ANALYSIS OF SPOILED FRUIT SAMPLES FOR THE PREVALENCE OF BACTERIA

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INTRODUCTION

Microorganisms are found everywhere , since the conditions which bring about their growth are readily available like humans, they prefer a warm ,moist environment ,a supply of oxygen and food and because their nutritional requirements are similar to ours , they readily contaminate our food supplies .when food spoilage is caused by growth of micro organisms ,it becomes soft and smells and tastes bad (Filtenborg *et al* .,1996).

It has been known that fruits constitute commercially and nutritionally important indispensable food commodity. Fruits are consumed in large quantity in the world .Fruits are a source of nourishing substance that keep us alive and healthy .Fruits play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which helps to live a healthy life (Al-Hindi *et al* .,2011) .India is the fourth largest producer of fruits in the world ,yet due to losses in the field ,during handling processes of the crop from the grower to the wholesale dealer and to retailer and finally to consumers (chukwuka *et al* ,2010 and zubair,2009) ,they become inadequate (Barth *et al*.2013) .Most microorganisms that are initially observed on whole fruit or vegetable surfaces are soil inhabitants (Andrews and Harris ,2000;Janisiewicz ,and Korsten ,2002). Spoilage refers to any change in the condition of food in which the food becomes undesirable or un acceptable for human consumption (Akinmusire, 2011). The high concentration of various sugars ,minerals , amino acids , provides a good platform for successful growth and survival of various microorganisms (Bhale,2011) .

One of the limiting factors that influence the fruits economic value is the relatively short shelf life period caused by pathogens attacked .One of the limiting factors that influence the fruits economic value is the short shelf –life period caused by pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post – harvest handling even in developed countries (Droby, 2006; Zhu, 2006). Bacterial spoilage first causes softening of tissues as pectin's are degraded and the whole fruit may eventually degenerate into a slimy mass. Starch and sugars are metabolized next and unpleasant odours and flavours develop along with lactic acid and ethanol (Rawat, 2015). Spoilage of fresh fruits usually occurs during growth , harvest ,storage , transport ,and handling which is done both by

bacteria and fungi .Spoilage is manifested by a variety of sensory cues such as off-colours , off - odours ,softening of fruits .

The spoilage of most fruits is always associated with bacteria and fungi .The adverse effect of bacteria in fruits has resulted in shortage of fruits for consumption .In spoilage organisms ,

chemical reactions that cause offensive sensory changes in foods are mediated by variety of microbes that use food as carbon and energy source . Spoilage microbes are common inhabitant of soil , water ,or intestinal tract of animals and are dispersed through air and water and by the activities of small animals ,particularly insects .e.g. *Penicillium* spp cause visible rot on citrus ,Pear ,and Apple fruits . Healthy fruits have many microbes on their surface but can usually inhibit their growth until after harvest . Ripening weakens cell walls and decreases the amount of antifungal chemicals in fruits, and physical damage during harvesting causes breaks in outer protective layers of fruits that spoilage organisms can exploit. Some bacteria, including *staphylococcus* spp and *Bacillus* spp cause spoilage of fruits .Some spoilage microorganisms are capable of colonizing and creating lesions on healthy, undamaged plant tissues (Tournas and Katsoudas, 2005). In order to know the microorganisms present on spoiled fruits, the isolation of pathogens is done on standard media .The susceptibility of these organisms is studied to become acquainted whether the spoilage bacteria are resistant or sensitive to antibiotics.

The study is an attempt for isolation and characterisation of spoilage bacteria associated with different fruit samples .The objectives of the present work are »

- Collection of different rotting fruit samples.
- Isolation of bacteria .
- Identification of isolated bacteria
- Antibiotioc sensitivity test of isolated bacteria.

REVIEW OF LITERATURE

Fruits normally carry natural non –pathogenic epiphytic organisms ,but during growth , harvest ,transportation and further handling the produce can be contaminated with pathogens from animal and human sources .As most of these produce are eaten without further processing ,their microbial content may be a risk factor for the consumers health and therefore a food safety problem (Brandl ,2006 ,Hamilton *et al* ., 2006). Since the early 1990s awareness of the potential of fresh produce to cause food disease has increased and reported outbreaks associated with consumption of fresh fruits have grown steadily. Most of the reported outbreaks are associated with bacterial and fungal contamination.

Various pathogens including bacteria were derived from spoiled avacado. Avocado fruit was examined by culturing on nutrient agar for bacteria causing post -harvest spoilage on the fruits ; various bacterial species were isolated .The bacteria include *Erwinia sp, Streptococcus sp , E. coli* and *Staphylococcus sp , Pseudomonas sp* .

Spoilage of fresh fruits usually occurs during storage and transport . Species of bacteria from grapes taken from the Takestan , Shahrod and Hamedan cities. Bacteria, *Bacillus spp*., *Micrococcus spp*., *Clostridium spp*., were isolated (Maulani *et al*: 2011).

Increased attention has been given to the need for better methods to disinfect fresh produce containing human pathogens .Enteric pathogens such as *E.coli* and *Salmonell*a are among the greatest concerns during food –related outbreaks (*Buck et al* .,2003). Several cases of typhoid fever outbreaks have been associated with eating contaminated fruit grown in or fertilized with contaminated soil or sewage

(Beuchat, 1998). These increases in fruit borne infections may have resulted from increased consumption of contaminated fruits outside the home as most people spend long hours outside the home. In Nigeria , for instance , street vending of handy ready-to-eat sliced fruit has recently become very common and the market is thriving .

Food Borne Outbreaks

An outbreak is defined as 2 or more cases of the same illness in which an epidemiologic investigation implicates the same food item as the vechile (CDC 2000).

Vegetables and fruits have been associated with outbreaks of food-borne disease in many countries . Organisms involved include bacteria, fungi, viruses, and parasites (De Roever, 1998). Many outbreaks are never recognized because of their small size , long incubation period , or geographical or temporal dispersions . A wide variety of bacteria , fungi , and parasites have been linked to outbreaks of illness associated with fresh produce .Food –borne pathogens that are frequently associated with fresh produce generally originate from enteric environments –that is they are found in the intestinal tract and faecal material of humans or animals.

Produce may get contaminated with microbial pathogens during production, harvest, processing, and transporting, as well as in retail and food service establishments and in the home kitchen leading to food –borne illness. Contamination at any point in the food handling chain can be exacerbated by improper handling and storage of produce prior to consumption. It is important to know the point of contamination to control the outbreaks effectively. Contamination of raw fruits with pathogenic organisms of human health significance can occur directly or indirectly via animals or insects ,soil ,water ,dirty equipment ,and human handling ,for example ,fruit flies have been shown to transfer *E.coli* 0157;H7 to damaged apples under laboratory conditions .This may have implications during harvesting and in packing sheds or processing facilities , where damaged produce is inevitable and flies may be separated from fruit growing operations , wild animals , and birds can only be controlled to a limited extent . Human hygiene , including hand washing all along the food chain , is critical in reducing or eliminating contamination with faecal pathogens .

The reported fruit borne outbreak to Cantaloupe occurred in Mexico just over 10 years ago and of the over 50 people infected in this outbreak, about 9 were culture confirmed. The outbreak involved the death of two and was caused by Salmonella (Jacque Wilson, 2009).According to Castillo and Escartin (1994) , *Campylobacter jejuni* can survive on sliced watermelon and papaya for sufficient time to be a risk to a consumer . More recent data from the centre for science in the public Interest (CSPI) database indicate produce outbreaks accounted for 4% of all food-borne outbreaks reported from 2011 to 2005 in Australia (Kirk *et al* ., 2008) .

In the last decade, in North America over 1700 people have fallen ill after consuming juice . Most of these outbreaks involved unpasteurized juices such as apple juice, orange and lemonades. Other fresh fruit juice outbreaks included pineapple, carrot , banana , and mixed juices (CSPI, Outbreak Alert; Database).

The pathogens or biological agents, responsible for these illnesses and deaths include bacteria, fungi,

viruses, and parasites, as well as metal contaminants. The most comon pathogens were *E.coli* 0157 and 0111, *Salmonella*, and *Norovirus.* A few outbreaks *Vibrio cholera, Clostridium botulinium, Yeast* and Hepatitis A .

Sources of contamination in fruit

Pre harvest source of contamination: Fruit and vegetables are contaminated in the fields during growth. Various sources of contamination of fruits prior to harvest can be listed as manure , sewage ,sludge ,irrigation water , runoff water from livestock operations
,and wild and domestic animals (Steele and Odumeru 2004 ; Beuchat 2006 ;Delaquis *et al* 2007 ;Doyle and Erickson 2008). In addition , a number of other indirect sources of contamination have been identified and include trophic interactions between plants and plant foragers (Sasaki *et al* ., 2000 ;Sela *et al* ., 2005 ; Kenny *et al* .,2006 ; Sproston *et al*.,2006). Results of some of the studies that describes the contamination of papaya are summarised here . For centuries plant pathologists have been investigating the interactions of microbes and plants and have come to understand many of the strategies that microorganisms have developed for niche adaptation , host range specificity , nutrient acquisition and utilization and mechanism of pathogenicity . Using this broad knowledge base plant pathologists have , best known to most consumers and many producers, developed economically feasible and environmentally sustainable management strategies that have protected our crops from devastating disease such as papaya ring spot and most recently soybean rust . Research in plant pathology addresses issues of food security with a focus on fundamental research, teaching and extension outreach . Traditionally research has focussed on crop protection with respect to plant pathogens and toxins .

However, the occurrence of multiple research to include food safety issues related to fresh produce (Lewis and Melanie , 2011).

Various sources and kinds of risk factors can be associated with the contamination of fresh produce by both plants and food borne pathogens, and all must be managed adequately such that diseases are minimized and no harm comes to the consumer .

The primary method to reduce spoilage and food borne pathogens on produce is adherence to management strategies that prevent contamination, however post harvest contamination remains a critical step in the production of high microbial quality, safe produce.The microbial quality of fresh cut and whole head lettuce, whole tomatoes and detached grapes after exposure to micro droplets of the sanitizing agents Storx (hydrogen dioxide) or KleenGrow (quaternary ammonium) produced using de-correlation humidification technology was evacuated in this research . High concentrations of both disinfectants eradicated *Salmonella enterica Serovar Typhimurium* on whole tomatoes but not *E. coli* 0157 populations on whole or cut heads of lettuce. Coliform and *Lactobacill*i, *E.coli* were not detected

on mon-treated grapes and aerobic bacteria were less than or equal to log10 2.7.Populations of aerobic and *Lactobacilli* bacteria were significantly higher on grapes exposed for 10 sec to 1% and 4% storOx than the non-treated controls, but were not higher after a 30 sec exposure time at the same concentrations (Lewis and Melanie , 2011).

Postharvest sources of contamination: Upon harvest of field crops , a series of processing steps are applied to the product that in general includes trimming , peeling , shredding , washing , dewatering ,and packaging . Such fresh cut processing operations are critical to converting the crop into a consumable product ; however , they also create surfaces upon which enteric pathogen can more easily attach as well as release large amounts of nutrient rich liquids that are more readily utilized by the attached microbes .

For example , Anthracnose of bananas , caused by *Colletotrichum musae* and crown rot of bananas , caused by broad unspecific parasitic complex , are the most important post – harvest disease affecting the quality of exported bananas , These diseases develop during fruit transportation , conservation , ripening ,and marketing .

Like for most post-harvest diseases , the control of these diseases relies mainly on post- harvest practices like fungicide applications , fruit handling , cooling , CA OR MA
...Nevertheless , the regular observation of seasonal and spatial variations in the performance of these practices highlights the strong influence of pre- harvest factors .These pre-harvest factors determine a fruit potential quality that is elaborated at field level . This potential of fruit quality is constituted by a physiological component(the fruit susceptibility) and by a parasitic component (the level of fruit contamination).

The parasitic component is defined as the level of fruit contamination by the pathogens . In the case of anthracnose , this level is the quantity of quiescent appressoria at the fruit surface and a specific method has been established for the quantitative assessment before harvest .

It has been shown that floral remnants are the main inoculum sources for the fruit contamination by *Colletotrichum musae* . Rainwater is essential for the dissemination of conidia , so the early protection of bunches with a plastic sleeve reduces strongly fruit contamination .

The physiologic component is defined as the level of fruit susceptibility to the post-harvest disease and is evaluated through artificial inoculations. Fruit grown in highland areas are less susceptible to anthracnose and also to crown rot than fruit grown in lowland areas . The physiological age of fruit at harvest has a strong influence on fruit susceptibility to both diseases: the youngest the fruits, the less

susceptible . The modification of source – sink ratio (fruit trimming), influences fruit susceptibility to crown rot but has no influence on fruit susceptibility to anthracnose.

Fungal spoilage forms an increasing economic problem in the food industry .Chemical antifungals are becoming less attractive as food preservatives and hygiene agents due to development of resistance and due to stricter legal regulations concerning the permitted concentrations .Finally , consumers tend to demand more 'naturally preserved ' or preservative free product (Brul and Klis, 1999).

Filamentous fungi may grow on many different types of food products and it is estimated that 5-10% of foods are lost post- harvest due to the growth of filamentous fungi . They may affect sensory quality of the product in several ways ; through visible filamentous growth , through production of exo-enzymes . They may be involved in spoilage of a very broad range of food commodities but are commonly involved in spoilage of low water activity products .

MATERIALS AND METHODS

The present study entitled ''**Analysis of spoiled fruit samples for the prevalence of bacteria** ''was carried out in the Department of microbiology Sardar Bhagwan Singh University, Balawala , Dehradun .

Collection of samples

Fruits like banana , apple ,papaya , orange , pomegranate ,mango , grapes , lemon , were collected from different places (Table 1) . The samples of fruits were collected in sterile poly bags and taken to the laboratory as earliest as possible. The samples were analyzed for the presence of bacteria.

Table 1: Sample collection

Sample No.	List of Samples
1	Apple
2	Orange
3	Banana

4	Papaya
5	Mango
6	Grapes
7	Lemon
8	Pomegranate

Media used

Various media for the isolation and characterization of bacteria have been used such as Nutrient Agar, Mueller Hinton Agar, Mannitol Salt Agar, Starch agar medium, Glucose broth, Na+ Nutrient Agar Medium, VP broth, Pseudo F Agar were prepared and sterilized by autoclaving at 121°C for 15-20 min at 15 lbs psi pressure .

Media Composition

a) Nutrient Agar

Ingredients	Amount (g/l)
Peptone Beef extract	5.0
Sodium chloride	3.0
Agar	8.0
	16.0

B) Mueller Hinton Agar

Ingredients	Amount (g/l)

Beef infusion	300.0
Casein acid hydrolysateStarch	17.50
Agar	1.50
	16.0

C) Mannitol Salt Agar

Ingredients	Amount (g/l)
Enzymatic Digest of Casein	5.00
Beef extract	1.00
Sodium chloride	75.00
D- Mannitol	10.00
Agar	15.00
Phenol Red	0.025

D) Starch Agar Medium

Ingredients	Amount (g/l)
Meat extract	3.000
Peptic digest of animal tissue Starch,	5.000
soluble	2.000
Agar	15.000

E) Glucose broth

Ingredients	Amount (g/l)
Casein enzymic hydrolysateGlucose	10.000
Sodium chloride	5.000
	5.000

F) VP broth

Ingredients	Amount (g/ l)
PeptoneGlucose	7.00
Di potassium hydrogen phosphate	5.00
	5.00

G) Pseudo F Agar

Ingredients	Amount (g/l)
Casein Peptone	10.00
Meat Peptone	10.00
Di potassium Phosphate	1.5
Magnesium	1.5
Glycerol	10.0 ml
Agar	15.0

METHODOLOGY

Samples of fruits collected from different sources were brought to the laboratory for the isolation of microorganisms.

The spoiled fruit samples were first crushed by the help of sterile mortar and pistle and 1 ml of sample was taken from each fruit sample and serial dilution upto (10^1 to $10^{7)}$ were prepared .0.1 ml from 3 dilutions 10^3 , 10^4 , 10^5 were evenly spreaded on the nutrient agar medium and incubated at 37°C for 24 hours . Plates were screened for the presence of isolated colonies after incubation period and characteristics were noted. After incubation, observations were made, and appearances of isolated colonies and their characteristics were noted
.Different selective medium showed the presence of different organisms .

Morphological Characterization

The colonies were observed for their colour, opacity, outline, texture, etc .The colonies were then picked and processed with Gram´s staining technique to differentiate between Gram positive and Gram negative .

Bio chemical Characterization of Isolates

The identities of the isolates were confirmed by different cultural and bio chemical tests. Different tests were performed and various characteristics for different pathogens were :

For Gram Positive Cocci, following tests were done to differentiate the particular species

a) Catalase test

Catalase is an enzyme produced by many bacteria .The enzyme splits hydrogen peroxide into water and oxygen .Hydrogen peroxide is a byproduct of aerobic respiration and is lethal if it accumulates in the bacterial cell . Catalase degrades the hydrogen peroxide in the bacterial cell before it can do any damage to the bacterial cell.

Transfer pure growth of the organism from the agar to a clean slide with a loop or glass rod .

Immediately add a drop of 3% hydrogen peroxide to the growth .

Observe for bubble formation .

In positive result ,gas bubbles are formed immediately when 3% Hydrogen peroxide is added to the colony .

b) Mannitol fermentation

Mannitol salt agar test is used to isolate and identify the presence of ***Staphylococcus aureus*** in a clinical specimen , which makes it both a selective , differential ,and indicator medium . The peptones and beef extract present in the mannitol salt agar supply all the essential nutrients for growth of the medium such as vitamins , nitrogen , minerals , and amino acids .A
7.5 % Nacl will result in the inhibition of species other than the *Staphylococci* .

A colony from pure culture was transferred aseptically to a sterile petri plate of mannitol saltagar .

The inoculated tube was incubated at 37c ° for 24 hrs and the results were determined . A positive test consists of a colour change from red to yellow

C) Novobiocin sensitivity

Novobiocin test is used to differentiate coagulase – negative *staphylococci* (CONS) and presumptively identify the isolate as *staphylococcus saprophyticus* (novobiocin resistant) .

First of all containers were allowed to come to room temperature before use . Using a pure 18-24 hour culture ,suspension of the organism was prepared .

Mueller Hinton Agar was inoculated with a sterile swab to obtain confluent growth .

Aseptically 1ug novobiocin disk was inoculated onto agar surface and lightly press down to ensure full contact with the medium .

Plates were incubated aerobically for 18 to 24 hours at 35-37°C .

The diameter of the zone of inhibition around the disk was measured (in milimeters) and record as susceptible or resistant .

Gram Positive Rods

Spore test

Bacterial endospores are metabolically inactive, highly resistant structures produced by some bacteria as a defensive strategy against unfavourable environmental conditions .In the Schaeffer-Fulton's method, a primary stain –malachite green is forced into the spore by steaming the bacterial emulsion. water is used to decolourise the Vegetative cells as Malachite green is water soluble and has low affinity for cellular material .Safranine is then applied to counter stain any cells which have been decolourized .At the end of staining
,Vegetative cells will be Pink , and endospores will be dark green .

A clean grease free slide was taken and smear was made using sterile technique.

The slide was air dried and the organism was heat fixed on glass slide and was covered with square of blotting paper.

The blotting paper was saturated with malachite green stain solution and was steamed for 5 minutes, and the dye was added regularly as required by keeping the paper moist.

The slide was then washed with tap water.

0.5% safranin was used as counter stain for 30 seconds .slide was again washed with tap water and blot dry.

The slide was then examined under microscope for the presence of endospores.

b) Starch hydrolysis

This test is used to determine the ability of an organism to hydrolyze starch .In this test the test bacteria are grown on agar plates containing starch , if bacteria has ability to hydrolyze starch , it does so in the medium ,particularly in the areas surrounding their growth while the rest of the area of plate still contain non-hydrolyzed starch . Since no colour change occurs in the medium when organisms hydrolyze starch, iodine solution is added as an indicator to the plate after incubation.

Using sterile technique, a single streak inoculation of organism to be tested was done into the centre of labelled plate.

The bacterial inoculated plates were then incubated for 24-48 hours at 37°C.

After incubation, the surfaces of the plates were flooded with iodine solution with a dropper for 30 seconds.

Excess iodine was poured off.

The plated were then examined for clear zone around the line of bacterial growth

In a **positive test** , a clear zone around the line of growth after addition of iodine indicates that the organism has hydrolyzed starch ,while a black colour shows **negative test** .

Gram Negative Rods

a) Oxidase test

The enzyme oxidase plays a vital role in the operation of the electron transport system during aerobic respiration .Cytochrome oxidase catalyses the oxidation of a reduced cytochrome by molecular oxygen, resulting in the formation of water or hydrogen peroxide, Aerobic bacteria
, as well as some facultative anaerobes and microaerophiles exhibit oxidase activity **.**

Oxidase disc was taken

The colony to be tested was picked up by help of plastic loop or platinum wire ,and touched on the oxidase disc .

The change in the colour was noted within 10 seconds .

In a positive test , a deep blue colour develops at the site of smear on the oxidase disc ,within 10 seconds . In the negative test , the colour of the smear on the zone of oxidase disc remain unchanged .

b) Glucose fermentation

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate . It tests for the presence of acid / or gas produced from carbohydrate source such as Glucose , Lactose , Sucrose or any other carbohydrate is used for this purpose . A pH indicator (such as Bromothymol blue (BTB) is also pesent in medium which detects the lowering of pH of medium due to acid production . Small inverted tubes Durham tube is immersed in medium to test for the production of gas (hydrogen or carbondioxide).

Five ml of glucose broth was innoculated with the test culture and incubated for 24- 48 hrs at 37°C .

If the medium in the tube turns yellow , then the bacteria are fermenting glucose .

If the medium in the tube remains red or unchanged , it indicates the bacteria cannot ferment that particular carbohydrate source in the media .

C) Voges – Proskauer (VP) Test

The Voges Proskauer purpose is to determine whether an organism can produce acetyl methyl carbinol (acetoin) from fermentation of glucose . This test is simultaneously used if MR +ve VP –ve , MR –VE VP +VE . In the presence of atmospheric oxygen and alkali (40% potassium hydroxide), the small amount of acetyl methyl carbinol present in the medium is converted to diacetyl which reacts with the peptone of the broth to produce a red colour

Reagents Naphthol solution

Ingredients	**Amount**
α-napthol	5 gm
Ethyl alcohal	100 ml

Potassium hydroxide

Ingredients	**Amount**
Potassium hydroxide	40.0Distilled water 100 ml

Five ml of VP broth medium was inoculated with the test organism and incubated at 37° for 24-48 hrs

To the culture ,0.6 ml of napthol solution and 2-3 drops of KOH were added and observed after 15-30 minutes .

The development of pink colour within 15-20 constituted a positive test .

D) Fluorescent Diffusible Yellow Pigment (Fluorescein)

Pseudomonas F Agar is a solid medium used for the detection and differentiation of P*seudomona*s on the basis of fluorescein production . Magnesium sulfate stimulate fluorescein production .If growth is observed on *Pseudomonas* F Agar fluorescein production is determined by visual examination of the plates under ultraviolet lighting .A positive result is the observance of greenish- yellow pigment in the agar which fluoresces under UV lightning .

Using a sterile inoculating loop , the sample was streaked over the surface of the agar .

The agar plate was then incubated at 35-37°c for 18-24 hrs .If the sample fails to grow , re incubate sample at 25-30°C , for 1-2 days .

The culture was then observed for growth and pigment production under a long wavelength UV light .

Antibiotic Sensitivity Tests

Antimicrobial sensitivity tests were conducted according to the method specified by NCCLS (Now CLSI, Clinical Laboratory Standard Institute) and as described by Collins et al., (1995). The antibiotic disc were stored at 4˚C till use. Sensitivity of isolates of *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Lactobacillus delbreuckii, Corynebacterium kutsceri* were

tested for Ciprofloxacin, Vancomycin, Erythromycin, Amoxyclav, Ampicillin, Ceftazidime, Amikacin

Antibiotic sensitivity of different isolates was tested on Mueller Hinton Agar . 25 ml of the medium was added into the sterilized petriplates and allowed to solidify .

First of all the bacteria inoculums were prepared by inoculating it to 10 ml nutrient broth and by taking a loopful sample in 2 ml distilled water and antibiotic discs were placed on surface of agar . ml of inoculum was spreaded with the help of glass spreader on the surface of MHA plates . Plates were incubated for 24 hrs at 37˚C .

Discs containing different antibiotics were placed on the surface of the agar using a pair of sterilized forceps at about equal distance from each other . The plates were incubated at 37˚C for 24 hrs . Each plate was observed for zone of inhibition around discs, which were measured in millimetres .

RESULTS

With the increase in awareness of nutritive value of fresh fruits, their consumption has increased tremendously. However the increased consumption of fresh produce has resulted in the emergence of food borne disease outbreaks associated with fruits (Hedberg, *et al*., 1994). In the present study an attempt has been made to determine the micro flora of fruits collected from different sources and the observation are discussed below.

Isolation and identification of Bacteria from fruitsMorphological Identification

Isolation on Nutrient agar, various mucoid, yellow, creamy, transparent, dull white, orange, wavy margin, colonies were obtained. The colonies were further taken for grams staining which resulted mostly in purple coloured gram positive cocci, gram positive rods, gram positive bacilli, and pink coloured gram negative rod. These were further checked for various tests including catalase test , spore test , acid fast staining etc ,these isolation tests were confirmed by morphological characteristics like the yellow coloured mucoid colonies indicated that it may be *Staphylococci* .

1. Gram Positive Cocci

For **Gram positive cocci** isolates ,**catalase test** was done at first which showed all isolates as catalase positive .Further **mannitol fermentation** was done to all isolates which showed one isolate fermenting mannitol producing yellow colour confirming *Staphylococcus aureus* , rest of isolates were done for **Yellow pigment (colony)** which gave negative results indicating *Staphylococcus spp* . These *Staphylococcus spp* were further done for **Novobiocin sensitivity test**. The novobiocin sensitivity test showed both novobiocin resistant as well as novobiocin sensitive spp. The novobiocin sensitive isolates showed *Staphyloccus epidermidis* while novobiocin resistant isolates showed *Staphylococcus saprophyticus*.

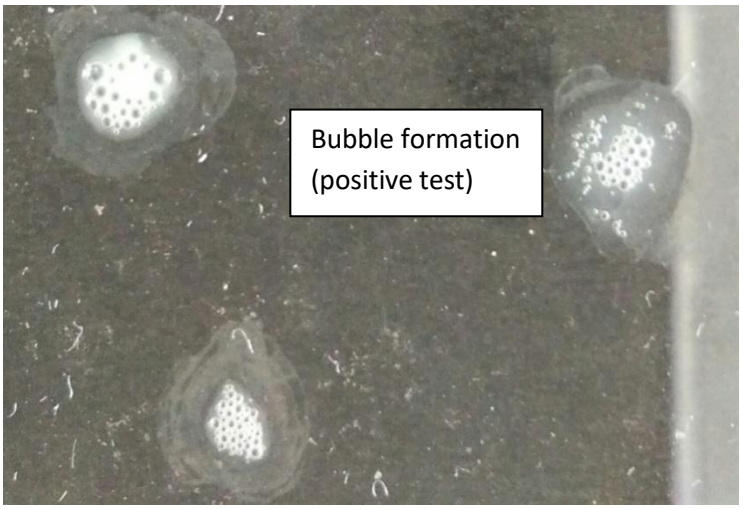


Fig.1: Catalase test showing bubble formation indicating Catalasepositive result

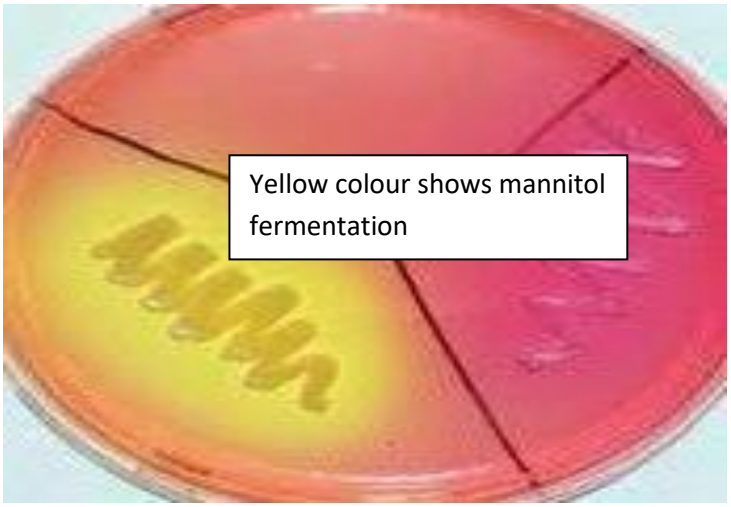


Fig.2: Mannitol fermentation of Isolate (PS1) producing yellow coloration indicates the presence of *Staphylococcus aureus*

Gram Positive Rods

For **Gram positive Rods**, **Endospore staining** was done which showed negative results .Further **acid fast staining** was done to endospore negative isolates which again showed negative results indicating *Corynebacterium* spp and *Lactobacillus spp.* Further Catalase test was done, in which 20 Isolates showed catalase positive result indicating *Corynebacterium* spp ,while as 1 isolate showed Catalase negative result indicating *Lactobacillus* spp **Starch hydrolysis test** was further done to catalase positive isolates, in which all isolates showed starch hydrolysis positive results and confirmed the presence of *Corynebacterium kutsceri* .

Glucose fermentation activity was further checked for Catalase negative isolate which showed acid production indicating *Lactobacillus casei* and *Lactobacillus delbrueckii*. Finally Mannitol fermentation

test was done which showed negative results confirming the presence of *Lactobacillus delbrueckii*.

Fig.3: Starch hydrolysis test showing the presence of *Corynebacterium kutsceri.*

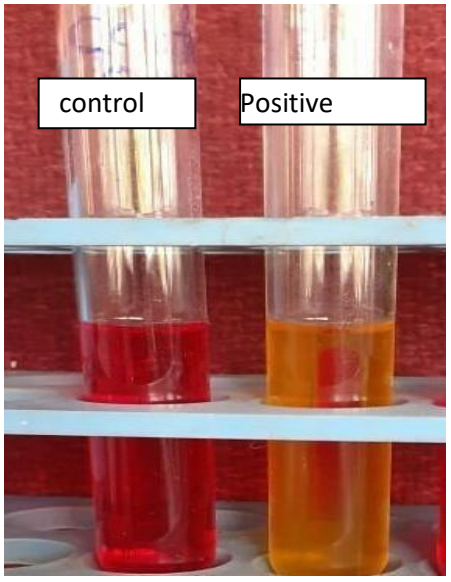


Fig.4: Glucose fermentation test performed for *Lactobacillus* spp shows yellow colour production for isolate(OS4) indicating positive result .

Gram Negative Rods

For **Gram Negative Rods**, Oxidase test was done in which all isolates showed positive result indicating *Aeromonas spp, Pseudomonas spp, Vibrio spp.* Further **Glucose** fermentation test was done in which one isolate showed positive result**(+ve Acid)** while another one showed negative result**(-ve Acid)** .The positive acid isolate was further taken and grown on **Na^+ nutrient agar medium** which showed negative result indicating *Aeromonas spp*. **VP test** was further done to *Aeromonas spp* which showed VP negative result showing Other *Aeromonas spp* .

On the other hand the**(–ve Acid)** *Pseudomonas spp* were grown on **Psedo F Agar** to check Fluorescent Diffusible Yellow Pigment produced by *Pseudomonas* which showed negative result as no green pigment production was seen on the plate .The negative result showed the presence of other *pseudomonas spp* .

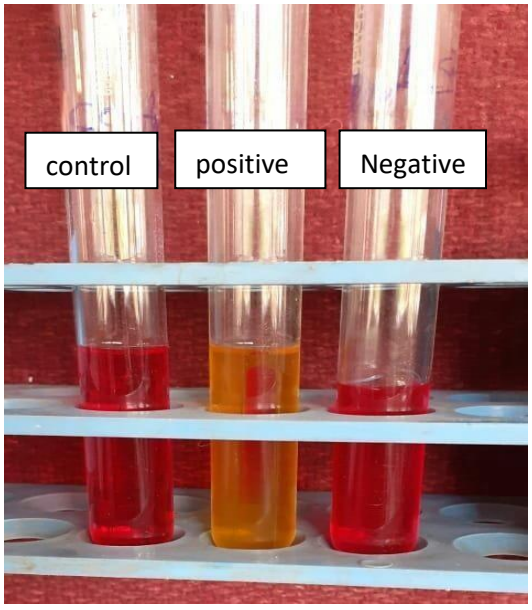


Fig.5: Glucose fermentation test showing positive result (yellow colour production) for isolate(GS3) and negative result for isolate (LS2).

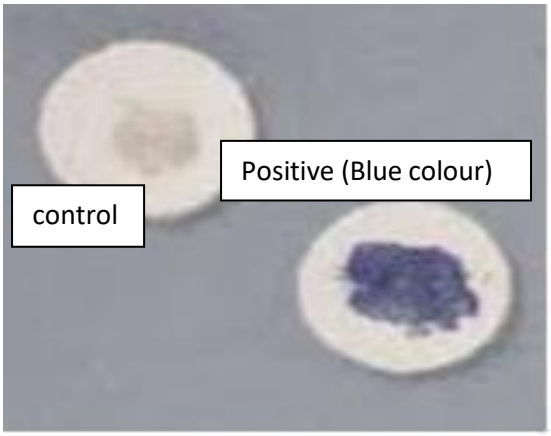


Fig.6: Oxidase test showing (blue colour production) on oxidase disc indicating oxidase positive result

Table 2 : Colony characteristics of Bacterial Isolation

Sampleno.	Samplename	Colour of Colonies	Isolated Organism
1	Apple	Yellow coloured mucoid colonies	*Staphylococcus epidermidis*
2	Banana	White coloured mucoid colonies	*Staphylococcus Saprophyticus*
3	Grapes	White coloured mucoid colonies	*Corynebacterium Kutsceri*
4	Lemon	Cream coloured small ,mucoid colonies Small dull white colonies	*Pseudomonas spp. Corynebacterium kutsceri*
5	Mango	Cream coloured small,round mucoid colonies	*Corynebacterium Kutsceri*
6	Orange	Red coloured colonies , Cream coloured colonies	*Lactobacillus delbrueckii Staphylococcus Epidermidis*
7	Papaya	Cream coloured large colonies	*StaphylococcusAureus*
8	Pomegranate	white coloured small mucoid colonies	*Corynebacterium Kutsceri*

Antibiotic Sensitivity Test

The results of antibiotic sensitivity test were interpreted on the basis of Disc Diffusion Method or in accordance to Performance Standards for Antimicrobial Disk Susceptibility Test, CLSI (formerly NCCLS) and are presented as the resistance of bacterial isolates and sensitivity among the bacterial isolates .

Among the **β-lactam antibiotics** : Ampicillin was showing sensitivity for 10 isolates out of 30 i.e 20 isolates were resistant for this antibiotic.

Amoxyclav was showing sensitivity for 4 isolates out 30, rest 20 isolates were resistant for this antibiotic.

The **Macrolide antibiotics**: Erythromycin was showing sensitivity for 13 isolates out of 30; rest 17 isolates were resistant for this antibiotic.

The **Glycopeptide group of antibiotics:** Vancomycin showed sensitivity for 12 isolates out of 30 isolates rest 18 isolates were resistant for this antibiotic

Quinolone group: Ciprofloxacin showed highly sensitive to all the isolates of every strain.

Cephalosporins group of antibiotics: Ceftazidime was active towards most of the isolates Sexcept 8 isolates which were showing resistance to this antibiotic.

Aminoglycoside antibiotics: Amikacin showed highly sensitive to all the isolates of everystrain.

Table 4 : Antibiotic Sensitivity Test of Bacterial Isolates

Isolateno	**zone diameter(in mm)**						
	CIP	**AMC**	**AMP**	**E**	**VA**	**AK**	**CAZ**
AS1	27	_	_	15	19	31	28
AS2	34	_	_	_	_	16	21
AS3	32	_	_	_	_	14	22
AS4	36	30	32	36	32	24	18
BS1	27	_	_	_	_	4	4
BS2	30	_	14	_	22	6	8
BS3	28	_	12	16	_	18	_
BS4	30	_	16	_	18	18	14
OS1	36	_	_	16	17	10	12
OS2	33	_	_	16	34	11	16

Antibiotics used in Antibiotic Sensitivity test .

CIP = Ciprofloxacin (10mcg). AMC = Amoxyclav (30mcg). AMP = Ampicillin (10mcg). E = Erythromycin (15mcg). VA = Vancomycin (30mcg).AK = Amikacin (30mcg).

CAZ = Ceftazidime (30mcg).

Antibiotic Sensitivity Test of Bacterial Isolates

Isolateno	zone diameter(in mm)						
	CIP	AMC	AMP	E	VA	AK	CAZ
OS3	36	32	34	34	33	32	28
OS4	34	_	04	12	31	18	16
MS1	28	12	16	_	26	22	10
MS2	24	_	24	12	22	12	_
MS3	26	_	17	_	19	8	10
PS1	24	14	13	14	10	14	_
PS2	25	_	17	_	24	12	12
PS3	28	_	18	_	15	12	14
PS4	19	_	22	_	21	14	_
PGS1	32	_	16	_	24	16	12

Antibiotics used in Antibiotic Sensitivity test .

CIP = Ciprofloxacin (10mcg). AMC = Amoxyclav (30mcg). AMP = Ampicillin (10mcg). E = Erythromycin (15mcg). VA = Vancomycin (30mcg). AK = Amikacin (30mcg).

CAZ = Ceftazidime (30mcg).

Table 4 : Antibiotic Sensitivity Test of Bacterial Isolates

Isolateno	zone diameter(in mm)						
	CIP	AMC	AMP	E	VA	AK	CAZ
PGS2	24	_	14	_	13	17	14
PGS3	25	_	15	_	25	16	12

PGS4	22	_	18	10	16	20	12
LS1	26	_	22	_	22	12	12
LS2	19	_	23	_	20	_	_
LS3	20	_	11	_	19	12	_
GS1	23	_	16	_	18	18	12
GS2	31	_	24	_	21	22	12
GS3	30	_	17	12	19	12	12

Antibiotics used in Antibiotic Sensitivity test .

CIP = Ciprofloxacin (10mcg). AMC = Amoxyclav (30mcg). AMP = Ampicillin (10mcg). E = Erythromycin (15mcg). VA = Vancomycin (30mcg).AK = Amikacin (30mcg).

CAZ = Ceftazidime (30mcg

Antibiotic Susceptibility profile of bacteria isolated from rotten fruit sample .

Staphylococcus epidermidis and *Staphylococcus saprophyticus* isolates obtained from fruit samples were tested for their sensitivity to Ciprofloxacin (10 mcg) Amoxyclav (30mcg) Vancomycin (30 mcg) Ampicillin (10 mcg) Erythromycin (15 mcg) . all the isolates of Staphylococcus epidermidis were found to be susceptible to to three antibiotics viz., Ciprofloxacin, Vancomycin, Erythromycin, the only isolate (AS1) was found resistant to Amoxyclav, Ampicillin .

For *Staphylococcus saprophyticus*, all the isolates were found to be susceptible to these antibiotics, the only isolate (BS1) was found resistant to Vancomycin, Erythromycin, Amoxyclav, Ampicillin .

Corynebacterium kutsceri and *Staphylococcus aureus* isolates obtained from fruit samples were tested for their sensitivity to Amoxyclav (30 mcg), Erythromycin (15 mcg), Ceftazidime (30 mcg), Amikacin (30 mcg) . All the isolates were found to be susceptible to these antibiotics except the isolates (PS2) and (MS3) were found resistant to Amoxyclav, Erythromycin, Ceftazidime, Amikacin

Lactobacillus delbrueckii isolates obtained from fruit samples were tested for their sensitivity to Ciprofloxacin (10 mcg), Ampicillin (10 mcg), Amoxyclav (30 mcg), Vancomycin (30 mcg), Erythromycin (15 mcg) .All isolates of Lactobacillus delbrueckii were susceptible to all antibiotics except Amoxyclav and Erythromycin which were found resistant .

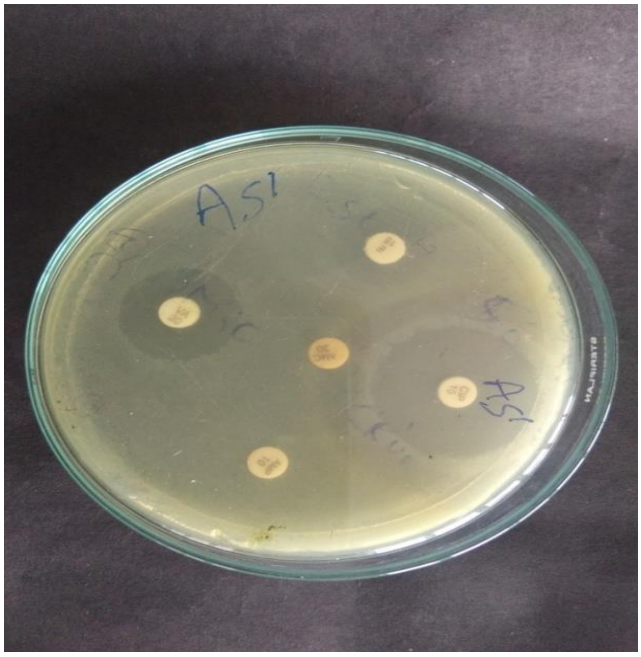
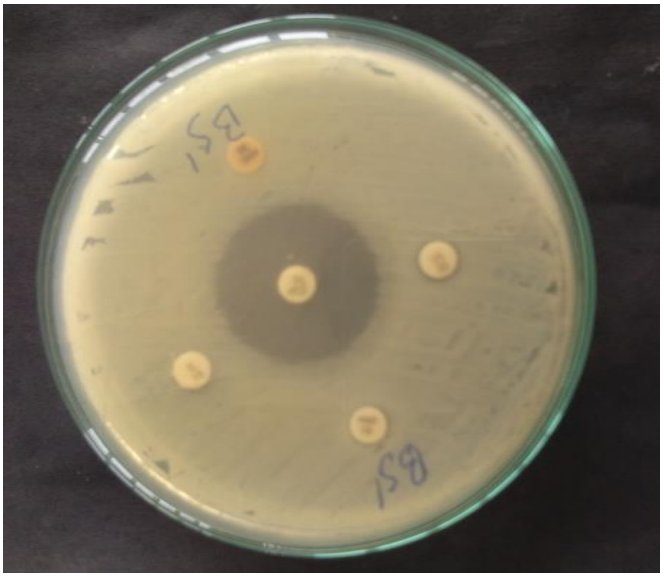
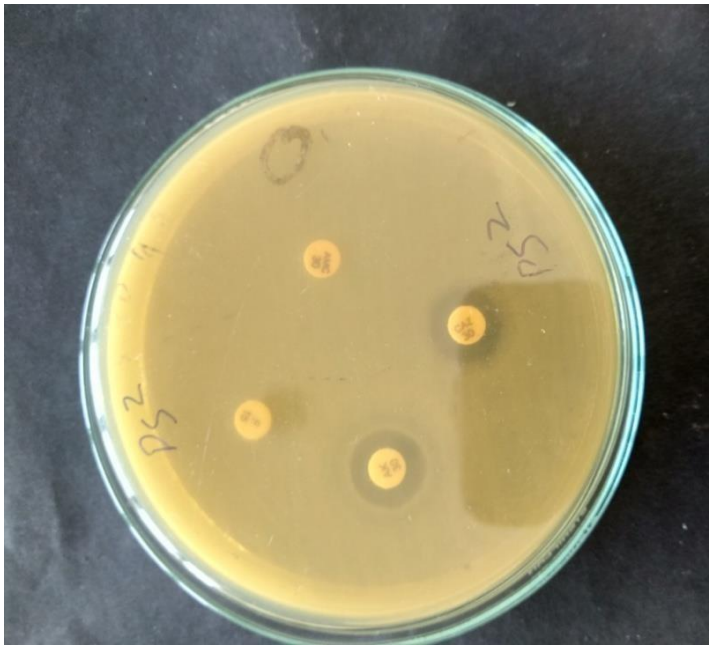


Fig (A) : Isolate (Asi) Showing Resistance To Amoxyclav, Ampicillin

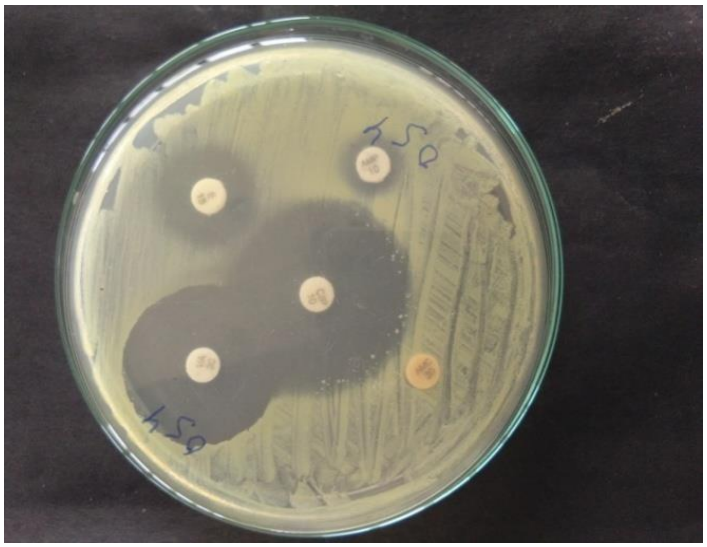


Fig(B):Isolate (Bsi) Showing Resistance To Vancomycin, Erythromycin,Amoxyclav, Ampicillin

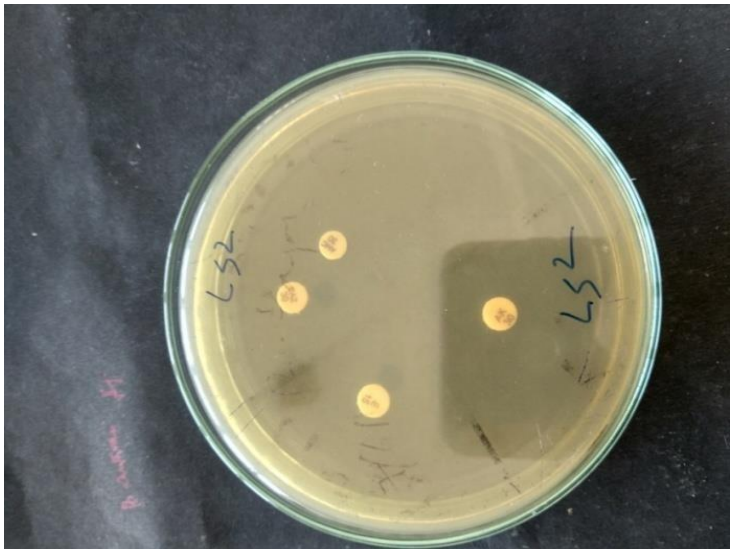


Fig(C): Isolate (Ps2) Showing Resistance To Amoxyclav, Erythromycin, Ceftazidime,Amikacin

Fig(D): Isolate (Ms3) Showing Resistance To Amoxyclav, Erythromycin, Ceftazidime, Amikacin .



Fig(E): Isolate (Os4) Showing Resistance To Amoxyclav, Erythromycin, Ampicillin



Isolate (Ls2) Showing Resistance To Amoxyclav, Erythromycin, Ceftazidime, Amikacin

(-) = No Zone Inhibition .

R = Resistance (if Zone size is below 13)

M = Moderate (if zone size is in between 14-17)S = Sensitive (if Zone size is above 20)

Fruits are consumed widely because of their nutritive value, mineral and vitamin content .The microorganisms normally present on the surface of the raw and minimally processed fruits

may consist of chances of contamination from soil or dust, irrigation water or various operations like harvesting, transportation, storage, and processing of the produce (Beuchat, 1996 ; Ray and Bhuma, 2007). Among the various groups of bacteria commonly found on fruits may include coliforms or faecal coliforms such as *Klebsella* and *Enterobacter* (Duncan and Razzell, 1972). However the surface microflora may include microorganisms capable of causing human diseases which may pose a threat to human health (Beuchat, 1996;Doyle 1990).

The current study was aimed at determining the microbiological quality of fruits from different sources and total 29 bacterial isolates were obtained from them. The isolates were then identified on the basis of bio chemical characteristics and the *Staphylococcus, Corynebacterium, Lactobacillus,* were isolated from fruits . Antibiotic resistance among the isolates was also evaluated using 7 antibacterial antibiotics Amoxyclav, Ampicilin,Ciprofloxacin, Erythromycin, Vancomycin, Amikacin, Ceftazidime .

In the same way, previously reported 32 isolates were taken from avocado fruits were found to be *Staphylococcus sciuri* , Rahnella and *Klebsella pneumonia* (Oladoye C.O.*et al* ;).

In present study, although the number of bacteria present in fruits is not high but is certainly indicative of unhygienic handling of vegetable from farm to market . The result obtained reveals that bacteria were abundant on the surafce of fruits .

Earlier workers have also reported the presence of these bacteria on fruits surfaces (Alivy *et al* ; 2005). Since the number of microorganisms on the surface of fruits was found to be low and in agreement with the standards mentioned in management (HACC_ PTQM). These fruits may not pose a risk for the consumer . Moreever these fruits are safe and do not need any pre-treatment to further minimize the food borne pathogens on the suraface of these fruits.

The *Staphylococcus aureus* isolated was an indication of poor hygiene practices by both the farmers and sellers . Further , the morphological and biochemical characteristics of the isolates from different samples were performed . The morphological and biochemical characteristics indicates the presence of bacteria such as *Staphylococcus, Corynebacterium, Lactobacillus* etc .

SUMMARY AND CONCLUSION

The present study entitled " Analysis of spoiled fruits samples for the prevalence of bacteria " was conducted to examine the presence of pathogenic bacteria in fruits that are used for consumption . The samples were collected from different sources . The results of the studyare summarized as follows :

The bacterial isolation was performed on Nutrient Agar medium . The isolated colonies of bacteria were yellow coloured , white mucoid and pink coloured in appearance .

Total 29 isolates of bacteria were taken for Gram's staining , catalase test, mannitol fermentation, endospore staining, starch hydrolysis test, glucose fermentation test, oxidase test etc .

Out of 29 isolates ,one isolate was *Staphylococcus aureus*, four isolates were *Staphylococcus epidermidis*, two isolates were *Staphylococcus saprophyticus*, twenty isolates were *Corynebacterium kutsceri*, one isolate was *Lactobacillus delbrueckii*.

The isolates were then checked for antibiotic sensitivity, total 7 antibacterial antibiotics were taken for the test which were Ciprofloxacin, Vancomycin, Erythromycin, Amoxyclav, Ceftazidime, Ampicillin,

Amikacin . The test was performed on Mueller Hinton Agar .

All isolates of *Staphylococcus epidermidis* were found to be susceptible to three antibiotics viz, Ciprofloxacin, Vancomycin, Erythromycin . Some resistance was observed againist Amoxyclav, Ampicillin .

All isolates of *Staphylococcus saprophyticus* were found susceptible to Ciprofloxacin, Vancomycin, Erythromycin, Amoxyclav, Ceftazidime, Ampicillin, Amikacin. One isolate was found resistant to Vancomycin, Erythromycin, Amoxyclav, Ampicilin .

Corynebacterium kutsceri isolates were found to susceptible to Amoxyclav, Erythromycin, Ceftazidime, Amikacin . Two isolates were found resistant to Amoxyclav, Erythromycin, Ceftazidime, Amikacin .

Lactobacillus delbrueckii isolates were found susceptible to Erythromycin, Amoxyclav, Vancomycin, Ampicillin . Some resistance was observed againist Amoxyclav .

In conclusion, we can say that as ascertained from the previous studies, the number of bacteria present on the surface of these fruits is satisfactory and within limits from human health point of view, but the consumption of such fruits may cause serious threat to consumer safety .

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