

Persistence of various food contaminating bacteria in various food samples of Solan city

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Abstract

Food is consumed to provide nutritional support for the body. Food is a source of nutrients therefore it also provides suitable growth medium to pathogenic organisms for its proliferation as a result food get contaminated which can cause consumer illness. Unsanitary handling of the food by the some of the vendors has been commonly found to be the source of contamination. In the present study from 12 food samples 30 bacterial strains were isolated. Some of the isolates showed characteristic growth/pigment over Nutrient Agar, MacConkey Agar, Bismuth sulphite agar and EMB Agar. Further, biochemical identification showed that isolate OJ was *E. coli*, isolate PJ was *S. aureus*, isolate CJ was *Salmonella sp.* and isolate SJ was *P. aeruginosa*. Further, antibiotic susceptibility test results showed that minimum zone of inhibition about around 12mm showed by *Salmonella sp.* against Gentamicin. The isolate *S. aureus* was found resistant to Aztreonam and Penicillin whereas *E. coli* isolate was resistant to Cefotaxime and Ampicillin. On the other hand, *P. aeruginosa* was found resistant to Cefotaxime, Aztreonam and Cefoperazone. *Staphylococcus aureus* was found Coagulase positive while all other bacteria were Coagulase negative. *P. aeruginosa* and *Salmonella sp.* was found positive for proteolytic activity. Only *Pseudomonas aeruginosa* showed haemolytic activity. So, overall *Pseudomonas aeruginosa* showed maximum virulent traits alongwith antibiotic resistance. Therefore, it is necessary to monitor microbial community in the different food samples specially pathogenic bacteria by the govt. authority as the presence of pathogenic and multi-drug resistant bacteria may be a cause of epidemic.

Keywords: Antibiotic resistance, virulence, food safety, *E. coli*, *P. aeruginosa*, *S. aureus*, *Salmonella sp.*

INTRODUCTION

Food when consumed provides energy as well as various nutrients required for the body growth. It is mainly composed of carbohydrates, fats, proteins, vitamins or materials. It is mainly composed of carbohydrates, fats, proteins, vitamins or materials. The substance is ingested by an organism and assimilated by the machinery order to produce energy and maintaining life. The study of microorganisms that inhibit, create or contaminate food is called food microbiology [1].

A healthy food is good for health but on the other hand contaminated or unhealthy food is responsible for various diseases. Contaminated food not only leads to diarrhoeal diseases but also may lead to malnutrition. Therefore, to get good quality food hygienic practices should be strictly followed [2]. The various food associated pathogenic bacteria include: *B. cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringes*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus* [3].

Food borne illness usually arises from improper handling, preparation, or food storage. The various pathogens like bacteria, viruses, fungi or parasites if present in food may lead to serious human sickness. So, hygienic practices should be strictly obeyed during various food processing stages to overcome the foodborne illness. Besides foodborne pathogens sometime medicine in food, chemical compounds in pesticides or even toxic compounds naturally present may lead to foodborne illness [4]. Among various foodborne pathogens the common one is bacteria. Initially gastrointestinal infection with foodborne pathogen leads to vomiting, diarrhea and cramps but uncontrolled infection may spread to whole body

which leads to the death of host [5]. To maintain the food quality regular microbial surveillance should be done in food or food products. Keeping this in view present study was designed to check the microbial quality of different food products available in local market.

MATERIAL AND METHODS

Collection of food samples:

Samples of fruit juices (orange juice, grape juice, mausami juice, pomegranate juice, carrot juice, sugarcane juice, apple juice), mango shake, banana shake, panipuri and curd were collected from local market of Solan and examined for the presence of pathogenic bacteria. The different samples were collected in a sterilized plastic container with proper labeling. All the samples were brought directly to the laboratory and processed within 24 hours.

Sample processing and isolation of bacteria:

The samples were processed for the isolation of bacteria using serial dilution method on Eosine Methylene blue (EMB), MacConkey agar and Nutrient agar. Further the selected isolates were grown on Mannitol Salt agar (MSA) and Bismuth Sulphite agar (BSA).

Characterization of bacterial isolates:

The selected bacterial isolates were identified on the basis of morphological and biochemical characteristics as described in the Bergey's manual of systematic bacteriology [6].

In vitro antimicrobial susceptibility testing:

The selected isolates were tested for their susceptibility against various antibiotics by Disc diffusion method as per methodology described by [7]. The various antibiotics tested in the present study were Ampicillin, Amoxyclav, Aztreonam, Cefoperazone, Cefotaxime, Ceftriaxone, Ciprofloxacin, Erythromycin, Gentamycin, Nalidixic Acid, Ofloxacin, Oxacillin, Penicillin, Methicillin, Tetracycline, Vancomycin.

Inoculum preparation:-

Inoculum was prepared by inoculating bacterial culture in nutrient broth and broth was incubated at 37°C for 24 hours.

Antibiotic sensitivity pattern:-

Lawn from inoculum culture was spreaded with sterile L- shaped spreader on Mullen-Hinton agar. Different antibiotic discs were distributed evenly on the surface of agar plate, with at least 24mm distance between them. Discs were placed individually with sterile forceps and pressed gently down on to the agar surface. Plates were incubated at 37°C for 24-48 hours and observed for the appearance of zones of inhibition around the discs. The zone of inhibition was measured in mm.

Analysis of virulence factors:-

The selected strains were further analyzed for various virulence factors:

Coagulase test:-

For coagulase test, colony of test microorganism was mixed with the normal saline followed by addition of a loop ful of plasma suspension. The slide was examined for the immediate clumping within 10 seconds. An uninoculated slide containing plasma and saline only were kept as negative control [8].

Hemolysin production:-

The haemolytic activity of the selected strains were detected on blood agar plates. The spot inoculated plates were incubated at 37° C for 24 hours. The haemolytic activity was detected by the presence of clear zone around the colonies [9].

Detection of proteolytic activity:-

The protease activity was detected on nutrient agar amended with 2% casein. The isolates were spot inoculated on amended media and plates were incubated at 37°C for 24 hours. The positive result indicated by the presence of clear zone around the bacterial colony [10].

RESULTS AND DISSCUSION

Isolation and Identification of bacteria from food samples:-

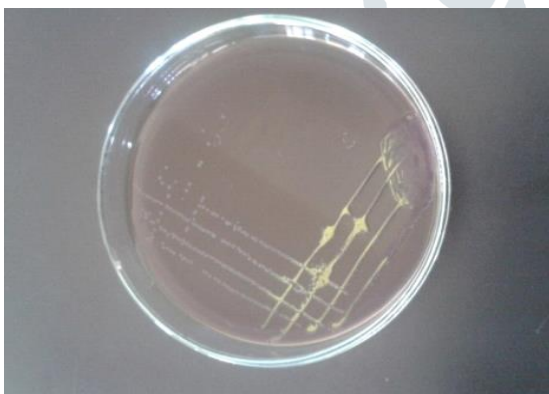
Food is biological in nature and is capable of supplying nutrients, food contain combination of nutrients and other healthful substances. Food is equally supporting the growth of contaminating microorganisms to the

food processor. Fruit juices are well recognized for their nutritive value, mineral and vitamin content. In India, especially in the metropolitan cities people they prefer to take juice of fresh fruits from the street vendors. These juices generally carry the contamination of *Shigella*, *E. coli*, *Staphylococcus aureus* and *Salmonella* [11].

The fruit and vegetable juices prepared by compromising hygienic practices are reported to be a major cause of food borne illness [12]. Throughout world enteropathogenic microbes like *Staphylococcus aureus*, *Vibrio cholera* and *Salmonella* leads to major food borne outbreaks [13]. These pathogenic bacteria are responsible for various diseases like enteric fever, dysentery and typhoid etc, therefore persistence of these pathogens in juices is unsafe for human health [14].

From twelve different food samples thirty isolates were isolated on Eosine Methylene blue (EMB), MacConkey agar and Nutrient agar. These three types of media were used to isolate the bacterial strains from different samples. EMB was used as the selective media for the isolation of *E. coli* whereas MacConkey agar as selective medium for the lactose fermenter. Only one isolate showed characteristic growth on EMB agar plate which was obtained from orange juice. This isolate showed metallic sheen on EMB plate and was Gram negative rods which confer that this was *E. coli* (Fig 1A). On MacConkey agar only one isolate showed pink coloured (lactose fermenter) colony which was obtained from the carrot juice. The one isolate obtained from sugarcane juice on Nutrient agar was Gram negative rods which showed greenish pigmentation indicated this isolate may be *P. aeruginosa* (Fig 1B). Bacterial colonies obtained on the Nutrient Agar and MacConkey agar plate were further streaked on other selective medium i.e. Mannitol Salt agar (MSA) for *Staphylococcus aureus*, BSA for *Salmonella* and EMB for *E. coli*.

It was observed that only one isolate obtained from pomegranate juice was Gram positive cocci in clusters which showed growth on Mannitol Salt Agar and converts pink colour of media into yellow and confirmed that the isolate was *Staphylococcus aureus* (Fig 1C). Whereas one isolate obtained from carrot juice was Gram negative rods showed black metallic shining colonies on Bismuth Sulphite Agar (Fig 1D) and was confirmed that it was belonged to *Salmonella spp.* Other isolates showed no characteristics growth over these selective media (MSA, BSA and EMB) and therefore all these isolates were screened out from the present study. Only four isolates which could be pathogenic were selected for further study.



(A)



(B)

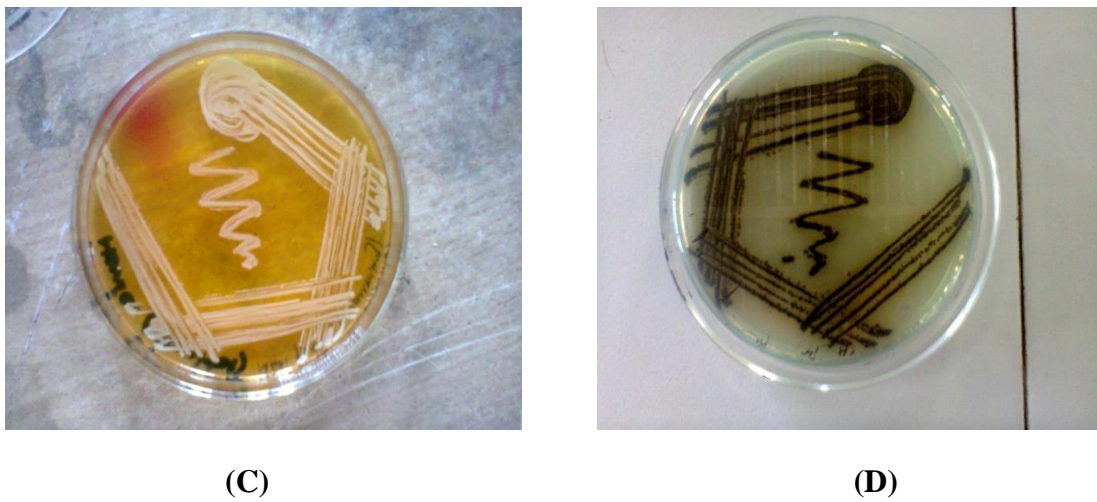


Fig 1:- Colony morphology of bacterial isolates of (A) *E. coli* on Eosine Methylene Blue Agar plate, (B) *Pseudomonas aeruginosa* on Nutrient Agar, (C) *Staphylococcus aureus* on Mannitol Salt Agar, (D) *Salmonella sp.* on Bismuth Sulphite Agar.

Biochemical characterization:-

Further four virulent strains were identified on the basis of biochemical characterization. To confirm the identity of bacteria Voges- Proskauer test, Indole test, Citrate test, Urease test and Methyl Red test were performed. Additionally, various sugars like Mannitol, Lactose, Dextrose and Sucrose were tested to be fermented by tested bacteria. Along with these test Motility and Urease test were also performed. All the isolates were motile except for isolate PJ. Isolate PJ was Indole –ve, MR +ve, VP –ve, Nitrate +ve, Citrate –ve, Urease +ve and identified as *S. aureus*. Isolate OJ was Indole +ve, MR +ve, VP –ve, Nitrate -ve, Citrate +ve, Urease –ve and identified as *E. coli*. Isolate CJ was Indole +ve, MR +ve, VP –ve, Nitrate -ve, Citrate +ve, Urease +ve and found to be *Salmonella sp.* Isolate SJ was Indole –ve, MR -ve, VP –ve, Nitrate +ve, Citrate +ve, Urease +ve and identified as *P. aeruginosa*.

In vitro Antimicrobial Susceptibility Testing:-

Antibiotic sensitivity test is generally used to determine the efficiency of antibiotic in controlling the microbial infection. The success of antibiotic in controlling the infection depends upon many factors like potential of bacteria to tolerate antibiotic, the infection location site and efficiency of antibiotic to reach infection site. Antibiotic resistant bacteria have evolved different types of mechanisms to overcome the lethal effect of antibiotics like inactive the antibiotics with the help of enzymes, removal of antibiotic from the cell using pump system, or alter the antibiotic attachment site etc [15].

In the present study the isolated pathogenic microorganisms i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella spp.* and *E. coli* were screened for *in vitro* antimicrobial susceptibility testing. The results showed that maximum zone of inhibition about around 35mm showed by Ceftriaxone antibiotic against *Salmonella sp.* (Table 1). Depending on antibiotics they may have several targets in the bacteria for killing. In the present study tested antibiotics kills the bacteria by several mechanisms and one of the mechanisms is enzyme inhibitor [16].

Antibiotics act on the active site and reach into intracellular targets and showed zone of inhibition. Likewise other bacteria also show sensitivity against some other antibiotics. In the present study it was found that among all antibiotics which were used against *Staphylococcus aureus* Amoxyclav (AMC) showed maximum sensitivity (24mm) while Penicillin (P-G) was resistant to the bacteria. In the case of *Pseudomonas aeruginosa* Ciprofloxacin (CIP) was the most sensitive (34mm) and Aztreonam (AT) showed resistant. Maximum sensitivity (35mm) in case of *Salmonella spp* was showed by Ciprofloxacin (CIP) and whereas Cefoperazone (CPZ) was found to be resistant. In case of *E. coli* maximum zone of inhibition (25mm) was showed by Ciprofloxacin (CIP) and Ampicillin (AMP) was found to be resistant.

Table No. 1. Zone of inhibition (mm) for various antibiotic against pathogenic organisms

S.NO.	Organisms	VA	P-G	GEN	MET	AT	OX	AMC	AMP
1)	<i>Staphylococcus aureus</i> (PJ1)	16mm (I)	8mm (R)	20mm (S)	10mm (I)	24mm (R)	6mm -	24mm (S)	12mm -
2)	<i>Pseudomonas aeruginosa</i> (SJ1)	CTR 20mm (I)	CIP 34mm (S)	CTX 20mm (R)	GEN 19mm (S)	CPZ 10mm (R)	AT 8mm (R)	E 14mm -	OF 18mm (S)
3)	<i>Salmonella sp.</i> (CJ1)	P-G 14mm (R)	GEN 20mm (S)	CPZ 12mm (R)	OF 24mm (S)	AT 14mm (R)	CTR 35mm (S)	CIP 35mm (S)	NA 22mm -
4)	<i>E. coli</i> (OJ1)	E 18mm -	TE 16mm (S)	CTX 17mm (R)	OF 20mm (S)	NA 14mm (I)	AMP 8mm (R)	CIP 25mm (S)	GEN 20mm (S)

AMP:Ampicillin, AMC:Amoxyclav, AT:Aztreonam, CPZ:Cefoperazone, CTX: Cefotaxime, CTR:Ceftriaxone, CIP:Ciprofloxacin, E:Erythromycin, GEN:Gentamycin, NA:Nalidixic Acid, OF:Ofloxacin, OX:Oxacillin, P:Penicillin-G, MET:Methicillin, TE:Tetracycline, VA: Vancomycin.

Analysis of virulence traits for different organism:-

Certain other tests were also performed to check the virulence traits of the bacteria. In this coagulase activity, proteolytic activity, haemolytic activity was tested (Table 2). While performing these test it was observed that out of four different organism *Pseudomonas aeruginosa* showed more virulence traits.

Table No. 2. Analysis of virulence traits for different organisms.

S.NO.	Name of the organism	Coagulase test	Proteolytic activity	Haemolytic activity
1)	<i>Staphylococcus aureus</i> (PJ1)	positive	negative	negative
2)	<i>Salmonella sp.</i> (CJ1)	negative	positive	negative
3)	<i>E. coli</i> (OJ1)	negative	negative	negative
4)	<i>Pseudomonas aeruginosa</i> (SJ1)	negative	positive	positive

Coagulase test is a confirmatory test for *Staphylococcus aureus* as *S. aureus* secrete free plasma coagulase which is a virulence factor and distinguishing it from CoNS (Coagulase Negative *Staphylococci*). Protease is an enzyme that is secreted by some microorganisms as it accounts for the virulence factor of bacteria. Proteases are the enzyme that breaks down specific bonds in polypeptides and proteins and they play very important role in many biological processes including in disease control like infection and stroke. In case

of haemolysin many bacteria shows haemolytic activity on Blood agar as it degrade the epithelial cells and grow intracellularly. Hemolysin is lipid and protein that causes lyses of red blood cells by destroying their cell membrane.

While performing all these tests, *Staphylococcus aureus* was observed as Coagulase positive while all other bacteria were Coagulase negative (Table 2). Proteolytic activity was shown by *Pseudomonas aeruginosa* (Fig 2A) and *Salmonella sp.* (Fig 2B) while other two bacteria showed negative result for porteolytic activity. In case of Haemolysin only *Pseudomonas aeruginosa* showed haemolytic activity (Fig 3) while all other bacteria were non haemolytic.



Fig 2 Proteolytic activity on Nutrient agar supplemented with casein showed by: (A) *Pseudomonas aeruginosa* (B) *Salmonella sp.*



Fig 3. *Pseudomonas aeruginosa* showing hemolytic activity on Blood agar plate

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