

Original Research Article

COMPARATIVE ANALYSIS OF BACTERIAL FLORA OF VEGETABLES COLLECTED DIRECTLY FROM THE SUPERMARKET AND VEGETABLE MARKET

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Abstract

To prevent food borne diseases, microbiological control is very important in the food industry. Therefore, the present investigation was undertaken to conduct a microbiological quality assessment of fresh vegetables that were collected from several regions of Chandigarh. The purpose of this study was to determine the bacterial load and safety of vegetables presented for sale in the marketplace. Vegetable samples (tomato and capsicum) collected from two different sites i.e. (vegetables market and supermarket), were analyzed for their bacteriological contaminants following different procedures. The maximum bacterial count present in the vegetable market was compared to that of the super market. The micro flora of these vegetable samples was found to be dominated by *Staphylococcus* and *Bacillus*. *Bacillus* and *Staphylococcus* were detected in tomato and Capsicum respectively. *Bacillus* and *Staphylococcus* were included as the most abundant species and as opportunistic pathogens of which may raise concern when working to improve the microbial quality of fresh vegetables. Our study demonstrated that the vegetables, sold in the vegetable market, were found to contain a higher microbial load that may represent a greater risk for human health. Hence, for many microorganisms, vegetables may act as a reservoir from where they will be colonized and infect the host.

Keywords

Microorganism, Identification, Biochemical test, Microbial load, Vegetables.

Introduction

Microbiology is the study of microorganism those which are so small that they cannot be seen with the naked eyes and observed only by microscope. Microbiology and all organisms include microbes that are divided into prokaryotes and eukaryotes. Prokaryotes are always single cell and DNA is not enclosed by a membrane, whereas eukaryotes are multi cellular organisms where DNA is closed by a membrane. Robert Hooke observes first microbes in fruiting bodies of moulds. Leuwenhoek is father of microbiology as he experimented with the microbiology in 1670. Scientific microbiology was developed in 19th century through the Louis Pasteur and Robert Koch work. Microbiology includes bacteria, archaea, virus, fungi, protozoa and algae. There are present

several pathogenic microbes that have found their way into fresh vegetables and fruits over the years leading to spoilage which are a great source of human healthy diet (Balali et al, 2020). Spoilage is the process in which fruits and vegetables (a great source of human healthy diet) is-are contaminated to the point that the and any change which reduce a product and-becomes unacceptable for human consumption. Some spoilage organisms such as yeast target ~~the~~ fruits and vegetables due to their low pH which allows the and-bacteria to can grow on the ~~fruits and vegetables~~ surface of the produce. Human infection and outbreaks is-are associated with the consumption of raw vegetables (Beuchat et al, 2022). Bacterial spoilage is the primary cause of tissue softening as the pectin are-is degraded. Micro flora is dominated by spoilage bacteria such as *Bacillus cereus*, *Salmonella*, *E.coli*, and *Clostridium*, and Staphylococcus etc (Tambekar et al, 2006). Food poisoning is the can become a major problem in case when items it-are was not stored properly. The e Commonly found bacteria that causes food poisoning are *Salmonella*, *Staphylococcus aureus*, and E.coli. Bacte. Common bacteria are often present on the surface of vegetables and fruits like *Staphylococcus*, *Bacillus*, *E.coli*, *Clostridium*, and *Agrobacterium*.

Nutrients are necessary for the growth of these the microbes. The substrate on which bacterial cultures are grown is done is known as the medium which provides both nutrition and pH to the organism. There are generally two different types of culture nutrient media such as a NAM, PDA, and YMA etc. On the basis of consistency, there were used two types of media media utilized i.e. solid media and liquid media. Solid media were-are used for studying the cloning character of the bacteria and liquid media are used for the profuse growth e.g. such as with a blood culture. On the basis of C chemical composition studies require two types of media a were used i.e. routine laboratory media and a synthetic media. Autoclave, dry heat, and radiation methods were used as are common laboratory sterilization methods. Human pathogens commonly associated with fruits and vegetables include Bacillus, Staphylococcus, E.coli, and Salmonella. Microbes found on kitchen surfaces may have originated from a produce source. Fruits and vegetable contamination may occur at any stage from production to consumption. Fresh apples, peppers, tomatoes and capsicum are known to harbor large bacterial populations. The presence of Staphylococcus in retail food has already been reported in China (Shubo et al, 2015).

This study evaluates the diversity of the Here are mentioned some bacterial community found associated with the surface of vegetables found in the marketplace utilizing microbial plate counts and biochemical testing. Fresh apples, peppers, tomatoes and capsicum are known to harbor large bacterial populations. We were started to explore the diversity of these communities. Human pathogens can be associated with fruits and vegetables examples *Bacillus*, *Staphylococcus*, *E.coli*, and *Salmonella*. Many of the microbes found on the kitchen surface appear to come from produce source. Fruits and vegetable contamination occur at any stage from the production to consumption. The presence of Staphylococcus in retail food has been reported in China (Shubo et al, 2015).

Materials and Methods

Location of samples

Bacteria samples were cultured from fruits and vegetables purchased in ~~carried out from~~ the vegetable market sector 40 Chandigarh and a supermarket SCF4 sector 9D Chandigarh. *Bacillus* collected from the surface of tomato was ~~cultured~~ ~~collected~~ from the vegetable market fruit and *Staphylococcus* falling microorganisms were collected from the surface of a capsicum ~~was collected~~ from the supermarket.

Composition of Nutrient agar medium

Bacteria falling on the surface of vegetables were measured ~~by-via~~ two types of agar media (nutrient agar media and peptone dextrose agar media) and two types of growth media (Mannitol salt agar and Luria Bertani). Nutrient agar media (Table 1) was ~~utilized~~ ~~used~~ for the bacterial growth counts ~~as-it~~ can effectively grow a variety of bacteria ~~types and as well as~~ fungi. Peptone dextrose agar was a general media utilized for the fungal isolation from plant samples (Table 2). ~~Here~~ ~~o~~ Organic compounds ~~were~~ ~~as~~ used as a source of energy. Yeast extract peptone dextrose was used as a growth medium to grow Yeast. Mannitol salt agar media contains a high salt concentration and ~~was~~ ~~ere~~ used ~~for~~ ~~as~~ a selective and differential growth medium in the laboratory (Table 3). ~~It~~ ~~contains~~ ~~high~~ ~~salt~~ ~~concentration~~. The identification of *Staphylococcus aureus* in clinical isolates requires a battery of tests ~~which~~ ~~are~~ ~~and~~ ~~costly~~ in resources in a limited setting. Luria Bertani (LB) is a nutritionally rich medium for culturing the bacteria (Table 4). LB media allows for the plating of bacterial cultures and the growth of colonies.

Table 1 Nutrient medium

Chemical name	g/l
Peptone	5
Beef extract	3
Sodium chloride	5
pH	5.5
Agar	15

Table 2 Peptone dextrose agar

Chemical name	g/l
Potato starch	4

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Comment [04]: Unless you are using different media than others, you do not need to list the recipe. Just erase anything that is not your own recipe and just state that you used nutrient agar, peptone dextrose, Mannitol salt agar, and LB. Shorter is better for journals..

Dextrose	20
Agar	15
pH	7.5

Table 3 Mannitol salt agar media

Chemical name	g/l
Peptone	10
Beef extract	1.00
Sodium chloride	75.0
D-Mannitol	10.00
Phenol Red	0.025
pH	7.3
Agar	15

UNDER PEER REVIEW

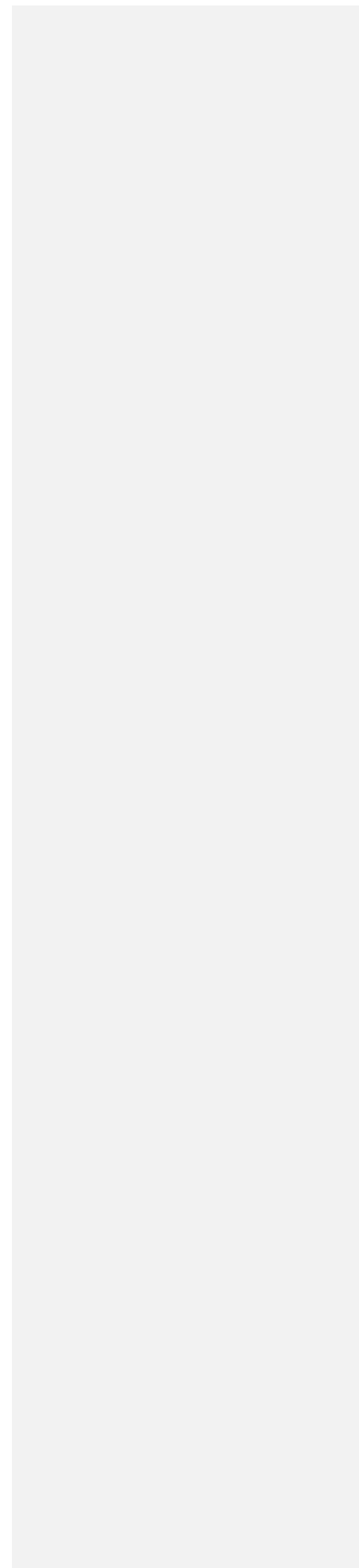


Table 4 Luria Bertani (LB)

Chemical name	g/l
Tryptone	0.25
Yeast extract	0.126
Sodium chloride	0.125
pH	7.5

Identification of bacteria

Identification of bacteria was ~~done conducted via by various different~~ physical and chemical methods.

A. Physical methods utilized

1. Shape of bacterial cell- rods or cocci

~~Antonivan Leeuwenhoek first show microbes through a microscopes in 1670 and differentiate it on basis of their shape and size. Basic shapes of bacteria are Coccus (spherical), Bacillus (rod shape), and Spiral (twisted).~~

2. Gram staining

~~Was used to Hans Christian's gram developed the gram stained technique in 1884. It is a common technique that were used to differentiate two large bacterial group based on their cell wall (Coico et al, 2005). The gram staining procedure distinguished between gram negative and gram positive groups by coloring the cell violet or red. Violet color appears due to presence of thick layer of peptidoglycan in bacterial cell wall which retains the crystal violet.~~ This technique is useful for bacterial sample contamination assessment and observation of morphological features of bacteria that were isolated from mixed cultures (Moyes et al, 2009).

B. Biochemical testing included the

1. Catalase test

1. Catalase is enzymes that detoxify hydrogen peroxide into molecular oxygen and water (Gagnon et al, 1959). A Catalase test is positive if bubbles are observed after adding hydrogen peroxide. It should appear as if simple water was dropped on the smear. Catalase test is used for gram positive bacteria and can utilize to distinguish Staphylococcus and Micrococcus.

2. Citrate test :

2. Citrate test is used to determine the bacterial ability to use sodium citrate as the source of carbon (Table 5). The citrate test is possible for those organisms which are capable of fermenting citrate. ~~Inoculate a tube having citrate medium with bacteria or it is also possible to streak a deep inoculation into "Simmons citrate tube" and incubate it.~~ Positive test ~~occurs was seen~~ when a particular color change ~~is was~~

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observed in the Simmons citrate tube and negative test was observed in case of no color change. The citrate test was used to distinguish between *Bacillus*, *Citrobacter*, *E.coli* and *Staphylococcus*.

Table 5 Citrate test

Chemical name	g/l
Sodium chloride	5
Sodium citrate	2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	0.2
Magnesium sulphate	0.2
Bromothymol blue	0.08

3. Vogues -Proskauer (VP) test

The Ability of bacteria to convert glucose to acetoin creates a red/purple color near top of tube. Bacteria producing a negative Voges-Proskauer result exhibit no color change (Hasan et al, 2015). When potassium hydroxide is added into acetoin then acetoin is converted to diacetyl that later reacts with alpha-naphthol and make pink color compound (Table 6). This test depends on the digestion of glucose to acetyl methyl- carbinol. Color changes to pink was observed in positive test and no color change was seen in negative test.

Table 6 Vogues-Proskauer (VP) test

Chemical name	g/l
Peptone	2
Dextrose	2
Potassium phosphate	2
Ph	6.1

4. Oxidase test

This test is used to determine the presence of bacterial enzyme cytochrome oxidase (Tarrand et al, 1982). Cytochrome are iron containing hemoproteins that transfer electrons to oxygen to form water in aerobic respiration. Cytochrome oxidase is a transmembrane protein complex, also present in the mitochondrial cytoplasmic membrane. Use a loop and pick the isolated colony from the fresh bacterial plate and rub it into filter paper after it add 2 drop of oxidase reagent on the organism smear and observe for color change. In Positive test, there was dark purple color and no color in case of negative test.

5. Urease test

Urease test identifies that organism that is capable of hydrolyzing urea to produce ammonia and carbon dioxide (Brink et al, 2019). Some bacteria have the enzyme that convert urea to ammonia and carbon dioxide which form ammonium carbonate in the presence of water. Urease test is a biochemical test that

detect the alkaline fermentation of urine with the resultant production (Table7). Rapids positive organism turns the entire medium pink within 24 hours.Negative organism produces no color change or yellow as a react of acidic production.

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Table7Ureasetest

Chemicalname	g/l
Peptone	1
Dextrose	1
Sodiumchloride	5
Potassiumphosphate	2
Phenolred	0.01
Urea	20
Agar	15
pH	6.7

6. Methyl red test

Many bacteria have ability to utilize glucose and converted into a stable acidic acid like acetic acids, formic acid or lactic acid, as the end product.Methyl red changes the color immediately and help to predict rather it's basic or acidic. Bacteria metabolize the glucose in pyruvic acid and later pyruvic acid metabolize through the mixed acid pathway to produce stable acids.Theacid production is different from species to species and depends on the enzymatic pathway present in the bacteria.Red color and yellow color or colorless appear in case of positive and negative test respectively(Table 8) (In Atlasal oral microbiology).

Table8Methyl redtest

Chemicalname	g/l
Peptone	2
Dextrose	2
Potassiumphosphate	2
pH	6.1

7. Nitrate reduction test

Nitrate test is used to determine the presence and absence of nitrate in the solution (Table 9). Nitrate test differentiate those bacteria that have ability to produce nitrate reductase enzymes. This enzyme hydrolyze nitrate to nitrite and nitrite further degrade into various nitrogen product like nitrogen oxides, nitrous oxides and ammonia.It can be performed by adding sulfanilic acid that will react with the nitrous acid and produce diazotized sulfanilicacid. This diazotized sulfanilic acid react with alpha-naphthylamine and form a red color compound in positive test. While no color was detected in case of negative test (Tlaieakhon et al, 2018).

Table 9 Nitrate reduction test

Chemical name	g/l
Peptone	5
Beef extract	3
Potassium nitrate	1
Sodium chloride	30
pH	7

8. Carbohydrate test

The microbes utilize carbohydrate to produce energy in form of ATP (Table 10). Degradation of the monosaccharide, disaccharides and polysaccharides by microorganism under anaerobic condition is carried out in the fermentation. Its end product can either be organic acids like formic, lactic, or organic acids. The fermentation medium contains carbohydrate with PH indicator. After fermentation, organic acid products turn into yellow color with the reduction of PH.

A) Preparation of carbohydrate fermentation broth

Dissolve sodium chloride, trypticase and phenol red in 100 ml of distilled water and transfer into flasks. Add 0.5-1% of carbohydrate into the flask. Sterilize in autoclave at 115 degree Celsius for 10 min. The overheating may result in breakdown of molecule and browning of sugar. Transfer it into the fermentation tubes or screw-capped tubes and label it.

B) Bacterial culture inoculation into fermentation medium tube

Incubate fermentation tube for 18 hours at 37 degree Celsius and observe it for acids and gas production. When the medium changes to yellow color, it means organisms are able to ferment carbohydrate. If it retains red color, means organism do not utilize the carbohydrate (Weldezigina et al, 2016).

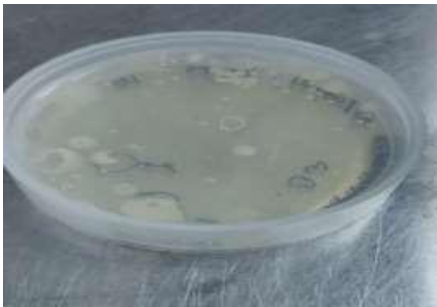
Table 10 Carbohydrate test

Chemical name	g/l
Peptone	5
Beef extract	3
Sodium chloride	5
pH	6.8

Result

The ~~Our experimented~~ Petri plates ~~exhibited~~ showed the ~~assorted~~ colonies of bacteria that were present on the surface of vegetables. ~~The bacterial growth on the~~ Vegetable Market ~~plates was had~~ high ~~number of bacterial presence as~~ compared to ~~that of the~~ supermarket ~~area~~ plates. The sector 40D vegetable markets ~~produced~~ had 20 bacterial colonies on NAM media & ~~while~~ the Supermarket ~~area~~ plates ~~had produced~~ 15 bacterial colonies (Fig. 1). We found *Bacillus* bacteria on tomato ~~that were taken~~ from the vegetable market and *Staphylococcus* bacteria was observed on the surface of capsicum annum ~~from the~~ of supermarket. ~~Confirmation of Bacillus and Staphylococcus bacteria was done by different type of tests as described below.~~

FIG.1 Bacterial colonies ~~appear~~ present on ~~the~~ Petri plates



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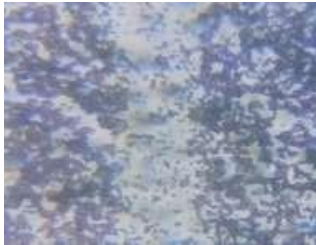
Bacillus bacteria collected from ~~the~~ vegetable bacteria collected market (tomato)

Staphylococcus from supermarket (Capsicum)

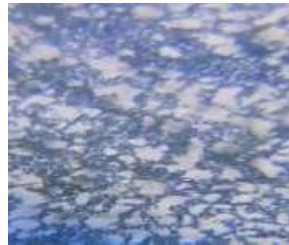
1. Gram staining

~~The bacteria react to the Gram stain and gram stain is purple colored. After the stain combine with bacteria, the bacteria will stay purple or turn pink.~~ In our experiment, ~~both vegetables~~ plates (market and supermarket) ~~samples~~ containing *bacillus* and *staphylococcus* respectively were found ~~to be~~ Gram positive ~~as they exhibited a~~ with purple color (Fig. 2).

Fig.2 Gram staining experiments



SampleA(*Bacillus*)



SampleB(*Staphylococcus*)

2. Catalase Test

The Catalase test is used to differentiate bacteria. ~~The~~ ~~B~~ bacteria produce ~~the~~ enzyme Catalase ~~by that~~ ~~by~~ using ~~the~~ oxygen, ~~to~~ protect themselves from the toxic by-products of oxygen metabolism. ~~In Both~~ ~~the~~ *Bacillus* and ~~Staphylococcus~~ samples bubbles ~~present~~ confirmed that the bacteria ~~was~~ Catalase positive (Fig. 3).

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Fig.3 Catalase test



SampleA(*Bacillus*)



SampleB(*Staphylococcus*)

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3. Citrate test

When an organism ~~utilizes~~ citrate then it ~~exhibits~~ ~~shows~~ positive reaction but not necessarily as the sole carbon source. The pH turns the pH indicator from green to blue. The blue color appears which indicate the test is positive. Both ~~the~~ *Bacillus* and *Staphylococcus* samples were found ~~to be~~ positive as shown in fig 4a. The observed color change ~~is was from~~ green ~~to~~ blue.

FIG.4(a) Citrate test



Sample A (*Bacillus*)



Sample B (*Staphylococcus*)

4. Nitrate test

When nitrate is present in the medium, then a red color will be produced. In our experiment the *Bacillus* and *Staphylococcus* cultures did not exhibit ~~don't show any a~~ color change so both samples were found to be negative (Fig 4b).

FIG.4(b) Nitrate test



Sample A (*Bacillus*)



Sample B (*Staphylococcus*)

5. Urease method

The product of decarboxylation of amino acids is urea. After the hydrolysis process, urea produces Carbon dioxide and Ammonia. In our experiment, both *Bacillus* and *Staphylococcus* sample showed a positive reaction by developing the an intense magenta to bright pink color in 15 min to 24 h (Fig5).

FIG.5 Urease test



6. Methyl red test (MR)

In this test, the test bacteria were grown in a broth medium that contained glucose. As we know, bacteria has the ability to utilize glucose via the production of an acid, the a color change d was observed from yellow to red in the broth cultures. *Staphylococcus* has the ability to utilize glucose and *Bacillus* does not have ability to utilize glucose, thus, so the bacillus test was showed positive and the staphylococcus was with negative result (Fig 6).

Fig.6 Methyl red test (MR)



Sample A (*Bacillus*)

Sample B (*Staphylococcus*)

7. Voges-Proskauer

Voges-Proskauer or VP is a test used for the detection of acetoin in a bacterial broth culture. This test is performed by adding potassium hydroxide and alpha-naphthol to this bacterial inoculated broth. Both samples *bacillus* and *staphylococcus* showed a negative reaction i.e via lack of a pink-red color (Fig 7).

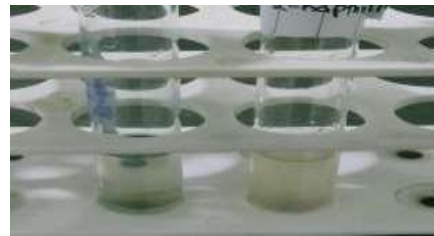


Fig.7Voges-Proskauer

SampleA(*Bacillus*)

SampleB(*Staphylococcus*)

8. Oxidase test

The presence of the cytochrome oxidase pathway is was detected by via the oxidase test. It catalyzes the transport of electrons between electron donors and a redox dye i.e. tetramethyl-p-phenylene-diamine in the bacteria. This dye is reduced to a purple color. BHere both *Bacillus* and *Staphylococcus* are were negative because of no color changes (Fig 8).

Fig.8Oxidasetest



9. Carbohydrate test

During the Carbohydrate test, the medium changed to a yellow color due to the production of acid. Microbes produce organic acids following after fermentation of the carbohydrates. Both *Bacillus* and *Staphylococcus* showed the carbohydrates test were positive (Fig 9).

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Fig9Carbohydratetest

Fig9.1NutrientMediawithcarbohydratesforLB



Fig9.2NutrientMediawithCarbohydratesforMSA

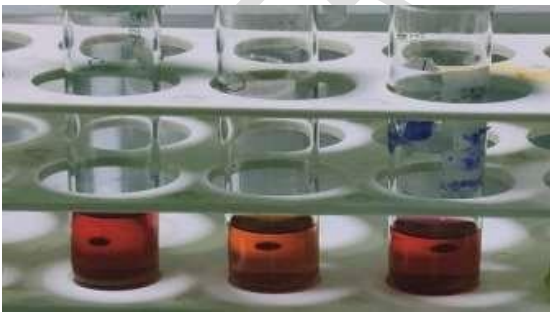


Fig9.3FollowingAfter inoculation, color changes occurred in sampleAandsampleB Media



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Discussion

The Vegetable market ~~area~~ samples contained a ~~more~~ greater abundance of bacterial flora compared to ~~that of~~ the supermarket. ~~Thus, area as we can see that~~ more contamination was ~~evident in the present on the~~ Vegetable market samples. ~~After observations from ng the results of the surface growth of microbial growth showed eount, we can state that their~~ supermarket ~~area~~ samples of *Staphylococcus* growth eount was less i.e. was 50.50 percent, which was less numerous ~~as compared than to the~~ vegetable market samples, where the *Bacillus* count was 90 percent. Further, ~~more eadditional~~ experimentation was ~~done~~ conducted to identify the microbes from the environment of different places. Mostly bacteria ~~were found of of~~ human and soil origin were found. The microbes ~~eome~~ from the humans ~~were are~~ mostly from the skin and respiratory tract, ~~were disbursed released~~ by sneezing or talking whereas the soil microbes were released ~~by via~~ dust. These infectious bacteria make us unhealthy by releasing toxins that damage our tissues, such as *Streptococcus*,

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Staphylococcus, *Bacillus*, and *E.coli*. Both *Bacillus* and *Staphylococcus* microbes are gastrointestinal pathogens, *Vibrio cholera* causes cholera, *E.coli* causes cystitis and *Bacillus* pathogen is capable to of causing disease due to the production of an enterotoxin or non-ribosomal peptide synthetase toxin. Tambekar and Mundhada in 2006 also reported pathogens detected from fresh vegetables included *Bacillus*, *Staphylococcus* and *E.coli*. Several studies have reported the presence of pathogenic bacteria in vegetables and fruits, however the bacterial but the counts were rarely documented (Olaimat et al, 2012). Kuramoto et al, 2006 Some other researchers reported that in 2005, reported that *Staphylococcus aureus* was the causal agent of a food poisoning outbreak in Japan in 2005 where pickled radish was one of its sources (Kuramoto et al, 2006). It was also observed that 50% of all vegetable samples that were collected from 39 major cities in China contained *B. cereus*. The level of contamination was more or less similar as to those of previous surveys in other different countries, i.e., 57% in Mexico City, 20–48% in Korea (Chon et al, 2015), and 52% in the southeast of Spain (Flores-Urban et al, 2014). These reports, together with our study, indicate that *Bacillus* and *Staphylococcus* contamination in vegetables is very common. This result suggests that the consumption of vegetables contaminated with these pathogenic bacteria is a potential health hazard. Treatment of these bacterial infections also includes good nutrition and intensive care. People who have had closed contact with a person with bacterial disease may also need to be treated for the disease. Thus during the preparation of vegetables some precautions may be required like washing your hands, kitchen utensils, chopping boards, and countertops before and after preparing fruits and vegetables. Moreover, it is very important to clean the vegetables and fruits prior to consumption before eating.

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Conclusion

This study experiment reported pathogenic bacteria found on the vegetables collected from the vegetables market and super market in Chandigarh. The hygienic quality of the vegetables and water in the area was seen very poor due to the higher mean bacterial counts found (many and it was recorded were beyond the standard safe limits). The Vegetable micro flora was dominated by *Staphylococcus* and *Bacillus*. The presence of *Bacillus* bacteria on the vegetable market was in high number than the *Staphylococcus* bacteria present in the supermarket. Washing vegetables and then storing them in food grade antibacterial chemicals may assist with reducing pathogens and other microbial flora. Thus, new and improved Good agricultural practices are required should be used along with various safety measures during the production of fresh fruits and vegetables in the region. It is better to wash vegetables and stored to them in food grade antibacterial chemicals for a good time to free pathogens and reduces the microbial flora.

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