

Microbiological surveillance of food and beverage services in a tertiary care hospital of Punjab, India

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Abstract

Microbiological surveillance of food and beverage services concerns the investigation and identification of various organisms, which cause the food borne disease in the human being. These diseases can vary from mild to severe in the gastrointestinal tract. The severity of diseases is depending on the load and type of the organism. Disease caused by foodborne pathogens can vary from diarrhoea to toxic shock syndrome. These diseases are caused by the variety of Gram-negative organism like *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio cholerae* and *Escherichia coli* O157:H7. Consumption of food, contaminated by their organism it can lead to profuse, painless, watery diarrhoea and vomiting, and in severe cases hypovolemic shock and death can occur in less than 2 hours. *Escherichia coli* O157:H7 can produce the deadly toxic after the consumption. The main source of their organism are raw milk, unpasteurized juice and raw sandwich. *Shigella* causes the infection in the gastrointestinal tract and their toxin can survive the gastric acidity better than another organism. *Salmonella* is the cause of gastroenteritis, food poisoning, enteric fever and septicaemia. *Campylobacter* is present in the raw and undercooked food, untreated water and raw milk. If the hospital kitchen and some food is preparing is an unhygienic manner, there are high chance that type of organism was spoil the food easily and may cause infections in the patients and hospital staff. Therefore, the food and beverage service in the hospital need to ensure every hygienic process in the preparation of the food. In the current study, we examined the presence of pathogenic microbes in food material, utensils and food handlers in a tertiary hospital of Mohali, Punjab, India.

Keywords: Food contamination, bacteria, public health, microbial surveillance, hospital environment

INTRODUCTION

Food microbiology is study of those organisms which contaminate the food and cause the spoilage of food, Pathogens may cause disease especially if food is improperly cooked and stored. Government authorities, hospitals and food manufacturers perform microbiological analysis for monitoring the level of contamination to detect the associated risks. Raw and cooked food carry certain microorganism that are mostly unharmed for the human body, but some may spoil the food. Food spoilage microorganisms produce undesirable flavour, odour, texture and appearance that make food unsuitable for human consumption. Food spoilage and food poisoning is a serious issue in the developing countries because of inadequate processing and refrigeration facilities [1]. The food spoiling microorganisms can be categorized as bacteria, virus, fungi and parasites.

Bacteria are mainly present everywhere on the earth and bacteria contribute more than 90% to infect the human body. Bacteria are most common cause of food poisoning. Food poisoning occurs when the person eats the contaminated food and this contamination can occur due to the bacterial flora. Gram negative organisms are the

cause of food poisoning. In this Gram-negative series of bacteria, the bacteria are *Vibrio cholerae*, *Escherichia coli* 0157:H7, *Shigella*, *Salmonella*, *Campylobacter*. *Vibrio cholerae* live in contaminated water and food [2]. When the healthy person ingests this contaminated food, the bacteria will reach the gastrointestinal tract and cause the symptoms [3-5]. This organism is mostly present in the fish, seafood such as prawns and tuna. The symptoms of cholera are diarrhea, vomiting and nausea. *Escherichia coli* 0157:H7. (*E. coli*) is Gram negative organism and cause the infections of digestive system. *E. coli* is the normal flora of the human and animals' intestine. Most of the time it is not cause the any infection in the body but if *E. coli* strain 0157:H7 are introduced to body it leads to the food poisoning. The main source of this strain is water, juice, unpasteurized milk, beef, raw hamburger and undercooked food. Symptoms of food poisoning includes diarrhea, vomiting, fever, headache, nausea, stomach pain etc. [6]. *Shigella* is Gram negative organism and causes the infect the gastrointestinal tract of human and animal. Infections caused by *Shigella* is called *Shigellosis* [2, 7]. The main source of this infection is contaminated food, water and direct contact with infected persons. This also present diarrhoea, vomiting, stomach pain, fever, headache etc.

Salmonella is food and waterborne pathogen that may result when contaminated food is consumed. The *Salmonella* family have more than 2300 serotype but *Salmonella enteritidis* and *Salmonella typhimurium* are most common. The main sources of this organism are water, rice, egg-based food, salads and milk products. The symptoms of this infection are stomach pain, diarrhoea, nausea, fever and chills [5, 8]. *Campylobacter* causes disease called campylobacteriosis. *Campylobacter* is gram negative bacteria. Mostly *Campylobacter jejuni* cause the food poisoning in human digestive system. The main source of this bacteria is raw water, uncooked and under cooked food, raw milk, chicken, raw hamburger etc. [8, 9]. Symptoms of this infection induces diarrhoea, stomach pain, nausea, vomiting, leg cramps, muscle pain and headache [9, 10].

Viruses are intracellular particles and cause the variety of the infection in the human body. Food poisoning is one of the most common infection caused by Virus in the human body. Many of virus are transmitted by consuming contamination food and water. Example of food borne virus is *Hepatitis E virus (HEV)*, *Hepatitis A virus (HAV)*, *Norovirus*, *Rotavirus* [2, 11, 12].

Fungi are eukaryotic microorganism and play a role in food poisoning. *Aspergillus flavus*, *penicillin verrucosum* are the two species are caused the food poisoning in human being. Fungi cause more complication in human than the bacteria and virus. *Aspergillus* is a group of moulds. Their potential contamination of food stuff and animal feeds is widespread under favorable environmental condition [13].

Parasites derive nourishment and protection from the host. It is transmitted from animal to human and human to human. The main source of is food and water. Many of parasites are identified which are transmitted to humans from the animal meat and direct connect. For example: *Taenia saginata* transmit from the beef meat, *Taenia solium* from the pig, *Giardia lamblia* from the water, *Entamoeba histolytica* from the water etc. Parasites are transmitted from uncooked food, undercooked food and also from water [2, 9, 14].

The main objective of the current study was microbiological surveillance of food and beverage services in a tertiary care hospital and identification of the foodborne pathogens in the hospital care facility. For the purpose, we

examined the microbial flora from food (raw, cooked and ready to eat food), water, utensils, serving area and hand soaps. We also inspected the stool of food worker for the presence of pathogenic organism.

MATERIALS AND METHODS

The study was conducted from 1st January, 2018 to 30th April, 2018 in a tertiary care hospital of Mohali, Punjab, India. Following samples were collect from the hospital's kitchen in triplicate by using standard microbiological technique:

A. Raw food

Cheese, Salad (cucumber, beat root, onion, radish), carrot, peas, patient natural feed, Patient powder feed (Peptamen powder), fruits (apple, papaya, watermelon).

B. Cooked food

Chana dal, paneer sabji, gazar, white chana dal, boiled rice, moong dal, khichdi, moong khichdi, besan sierra, kheer, light dal (mix)

C. Ready to eat food

Milk, yogurt, ice-cream, juices, tea.

D. Others

Utensils (Before use), kitchen air sample, kitchen water from main supply, hand soap, food handlers finger samples, food handlers stool samples

The specimen was collected in the sterile container with all standard precautions and processed without any delay. According to the sample type, they were inoculated culture media. Combination of solid and enriched medium was used for identification. The media used were MacConkey agar (HiMedia, Germany), blood agar (HiMedia, Germany), ylose-lysine deoxycholate (XLD, HiMedia, Germany), selanite broth (Selanite F broth, HiMedia, Germany) and Muller Hinton broth (HiMedia, Germany). Specimens were inoculated with Hi-FlexiLoop (HiMedia, Germany). Plates were incubated at 37°C in an incubator for 24- 48 hours. After incubation of 24-48 hours culture plates were examined for bacterial growth and identification by the standard microbiological technique. Identification was done by manual methods [15].

RESULTS

The study was conducted in Lab Medicine Fortis Hospital, Mohali from 1st January 2018 to 30th April 2018. In this study, a total of 209 samples were collected and processed for identification and sensitivity testing. Pathogenic organisms were looked for-

1. *Vibrio cholerae*
2. *Shigella spp.*
3. *Salmonella spp.*
4. *Escherichia coli 0157:H7*
5. *Campylobacter spp.*
6. *Clostridium botulinum*

The results are demonstrated in Table 1-6.

Table 1: Microbial population in raw food sample

Types of sample		No. of samples	Gram Stain	Result
Cheese		3	Gram Positive bacilli	<i>Enterococcus spp.</i> , <i>Bacillus spp.</i>
Salad	Cucumber	3	Gram Positive cocci Gram Positive bacilli	<i>Bacillus spp.</i> , <i>Staphylococcus aureus</i>
	Beetroot	3	Gram Positive bacilli	<i>Kluyvera cryocrescens</i> , <i>Acinetobacter junii</i>
	Onion	3	Gram Positive bacilli	<i>Enterobacter cloacae</i>
	Radish	3	Gram Positive bacilli	<i>Bacillus spp.</i>
Patient Natural feed		6	Gram Positive cocci + Gram Negative bacilli	<i>Bacillus spp.</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i>
Patient powder feed		3	Gram Negative Cocci	<i>Bacillus spp.</i>
Fruits	Apple	3	Gram Negative bacilli	<i>Bacillus spp.</i> , <i>Staphylococcus aureus</i>
	Papaya	3	Gram Negative bacilli	<i>Bacillus spp.</i> , <i>Pseudomonas aeruginosa</i>
	Watermelon	3	Gram Negative bacilli	<i>Bacillus spp.</i>

Table 2: Microbial population in cooked food sample

Type of sample	No. of samples	Gram Stain	Result
Chnna dal	9	Gram Negative Bacilli	<i>Enterobacter spp.</i>
Paneer Sabji	9		Sterile
Gazer Matar	6	Gram Negative Bacilli	<i>Bacillus spp.</i>
White Chana Dal	6		Sterile
Boiled rice	9		Sterile
Moong Dal	6		Sterile
Khicri	6		Sterile
Moong Khicri	9		Sterile
Besan Shera	3	Gram Positive cocci, Gram Positive bacilli	<i>Bacillus spp.</i> , <i>Staphylococcus aureus</i>
Dalia	3		Sterile
Kheer	6		Sterile
Light moong dal	9		<i>Bacillus spp.</i>
Sabji Bhurji	6		Sterile

Table 3: Microbial load in ready to eat food sample

Type of Sample	No. of Samples	Microscope Report	Result	
Milk (pack)	3	Gram Positive Bacilli	<i>Bacillus spp.</i>	
YOGURT	3		Sterile	
ICE-CREAM	STRAWBERRY	3	Gram Negative Bacilli	<i>Klebsiella pneumoniae</i>
	VANILLA	3	Gram Positive Bacilli	<i>Bacillus spp.</i>
JUICE (Pack)	3		Sterile	
TEA	3	Gram Positive Bacilli	<i>Bacillus spp.</i>	

Table 4: Prevalence of microbes among utensils used for serving food

Types of Sample	No of samples	Gram Stain	Result
Tea Flask	1	Gram positive cocci, Gram positive bacilli	<i>Staphylococcus aureus</i> , <i>Bacillus spp.</i>
Tea cattle	1	Gram positive bacilli	<i>Bacillus spp</i>
Chapati box	1	Gram positive bacilli, Gram positive cocci	<i>Bacillus spp.</i> , CONS (Coagulase negative <i>Staphylococcus aureus</i>)
Serving bowl	1	Gram positive cocci	<i>Staphylococcus aureus</i>
Spoon	1	Gram positive cocci	CONS
Cup	1	Gram positive cocci	<i>Staphylococcus aureus</i>
Sosser plate	1	Gram positive cocci	<i>Staphylococcus aureus</i> , CONS, <i>Bacillus spp.</i>
Big plate	1	Gram positive cocci	<i>Staphylococcus aureus</i> , CONS
Serving area	1	Gram positive bacilli	<i>Bacillus spp.</i> , CONS
Chopping surface area	1	Gram positive cocci	<i>Staphylococcus aureus</i> , CONS, <i>Bacillus spp.</i> , <i>Enterococcus</i>
Chopping board	1	Gram positive cocci	CONS
Medium bowl	1	Gram positive cocci	CONS, <i>Enterococcus spp.</i>
Large bowl	1	Gram positive cocci	CONS, <i>Enterococcus</i> , <i>Bacillus</i>
Small bowl	1	Gram positive cocci, Gram positive bacilli	<i>Staphylococcus aureus</i> , <i>Bacillus</i>
Tray	1	Gram positive cocci	CONS

Table 5: Microbial load in water supply and hand soap

Type of Sample	No. of samples	Gram stain	Result
Water from main supply	3	Gram Negative cocci, Gram positive bacilli	<i>Pseudomonas aeruginosa</i> , <i>Bacillus spp.</i>

Hand soap	2	Gram Negative cocci, Gram Negative Bacilli	<i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i>
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Table 6: Microbial load in the hands of food handler staff

Type of Sample	No of samples	Gram stain	Result
Hand Fingers (1)	2	Gram Positive cocci, Gram positive bacilli	CONS, <i>Bacillus spp.</i>
Hand Fingers (2)	2	Gram positive bacilli, Gram positive bacilli	<i>Bacillus spp.</i> , CONS
Hand Fingers (3)	2	Gram positive cocci, Gram Negative cocci	Methicillin-susceptible <i>Staphylococcus aureus</i> (MSSA), <i>Bacillus spp.</i>
Hand Fingers (4)	2	Gram positive cocci, Gram positive bacilli	MSSA, <i>Diphtheroid</i>
Hand Fingers (5)	2	Gram positive bacilli, Gram Negative bacilli	MSSA, <i>Bacillus spp.</i> , <i>Diphtheroid</i>
Hand Fingers (6)	2	Gram Negative cocci	MSSA, <i>Bacillus spp.</i>
Hand Fingers (7)	2	Gram Negative bacilli	MSSA, <i>Bacillus spp.</i>
Hand Fingers (8)	2	Gram positive cocci, Gram Negative bacilli	MSSA, <i>Bacillus spp.</i>
Hand Fingers (9)	2	Gram positive cocci, Gram Negative cocci	MSSA, <i>Bacillus spp.</i>
Hand Fingers (10)	2	Gram Negative bacilli, Gram positive bacilli, Gram positive cocci	MSSA, <i>Bacillus spp.</i> , CONS
Hand Fingers (11)	2	Gram Positive cocci	MSSA, <i>Bacillus spp.</i>
Hand Fingers (12)	2	Gram Positive cocci, Gram positive bacilli	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), <i>Bacillus spp.</i> , <i>Diphtheroid</i>

Hand Fingers (13)	2	Gram Positive cocci Gram Negative bacillis	MSSA, <i>Bacillus spp.</i> , CONS
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Total 26 samples were collected from the 13-different workers of kitchen. The normal flora (*Staphylococcus aureus*) was present on the skin. We performed the coagulase test to identify the coagulase positive *Staphylococcus aureus* and methicillin or oxacillin resistant *Staphylococcus aureus* (MRSA) and methicillin or oxacillin sensitive *Staphylococcus aureus* (MSSA) identified by use disk diffusion method. In case of MRSA, *Staphylococcus aureus* cause the skin infection. In kitchen if worker carry this MRSA bacteria it may transfer in to patient feed and patient get infection by the contamination food.

Stool sample of food handler

Examination of stool sample from food handler, reason behind it to identified the pathogenic organism in stool because if human being carries any pathogenic organism the chance to contaminate any other human and food by direct contact.

In this study, 20 stools samples were collected of food handler. After identification, I was found only normal flora of gastrointestinal tract i.e. *Escherichia coli*, and *Klebsiella spp.* no any food borne pathogenic organism was found.

DISCUSSION

The study on Microbiological surveillance of food and beverage services in tertiary care hospitals helps to identify and detection of pathogens organism in the kitchen's food and environment [16, 17]. Hospital kitchen serve the food to patient as well as hospital staff. In case of food contamination leads to more complication or hospital acquired infection to patient and cause illness in the hospital staff [18, 19]. Therefore, there is a need of training to the food handler staff regarding food hygiene [20].

During this study, we have collected the samples of raw food (cheese, salad, fruits etc.), cooked food (rice, sweet dish, cooked vegetables etc.), ready to use food (milk, yogurt, ice-cream and juice etc.), utensils before use, staff's and worker hands and stools samples. No pathogenic organisms were isolated from any of the sample, but few pathogen organisms were present on the workers hands like *MRSA*, *Klebsiella pneumoniae*, *pseudomonas aeruginosa*. This organism is not foodborne pathogens but cause the other infection in the human being. After this study, we met the head staff and discuss about this problem and discuss about the 5 steps of hand hygiene for maintaining healthy environment [21, 22]. After this discussion staff and worker follow these 5 steps for hand wash and after this hand the food. After 1 week, again sample were collected from same worker hand sample and identified only normal skin flora are present. No Gram-negative organism would be obtained in the kitchen articles. Raw food will contain some microorganism and hence the need for thorough clean is of at most important. However, presence of *E. coli* and *Klebsiella* from such sample may be used as a surrogate marker of food

contamination [23, 24]. Hygienic of food articles is of most important. They may be the cause of some pathogen like *Salmonella* and some parasite like *Giardia*. In our study we did not find any of the carrier of these microorganism.

Staphylococcus aureus was found on the hands of the worker. It can be a source of infection to the bear themselves.

All the water sample taken we found to be satisfactory result.

However, there is a need to look into the quality of the soap.

Presence of bacillus in cooked food may point to inappropriate storage.

CONCLUSION

Foodborne illness is cauterized by diarrhoea, vomiting, stomach pain, fever and fatigue, this illness is caused foodborne pathogen. This pathogen transmits from food and water by oral faecal route. In hospital contamination of food leads to more illness in patient and hospital staff.

In this study, a total number 209 specimen were collected and processed for pathogenic organism. In this study some sample show only normal environmental flora and some sample is sterile, but food handler finger sample and some food sample show growth of the *MRSA*, *Klebsiella spp.*, *Pseudomonas spp.* This study revealed cooked and raw food is sterile but food handler carries some pathogenic organism.

REFERENCES

- [1] S. Rawat, "Food Spoilage: Microorganisms and their prevention," Asian Journal of Plant Science and Research, vol. 5, no. 4, pp. 47-56. 2015.
- [2] T. Bintsis, "Foodborne pathogens," AIMS microbiology, vol. 3, no. 3, pp. 529-563. 2017.
- [3] J. H. J. Huis in't Veld, "Microbial and biochemical spoilage of foods: an overview," International Journal of Food Microbiology, vol. 33, no. 1, pp. 1-18, 1996/11/01/. 1996.
- [4] Z. A. A. Azad, M. F. Ahmad, and W. A. Siddiqui, "Food Spoilage and Food Contamination," Health and Safety Aspects of Food Processing Technologies, pp. 9-28: Springer, 2019.
- [5] P. S. Mead, L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe, "Food-related illness and death in the United States," Emerging infectious diseases, vol. 5, no. 5, pp. 607-625, Sep-Oct. 1999.
- [6] C. M. Burgess, C. Arroyo, D. J. Bolton, M. Danaher, L. O'Connor, P. J. O'Mahony, and C. Tlustos, "Food Safety: A Public Health Issue of Growing Importance," Introduction to Human Nutrition, pp. 388. 2019.
- [7] I. C. o. M. S. f. Foods, Microorganisms in foods 5: Characteristics of microbial pathogens: Springer Science & Business Media, 1996.

- [8] E. Scallan, R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin, "Foodborne illness acquired in the United States--major pathogens," *Emerging infectious diseases*, vol. 17, no. 1, pp. 7-15. 2011.
- [9] E. F. S. Authority, E. C. f. D. Prevention, and Control, "The European Union summary report on trends and sources of zoonoses, zoonotic agents and food- borne outbreaks in 2017," *EFSa Journal*, vol. 16, no. 12, pp. e05500. 2018.
- [10] T. Humphrey, S. O'Brien, and M. Madsen, "Campylobacters as zoonotic pathogens: A food production perspective," *International Journal of Food Microbiology*, vol. 117, no. 3, pp. 237-257, 2007/07/15/. 2007.
- [11] A. Sinha, and S. Dutta, "Waterborne & foodborne viral hepatitis: A public health perspective," *Indian Journal of Medical Research*, vol. 150, no. 5, pp. 432. 2019.
- [12] W. H. Organization, WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015: World Health Organization, 2015.
- [13] J. Houbraeken, and R. A. Samson, "Current taxonomy and identification of foodborne fungi," *Current Opinion in Food Science*, vol. 17, pp. 84-88. 2017.
- [14] K. Chakrabarty, and A. S. Chakrabarty, "Poor Maintenance of Food Hygiene and Food Safety," *Textbook of Nutrition in Health and Disease*, pp. 265-286, Singapore: Springer Singapore, 2019.
- [15] K. Aneja, *Experiments in microbiology, plant pathology and biotechnology*: New Age International, 2007.
- [16] C. Buccheri, A. Casuccio, S. Giammanco, M. Giammanco, M. La Guardia, and C. Mammina, "Food safety in hospital: knowledge, attitudes and practices of nursing staff of two hospitals in Sicily, Italy," *BMC Health Services Research*, vol. 7, no. 1, pp. 45, 2007/04/03. 2007.
- [17] J. Richards, E. Parr, and P. Riseborough, "Hospital food hygiene: The application of hazard analysis critical control points to conventional hospital catering," *Journal of Hospital Infection*, vol. 24, no. 4, pp. 273-282, 1993/08/01/. 1993.
- [18] T. Oteri, and E. E. Ekanem, "Food hygiene behaviour among hospital food handlers," *Public Health*, vol. 103, no. 3, pp. 153-159, 1989/05/01/. 1989.
- [19] E. M. Cooke, T. Sazegar, A. S. Edmondson, J. C. Brayson, and D. Hall, "Klebsiella species in hospital food and kitchens: a source of organisms in the bowel of patients," *Journal of Hygiene*, vol. 84, no. 1, pp. 97-101. 2009.
- [20] C. H. Acikel, R. Ogur, H. Yaren, E. Gocgeldi, M. Ucar, and T. Kir, "The hygiene training of food handlers at a teaching hospital," *Food Control*, vol. 19, no. 2, pp. 186-190, 2008/02/01/. 2008.
- [21] D. Pittet, "Hand hygiene promotion: 5 moments, 5 components, 5 steps, and 5 May 2009," *Int J Infect Contr*, vol. 5, pp. 1-3. 2009.
- [22] J. Mann, "Handwashing: 5 Steps to a Best-Practice Pay-Off." 2005.

- [23] H. L. Koo, Z.-D. Jiang, E. Brown, C. Garcia, H. Qi, and H. L. DuPont, "Coliform contamination of vegetables obtained from popular restaurants in Guadalajara, Mexico, and Houston, Texas," *Clinical infectious diseases*, vol. 47, no. 2, pp. 221-221. 2008.
- [24] Z. Taha, and N. Yassin, "Prevalence of diarrheagenic *Escherichia coli* in animal products in Duhok province, Iraq," *Iranian Journal of Veterinary Research*, vol. 20, no. 4, pp. 255-262. 2019.

