpha-naphthol is that of a catalyst and a colour intensifier. **Citrate utilization Test:** This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing Simmons citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen. Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO2. Production of Na2CO3 as well as NH3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium’s colour from green to blue. Urease test: The urease test identifies that organism that are capable of hydrolysing urea to produce ammonia and carbon dioxide. Urea agar is used to detect urease activity in a variety of microorganism. For urea agar, urease production is indicated by a bright pink colour on the slant and incubated at 370C for 24 hours. The culture medium will remain a yellowish colour if the organism is urease negative, and the culture medium will remain a pink colour if the organism is urease positive. **Antibiotic sensitivity test:** The antibiotic sensitivity test was done using the different antibiotic drug. Such as penicillin, ofloxacin, ceftriaxone, oxacillin, gentamycin, erythromycin, doxycycline, ciprofloxacin, vancomycine, ampicillin. The bacterial suspensions were prepared and 0.1 ml of sample was spread separately on the NAM media. Then antibiotic disc was applied over it. Kept the plates on incubator at 370C for 24 hours.

RESULT

According to the above conducted experiment, Bacillus, Staphylococcus, and Lactobacillus isolated from the Mosambi juices. Staphylococcus aureus isolated from the pineapple juices. Bacillus cereus, Streptococcus, Staphylococcus epidermidis isolated from the orange juices. Bacillus, Streptobacillus isolated from the Grapes juices. Bacillus anthrecis, Staphylococcus aureus isolated from the pomegranate juices. Bacillus and Staphylococcus are the most preveling bacteria from all the juices samples.

Table 1: Colony characterization of Bacteria.

S.no	Margin	Color	Elevation	Texture	Shape
1.	Wave undulate	Milky	Flat Shape	Mucoid	Round
2.	Smooth	Brown	Flat	Slimy	Punctiform
3.	Entire smooth	White	umbonate	Dry, mucoid	Irregular
4.	filamentous	White	Flat	Dry	Root like
5.	Entire smooth	Opaque/white	Convex	Slimy	punctiform

Table 2: Microscopic examination of bacteria.

Sr.no.	Juices Sample	Gram's staining	colour	Shape	Bacteria
1.	Pomegranate	Gram"s staining Gram"s staining	Purple purple	Rod Coccus	Bacillus anthracis Staphylococcus aureus
2.	Pineapple	Gram"s staining	Purple	Coccus	Staphylococcus aureus
3.	Orange	Gram"s staining Gram"s staining	Purple Purple Purple	Short rod Coccus in cluster Coccus	Strepto-Bacillus Rod
4.	Grapes	Gram"s staining Gram"s staining	Purple Purple	Rod Rod in chain	Bacillus cereus Streptococci Staphylococcus epidermidis
5.	Mosambi	Gram"s staining Gram"s staining Gram"s staining	Purple Purple Purple	Rod Coccus Long rod	Bacillus spp. Staphylococcus spp. Lactobacillus spp.

Table 3: Biochemical characterization of Bacteria.

S.no.	Bacteria	Indole	MR	VP	Citrate	Catalase	Oxidase
1	Bacillus	-	-	+	+	+	+
2	Lactobacillus	-	-	-	-	-	-
3	Staphylococcus	-	+	+	+	+	-
4	Streptococci	-	+	-	+	-	-
5	Streptobacillus	-	-	-	-	-	-

DISCUSSION:

Fruit juices have both positive and negative effects. Fruit juices are very popular among the people of all ages around the world due to their high nutritive value and fresh flavour. The concerns over their safety and quality of fruit juices have been raised in spite of their potential benefits. They contain various organisms including many harmless yeasts and saprophytic bacteria. By detecting this bacterial load in the juice, it apparently gives an idea about the quality of the sample Sandeep et. al (2001). Freshly squeezed fruits have little or no process steps that reduce pathogen levels, if contaminated Barro et al (2006). In addition, lack of appreciation of basic safety issues by vendors augments the microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies, and airborne dust. A number of studies from different countries have shown presence of *staphylococcus spp*, *Bacillus spp*, and variety of microorganism like *Streptococcus spp, Lacto-bacillus spp, Streptobacillus spp, Bacillus cereus, Bacillus anthracis, staphylococcus aureus, staphylococcus epidermidis.*

CONCLUSION:

The MPN analysis showed high levels of contamination in juice samples sold along the road sides of Beed city. This would be possible because of the poor quality of water was used in juice preparation; moreover, water is one of major sources of sewage contamination. The results of the present findings clearly demonstrated that, the road side ready fresh juices did not meet public health standards and many kinds of enteropathogenic bacteria were found namely, Bacillus, Staphylococcus, Lactobacillus, Streptobacillus, and Streptococcus. Such foods lead to hazardous effects to the consumers. Government agencies must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines for juice preparations, especially street vended fruit juices.

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