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Microbiological quality assessment of fast food available near MMU campus Mullana

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ABSTRACT

Microbial food contamination is a serious concern to the mankind as it attributes to various foodborne diseases. Million of peoples worldwide are known to be afflicted with diseases associated with foodborne pathogens. Keeping in view of these facts we investigated multiple fast food samples for the assessment of microbial contamination. Spring Rolls and Chowmein were reported to have highest bacterial load ranging from 105 to 106 cfu/ml amongst all the samples studied. Samples like Bhel-Puri and Pani-Puri, had relatively low bacterial counts in the range of 102 to 104 cfu/ml. Biochemical tests further confirmed the presence of Bacillus sp. and Klebsiella sp. in Spring Roll sample while Escherichia coli in Chowmein sample. Bhel Puri and Pani Puri samples had the predominance of Salmonella sp. However, the Burger, and the Samosa samples were found to be contaminated with Pseudomonas sp and Enterobacter sp respectively. Besides, coliform bacteria reported to be present in Pani-Puri sample. These aspects clearly indicate the pre-assessment and awareness of potential sources of food contamination for a good nutrition.

Keywords: Fast Food, Microorganisms, Contamination, Bacterial Load, Microbiological Assessment

INTRODUCTION

Fast food is a type of food which can be cooked and served very quickly and is ready for immediate consumption.[1,2] In most cases it retains such ingredients which can supply various essential nutrients to many microorganisms for their optimum growth. There are several opportunities for microorganisms to contaminate fast foods, if the food is prepared, processed, handled, and stored under unhygienic conditions. Fast food includes food items like Pizza, Burger,French fries, Chinese food and Indian food such as poha, pani puri etc. Burger, pizza, french fries etc are the most commonly consumed fast food in Germany and America. These fast foods are made availabel to peoples by main food chains such as MacDonald's, KFC, Pizza Hut etc.[3] Some other popular fast food meals such as sausage with ketchup and mayonnaise and Kebab which are served with raw vegetables such as cucumber, lettuce and tomatoes.[3,4,5] Due to presence of different pathogenic microorganisms in fast food leads to food borne diseases. A study shows that about 9.4 million illnesses are caused each year in the United States. [6] In the year 2014, 864 foodborne disease outbreaks were also reported.[7]

Various food borne diseases were caused by pathogenic *E. coli,*Non typhodial *Salmonella, Bacillus* sp. and *Campylobacter* sp. All these bacterial species colonise in the gastrointestinal tract of animals and increase their number and some of them also produce toxins.[8] The food get contaminated with various pathogens at different stages such as during production, processing, distribution, retail marketing and handling of the prepared product.[3] Red meats, contaminated raw meats and undercooked meat and poulty products are also responsible for transmission of food borne pathogens. [9] Microbial contamination in food products take place due to environment, contact with animals and pets and also due to person to person transmission.[3] Pathogenic microorganisms have been reportedly found in soil, water, air, skin and internal organs of animals and humans. These microorganisms get entry in the food by hands, wiping cloths and

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utensils, especially chopping boards which leads to food borne illness.[3] Contamination of food also take place due to parasites also. Food borne parasitic zoonoses include both helminthic and protozoan infections.[10] Amongst one thousand five hundred known infectious agents for human being, 66 are protozoa and 287 are helminths. [11,12] The emerging infectious diseases are zoonotic which shows a 60.3% presence in food products.[13] Due to zoonoses different types of diseases occurs which leads to food borne outbreaks.[14,15] Microorganisms such as *Salmonella*, *Campylobacter*, *E. coli* which present in soft part of animals and eggs of the tape worm such as Taenia solium cause illness in human beings after consumption of raw or undercooked meat products.

The meat and meat products stored at low temperature are spoiled by psychtrotrophs such as *Pseudomonas, Achromobacter* etc and surface of meat is contaminated by mesophiles, such as *Clostridium perfringens, Coliforms, Salmonella* and *Staphylococcus* .[9]

Some microorganisms inhabit the intestine, some produce toxins which are absorbed through the bloodstream, and some of them directly invade the deeper body tissues. The fast food industry plays an important role in meeting the food demands of the urban dwellers in developing countries. Fast foods play important nutritional role for consumers, specially for middle and low-income sectors of the population, who depend on fast foods for their main meals. Food and Agriculture Organization (FAQ) reported that fast foods provide variety and choice options for consumers and these food also provide sufficient quantity of nutritionally balanced diets.[16] Studies shows that Verocytotoxin-producing *Escherichia coli* 0157 (VTEC 0157) emerged as a major food-borne zoonotic pathogen in the 1980s and 1990s. Various bacterial species such as *Salmonella typhimurium* and *Campylobacter* are more ubiquitous in the environment, colonising a greater variety of hosts and environmental niches. Various types of microorganisms such as *Mucor* sp*., Aspergillus fumigatus, Trichoderma, Neurospora crassa and Aspergillus niger* has been isolated from salads prepared using raw vegetables. [17] Fast foods have been prepared by using food additives, refined sugar, white flours and trans fats which provide a good medium for growth of microorganisms. The fast foods are lacking in proteins, vitamins and fibres.[18]

In developed countries diarrheal illness are more commonly associated with contaminated food consumption.[19] Fast foods are the best media for growth of pathogens but it is not necessary that all the gastroenteritis are food borne and all food borne diseases leads to gastroentritis. A number of studies are in progress which aims to provide a better understanding the burden of gastroenteritis in public health and different foodborne diseases.[20] Due to food contamination by various microorganisms the diseases produced by them are controlled while others diseases emerge as new threats to public health. Supply of food at global level leads to the rapid and widespread distribution of foods. In the year 1991 in America there is discharge of ballast water contaminated with *Vibrio cholerae* cause a number of deaths.[21]

This paper presents various microbial contamination transmitted through the fast food sold by street venders. Few of these microbes have been identified as *Klebsiella, Enterobacter, Escherichia coli*, *Bacillus cereus*, *Salmonella, Pseudomonas* etc.

MATERIALS AND METHOD

The food samples tested were collected from various randomly selected street vendors. All samples were collected under sterile condition for further microbial assays and analysis. One gram of each sample such as Burger, Spring Roll, Bhel Puri, Samosa was suspended in 10 ml sterile distilled water and centrifuged for 10 mins at 2000 rpm. Standard Spread Plate method was used to calculate the bacterial load per gram or per ml. Sample dilutions were serially prepared ranging from 1:100, 1:1000, $1:10^4$, $1:10^5$,$1:10^6$, and so on. One ml of each diluted sample was spread on nutrient agar plates and incubated the plates at 37˚C for 24 hrs to determine the Standard Plate Count (SPC) as follows:

SPC/ml or gm = colonies counted/dilution factor

The Minimum Probable Number (MPN) of coliforms was determined by multiple tube fermentation technique. This was calculated only for liquid samples that is water of Pani Puri sample. Change in color of medium from purple to yellow and evolution of gas bubble in Durham´s tube were considered positive for MPN.

SELECTIVE MEDIA USED:

Three selective media were used for identification of bacteria were Mannitol salt agar (MSA), Salmonella differential agar (SDA) and MacConkey agar (McCA). Mannitol Salt Agar (MSA) was used as selective media for *Staphylococcus aureus*, MacConkey agar media was used to differentiate lactose and non-lactose fermenting bacteria. Salmonella differential agar media was used for *salmonella* species for their identification and differentiation from members of Enterobacteriaceae especially *Proteus* species.

MICROBIAL CHARACTERIZATION:

Gram staining reaction was further carried out for microscopic characterization of Gram positive (purple colour) & Gram negative (pink colour) bacteria. Various biochemical tests such as indole, MR, VP, nitrate, citrate, TSI (Triple sugar iron agar), urease were performed for identification of microbial genus for isolated bacteria from samples. In the Indole test, development of bright red color ring at the interface of the medium and reagent was an indication of the presence of Indole shows the presence of *Enterobacteriacea*. Methyl red test was used to differentiate species of the family *Enterobacteriacea.* Positive result shows development of red colour (indicating pH below 6) and negative result is shown by yellow colour (indicating no acid production) which indicates the presence of *E. coli* and *Enterobacter*. Voges-proskauer (VP) test were performed to determine the presence of *Klebsiella* which produced pink colour from glucose fermentation. Citrate test identified the enterobacteria in the food samples. The test is based on the ability of an organism to use citrate as sole source of carbon and ammonia as its only source of nitrogen. A change in colour of the indicator from green to blue, due to alkaline reaction indicates citrate utilization by *Enterobacteracea*. Triple Sugar Iron (TSI) agar is used to determine whether the organisms utilizes glucose, lactose or sucrose and produce

hydrogen sulphide gas. The formation of CO_2 and H_2 is indicated by the presence of bubbles or cracks in the agar and change in colour of the media represents sugar utilization. This test indicated the presence of *E. coli, Salmonella typhi, Pseudomonas aeruginosa*. Nitrate reduction test is used to determine the ability of the organism to reduce nitrate to nitrites or free nitrogen gas. The development of red colour due to formation of diazonium dye within few seconds was taken as positive test. Urease test is done to determine the presence of urease enzyme in bacteria

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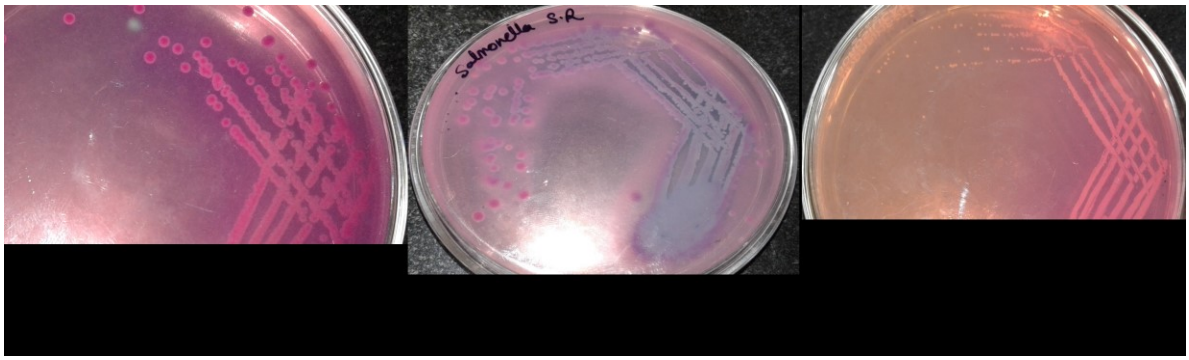

Figure 1: (A) Growth on MacConkey agar of spring roll sample. (B) Growth on Salmonella differential agar of spring roll sample. (C) Microbial growth on mannitol salt agar for spring roll sample.

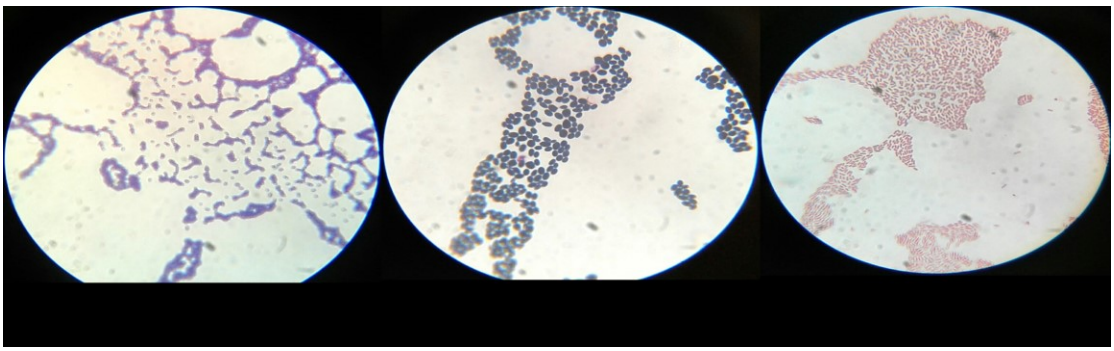

Figure 2: (A) Microscopic view on MSA agar shown gram positive cocci as seen in spring roll sample. (B) Microscopic view on salmonella differential agar shown gram positive coccobacilli for Pani Puri sample. (C) Microscopic view on Macconkey agar media shown gram negative bacilli of sample burger

RESULTS AND DISCUSSION

All the food samples were collected from the street vendors were subjected to microbial detection. These samples were Spring Roll, Chowmein, Burger, Samosa, Bhel puri, Pani Puri. Number of colonies observed at various dilutions of the tested food samples was counted per ml or gram. Mean of standard plate count (SPC) calculated for all the dilution for particular sample was taken as actual load of bacteria per ml or gram of the sample. Out of 6 samples that included Spring Roll and Chowmein had bacterial load very high ranging between 105 to 106 Cfu/ml while samples like Bhel Puri and Pani Puri, had relatively low bacterial counts in the range of 102 to 104 Cfu/ml. According to the recommendation of US public health service (1965), bacterial load for processed food samples should not exceed 105 Cfu/ml. Thus on the basis prescribed standards samples like Pani Puri and Bhel Puri were found to be fit for consumption. The high bacterial load in other samples implies a poor shelf life of the food. Hundred microliter of different sample supernatant were inoculated on MacConkey (MC) agar yielded lactose fermenting colonies of pink colour formed (Figure 1A). Mannitol Salt agar (MSA) was used to differentiate fermentation of mannitol. Colonies especially identified were of Staphylococcus aureus (Figure 1C). Salmonella differential agar was used for identification of Salmonella species (Figure 1B).

Bacterial isolates obtained on different selective media shows presence of different microorganisms. Bacteria observed in Mannitol Salt Agar and MacConkey agar from spring roll sample were gram positive cocci and gram positive Bacilli respectively (Figure 2). Isolate of Chowmein food sample on Salmonella differential agar and MacConkey agar were gram positive cocci and gram positive bacilli (Table 1). Bhel Puri sample shows gram positive bacilli on Mannitol salt agar and gram negative bacilli on SDA and MC agar media. Isolates from Burger and samosa sample shows presence of gram negative bacilli on SDA and MC agar media while gram positive cocco-bacilli on MSA media. Isolates of Pani-puri sample on MSA and SDA media were gram positive bacilli while on MC agar shows Gram negative bacilli.

Table 1: Microscopic and Biochemical analysis of microorganisms present in different food samples.

Sample No.	Name of Sample	Selective Media	Gram Staining	Biochemical Tests						
				Indol	MR	VP	Urea	Nitrate	Citrate	TSI
1	Spring Roll	MSA	+ve Cocci	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Macconkey	+ve Baciilli	-ve	+ve slightly red	-ve	-ve	-ve	+ve	+ve
		Salmonella	+ve Bacilli	-ve	-ve	-ve	-ve	-ve	+ve	+ve
2	Chowmein	MSA	+ve Cocci	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Macconkey	+ve Baciilli	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Salmonella	+ve Baciilli	-ve	+ve slightly red	-ve	-ve	-ve	-ve	+ve
3	Bhel Puri	MSA	+ve Bacilli	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Macconkey	-ve Bacilli	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Salmonella	-ve Bacilli	-ve	+ve slightly red	-ve	-ve	-ve	-ve	+ve
4	Pani Puri	MSA	+ve bacilli	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Macconkey	-ve bacilli	-ve	+ve slightly red	-ve	-ve	-ve	+ve	+ve
		Salmonella	+ve bacilli	-ve	+ve slightly red	-ve	-ve	-ve	-ve	-ve
5	Burger	MSA	+ve coccobacilli	-ve	-ve	-ve	-ve	+ve	-ve	+ve
		Macconkey	-ve Bacilli	-ve	-ve	-ve	-ve	+ve	-ve	+ve
		Salmonella	-ve Bacilli	-ve	-ve	-ve	-ve	+ve	-ve	+ve
6	Samosa	MSA	+ve bacilli and coccobacilli	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Macconkey	-ve bacilli	-ve	-ve	-ve	-ve	-ve	-ve	+ve
		Salmonella	-ve bacilli	-ve	-ve	-ve	-ve	-ve	-ve	+ve

Various biochemical tests were performed for identification of genus of isolated bacteria. Bacterial culture of Spring roll identified as Gram positive bacilli was found to belong to Bacillus. Biochemically, Spring roll sample was positive for MR, Citrate and Triple Sugar Iron agar (TSI), while Indole, VP, Urease, Nitrate were negative which indicates that microorganism belongs to Klebsiella genus. Sample Chowmein was positive for MR and TSI, while indole, VP, Urease, Nitrate, and Citrate were negative, infers that the microorganism was Escherichia coli. Gram negative bacilli from Bhel Puri and Pani Puri samples were Indole, VP, Urease, Nitrate, Citrate negative, while MR and TSI positive indicates that the microorganism belong to Salmonella (Table 1). Gram negative bacilli and gram positive cocco bacilli from Burger sample were Nitrate and TSI positive, while Indole, MR, VP, Urease, Citrate negative. In TSI test there was gas production in butt and slant broth. These characterize the microorganism as Pseudomonas. Gram negative bacilli of sample Samosa were Indole, MR, VP, Urease, Citrate, Nitrate negative, while TSI positive with gas production in butt and slant indicates the microorganism was Enterobacter. The results showed that the Spring Roll contains Klebsiella species, Chowmein contains E.coli, Burger contains Pseudomonas microbes, Bhel Puri and Pani Puri contaminated with Salmonella, while Samosa contains Enterobacter.

The coliform count in water of Pani Puri samples were reported the presence of gas in Durham's vial with change in colour of

medium from purple to yellow. This confirms the coliform bacterial contamination with combinations of 5-1-0 has MPN value per 100 ml is 33.

Growth of urban population leads to rise in number of street food vendors in many cities throughout the country. Street foods are consumed by an estimated 2.5 billion persons worldwide. The term "street foods" is used to describe wide range of ready to eat foods and beverages which are sold and sometimes prepared in public places. Vendor stalls are usually located outdoors or under the roof which are easily available in streets. Such food stalls lack the necessary conditions for storage, refrigeration and cooking to prevent contamination from microbes. [22]

Numerous reports shows the risks associated with consuming contaminated street-vended foods which have high levels of coliform bacteria and pathogenic bacteria.[23,24] Different types of food borne illness such as cholera, typhoid, salmonellosis, campylobacteriosis, shigellosis, amoebiasis and E. coli infections have been reported in many countries. Microbiological quality is directly related to quality of the water available to the vendors to prepare food and drinks. Access to the safe water supply goes long toward promoting food safety while the location in which street foods are prepared and significantly affects their safety. [22]

In the present study, samples were collected from various street vendors. All the samples were analyzed to access the total bacterial load and liquid samples for coliforms counts. Out of 6 samples that included spring roll and chawmein had bacterial load very high ranging from 105 to 106 Cfu/ml, while samples like Bhel Puri and Pani Puri, had relatively low bacterial counts (102 to 104 Cfu/ml). These findings were similar to a recent finding of 2.0 x 105 Cfu/ml in ready to eat salad vegetables. [25]

Attempts were also made to isolate and identify pathogenic bacteria from these samples. While processing the fast food samples for isolation of pathogenic organisms, strains of S. aureus , B. cereus, E.coli, Salmonella, Pseudomonas, Enterobacter, Klebsiella etc. were found. These microbial organisms belong to those genus which was recently reported from a study on sixty fast food samples [3], this paper also states prevention from microbial infection. [26] has shown more or less similar bacteria in their fast food sample, moreover, suggesting longer survival rate for E. coli and B. cereus. The high rate of human carriage of S.aureus is an important feature of the organism with respect to its role as a food-borne pathogen. The rate of staphylococcal carriage is estimated to be 10 to 40 percent in men. Induced staphylococcal intoxication is only major form of food poisoning in which food handlers play a significant role [27]. Staphylococcus may enter food through the skin of handlers. Especially pani puri and bhel puri samples were contaminated with Salmonella rather different from a study reporting no contamination from Salmonella and Vibrio cholerae. [28]

CONCLUSIONS

Most studies on street foods consumption concluded that these food could be harmful to health due to presence of harmful pathogenic microorganisms in them. The common reason for the unacceptable microbiological quality of street food was unhygienic food cooking and handling practices. [22] This study has shown that despite of the hygienic preparation of fast food sold by vendors, there were some pathogenic microorganisms present on the samples as their normal commensal and shows heavy growth in food. Through this study we have shown the presence of pathogenic bacteria in vendors food. The study also established the possible reasons of contamination of food samples and persistence of pathogens in the food sample. Attempts were also made to isolate and identify pathogenic bacteria from food samples. Pathogens isolated in this study, reported high bacterial load in Spring Roll and Chowmein. While processing the fast food samples for isolation of pathogenic organisms, strains of S. aureus, B. cereus, E. coli, Salmonella, Pseudomonas, Enterobacter, Klebsiella etc. were found. The critical points which prevent food from contamination are adequate time and temperature for cooking, prevent food from cross contamination, providing proper chilling temperature and food handlers should also be trained on hygienic food handling practices and safety.[29] WHO in 2007[,30,31] give important instructions by which we can handle and prevent food contamination by microorganisms. These were (1) We should wash and dry our hands before preparing a food. (2) Wash hands after handling raw foods such as meat, poultry, vegetables or fruits. (3)Equipment and food preparation area should be clean.(4) Entry of insects, pests and other animals should be prohibitted in kitchen or restaurants. (5) People suffering from gastrointestinal illness should not handle the food which is to be consumed by others.[3] We can also concluded that regular meals in suitable quantity and at right time leads to a good health with no health problems.To have a good health we should follow right nutrition, good food habits and healthy foods. The personal hygiene of vendor or worker is important in hygienic venting of fast food and it was found that poor personal hygiene contaminate the food item more.

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CONFLICT OF INTEREST

None

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