

A BACTERIOLOGICAL STUDY OF FOOD SERVED AT VARIOUS BUS STANDS OF CHANDIGARH AND NEARBY PLACES WITH REFERENCES TO E.coli.

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ABSTRACT

The study was undertaken to assess the microbial load of foods served at various Bus Stands of Chandigarh and nearby places. Some commonly served foods (channa, bhatura, bread pakoras, tomato sauce, orange juice, potato patty, black gram dal, samosa, sonpapri and water) were tested and E.coli was isolated from them. It was also isolated from possible sources of contamination (working area, serving area and wiping cloth). The total bacterial counts of food samples ranged between 3.3×10^5 CFU/gm to 8.9×10^8 CFU/gm whereas the gram negative counts ranged from 3.1×10^4 CFU/gm to 9.9×10^6 CFU/gm. The results of mean values of various food samples taken from different Bus Stands show higher values in case of water samples for both total bacterial and gram negative counts. E.coli was isolated in 77.6% of all the samples (food and contamination sources) screened. It was observed that E.coli isolation from food samples was 75% whereas isolation from all sources of contamination was 88%. Antibiotic sensitivity of E.coli isolates from food and swab samples was maximum (93.81%) for gentamycin and minimum for ampicillin (6.18%). Multi drug resistance was observed in 53.60% of the isolates. Salt aggregation test (SAT) for hydrophobicity of isolated E.coli revealed that 96.90% strains were hydrophobic. At the highest concentration of metal ions tested (100µg/ml), 11.34% were resistant to both silver nitrate and 93.81% to mercuric chloride. Hygiene and cleanliness practices followed by food shops were also surveyed in the study. Employees of only 13.33% food shops were trained in food hygiene practices before recruitment. Health checkups are being done annually in 40% outlets. 66.66% food shops follow daily overall cleanliness schedule. Soap or detergent or both were used for washing wiping cloth in 40% of the food shops, while remaining used plain water (26.66%) and disinfectant (20%).

INTRODUCTION

Food is one of the basic physiological necessities. Health and well balanced diet is essential for good health. Contaminated food represents one of the greatest health risks to a population and is a leading cause of diseases, outbreaks and transmission. Food safety issues have been the most wanted intervention in the field of Nutrition all over the world. The common man is busy with life and most often rely on the easily available and cheap food items gathered from vendors at Bus Stands and hence are prone for the food borne diseases. Food sanitation rests directly upon the state of personal hygiene and habits of the personnel working in the food stalls at the Bus Stands. The food served at bus stands are well appreciated by consumers because of their taste, low cost and ready availability for immediate consumption. Quality and safety are two common concerns cited with regards to these foods. The food served at bus stands are prepared and either sold for immediate consumption or consumed later without further processing and preparation. Further, the foods at bus stands are available where

there are limited sanitary facilities of running water, garbage disposal and clean toilets. In addition to this, the unhygienic use of simple facilities increases the risk of food contamination. The food served at bus stands are frequently associated with diarrheal diseases which occur due to improper use of additives, presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing practices (GMPs) and good hygienic practices (GHPs). Such foods have shown to be potential sources of bacterial pathogens notably E.coli. Therefore, it is essential to assess the knowledge and practice and help them to understand the importance of hygiene and teach regarding food hygiene. So, a research was conducted to make a bacteriological study of food served at various Bus Stands of Chandigarh and nearby places: with reference to E.coli.

METHODOLOGY

Sample Collection

The objective of the present study is to determine the microbial load of food samples served at Bus Stands of Chandigarh and nearby places and determine their hygiene standards. Ten bus stands of Chandigarh and close by places were surveyed to fulfill our objective. Ten food samples (Channe, Bhatura, Bread pakora, Sauce, Orange juice, Aloo patty, Black gram, Paneer, Son papri and water) were selected randomly. A questionnaire on hygiene practices carried out in these shops was prepared and filled by interview method. The shopkeepers and the workers were interviewed regarding the hygiene practices followed by them.

Bacteriological Analysis

Samples of food, water and swab were aseptically collected from the shops in sterilized tubes and immediately brought to the laboratory for analysis. Standard plate count method was used to determine the total and gram negative counts of samples. The inoculums from the samples were grown on Mac Conkey's agar for 24 hours at 37C. The dark pink, non-mucoid lactose fermenting colonies were picked up and the cultures were further purified by the plate streak method on EMB agar. The purified cultures were identified on the basis of colony morphology, gram staining and specific biochemical tests. The isolated colonies were picked up with the help of inoculating needle and incubated in sterile nutrient broth. The broth was incubated for 24hrs; the culture was streaked on nutrient agar slants and incubated at 37C for 24hrs. The cultures were maintained on nutrient agar slants, which were stored at 4C in the refrigeration. Sub culturing was done after every 15 days. The sensitivity pattern of E.coli isolates was determined selected antibiotics. Salt Aggregation Test was conducted to determine the hydrophobicity of E.coli isolated from food samples and swab samples from possible sources of contamination. Minimum inhibitory concentration was also determined.

Statistical Analysis

Mean values of bacterial counts of various food samples were calculated to analyze the most and least contaminated food. In order to determine the correlation between multi drug resistance and cell surface hydrophobicity, the chi-square test was applied.

RESULT

Hygienic quality of food served at Bus Stands has become an important public health issue and a great concern to everyone. The present study was undertaken to investigate the microbiological quality of food served at Bus Stands of Chandigarh and nearby places. Samples of various foods served and swab samples of working, serving area and wiping cloths used in the kitchen were procured from 10 Bus Stands of Chandigarh and nearby

places at random. Ten samples were collected for each food sample, the total number being 100. Ten swab samples were collected for working area, 10 for serving area and 5 for wiping cloth. A total of 25 swab samples were collected from different food shops of Bus Stands.

Microbial Load of Food Samples

All the samples were subjected to testing for their total and gram negative counts. The total counts were higher than gram negative counts. The total bacterial count of food samples ranged between 3.3×10^5 CFU/gm to 8.9×10^8 CFU/ml. The minimum counts were observed in Black gram dal followed by Son papri and Samosa, where as maximum counts were observed in Water, followed by Tomato sauce and Bread pakora. The gram negative count of the food samples ranged from 3.2×10^4 CFU/gm to 9.9×10^6 CFU/ml.

Isolation of E.coli

A total of 100 food samples were collected from various Bus Stands out of which 75 samples showed the isolation of E.coli. The maximum number of E.coli isolates were observed in Tomato sauce and Samosa, in which all the 10 samples showed E.coli isolation. An over all isolation percentage of E.coli from sources of contamination was 88%. Hundred percent isolation was observed in samples of working area where as samples from serving area and wiping cloth showed 80% isolation.

Antibiotic Sensitivity of E.coli

Ninety seven strains of E.coli were subjected to antibiotic sensitivity test. For this purpose 5 different antibiotics namely ampicillin, chloramphenicol, erythromycin, gentamycin and nalidixic acid were used. . In case of total E.coli from food samples (75), maximum sensitivity of isolates, i.e. 70 was noticed towards gentamycin, followed by chloramphenicol (63), erythromycin (62), nalidixic acid (60) and the least towards ampicillin (4). Sensitivity of 22 E.coli isolates taken from swab samples of sources of contamination towards different antibiotics show that maximum sensitivity (21) was seen towards gentamycin, followed by nalidixic acid (19), erythromycin (16), chloramphenicol (11) and a minimum of 1 towards ampicillin.

Cell Surface Hydrophobicity

A total of 97 strains of E.coli were subjected to the salt aggregation test (SAT) for determining the incidence and degree of cell surface hydrophobicity. Out of 97 strains being tested, 43 are highly hydrophobic, 29 optimally hydrophobic, 11 moderately hydrophobic, 9 show minimum hydrophobicity while 5 strains are not at all hydrophobic. In case, of the sources of isolation of E.coli, the percent aggregation of total strains at 0.02 M ammonium sulphate was 52.57%, followed by 73.19% at 0.2 M, 88.65% at 1.8 M and 96.90% aggregation was found at 3.2 M.

Metal Ion Resistance

The E.coli strains isolated were subjected to varying concentrations (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) of silver nitrate and mercuric chloride to determine their minimum inhibitory concentration towards E.coli.

Silver Resistance

The study shows that 95.87% strains of E.coli are resistant to 20µg AgNO₃/ml, 73.19% of strains are resistant to 40µg AgNO₃/ml, 46.39% to 60µg AgNO₃/ml, 25.77% and 11.34% of the total strains are resistant to 80µg AgNO₃/ml and 100µg AgNO₃/ml respectively

Mercury Resistance

Result indicates that 91 strains (93.81%) were resistant to 20µg HgCl₂/ml, 67 strains (69.07%) were resistant to 40µg HgCl₂/ml, 46 strains (47.42%) show resistance to 60µg HgCl₂/ml, 26 (26.80%) show resistance to 80µg HgCl₂/ml, and at the highest concentration of 100µg HgCl₂/ml, only 11 (11.34%) strains are resistant.

Hygiene Practices Followed By the Employees of Food Shops of Chandigarh Bus Stands

The employees of food shops of bus stands were interviewed regarding cleaning and hygienic practices followed by them. It is observed that 2 out of 15 food outlets (13.33%) have employees who are trained in food and hygiene and 33.33% of these food shops have written cleanliness recommendations which have been followed by their workers. In 60% outlets, it is mandatory for their employees to wear overalls while handling food and during washing of utensils and floor. None of them compel their workers to wear head covers while cooking and serving food. Eight out of 15 outlets (53.33%) use filtered water for drinking and cooking purposes. Kitchen safety against rodents, cockroaches, pests, flies and other insects are maintained by 6 food outlets (40%). Insect or fly trappers, rodent killing machines and chemical sprays to kill insects are being used to safeguard the kitchen. 73.33% cases use dustbin covered with lid. None of them use incinerator for disposal of kitchen leftover and garbage, whereas 80% of them use plates or lids to cover the food and water before and after consumption.

Means of Bacterial counts and their coefficient of variance for various food and swab samples taken from various Bus Stands:

The results show higher mean values in case of water samples for both total and gram negative counts. The trend of food contamination in decreasing order is as follows:-
Water > Channe > Bhatura > Tomato sauce > Samosa > Potato patty.

Statistical correlation of various properties of E.coli

Using chi-square test at 0.5 level, a significant correlation was observed between multi drug resistance and cell surface hydrophobicity.

SUMMARY

It is extremely difficult to pinpoint the precise beginning of human awareness of the presence and role of microorganisms in food but the available evidence indicates that this knowledge preceded the establishment of bacteriology or microbiology as a science. Foods are not only of nutritive value to those who consume them but often are ideal culture media for microbial growth. Thus, the greatest threat to the quality and safety of our food comes from microbial spoilage. Samples of food served at Bus Stands were screened for their bacterial count. The total counts of food samples range between 10⁵ and 10⁸ CFU/gm in case of Black gram dal and water, whereas gram negative counts lay between 10⁴ and 10⁶ CFU/gm in Potato patty and Water. Samples of water in our study showed the highest counts, ranging from 9.6 x 10⁶ to 1.93 x 10⁸ CFU/ml for total count, where as gram negative ranges between 6.8 x 10⁵ to 9.9 x 10⁶ CFU/gm. The lowest count was observed in Black gram dal

samples (i.e. 10^5 to 10^7 CFU/gm) while Potato patty (10^4 to 10^5 CFU/gm) samples gave the lowest gram negative counts. E.coli was isolated in 75 out of 100 and in 22 out of 25 food and swab samples respectively. The study showed that the isolation of E.coli is greater in the sample of Tomato sauce and Samosa. The swab samples taken from Bus Stands of Chandigarh were procured and screened for the presence of E.coli. The percent isolation of E.coli was maximum (100%) from working area due to its inadequate and unhygienic cleaning of working area. In order to determine, the virulence characteristics of E.coli isolates from food and swab (sources of contamination) samples of food served at Bus Stands, the properties studied were:-

- Antibiotic sensitivity
- Cell surface hydrophobicity
- Heavy metal ion resistance

Lack of adequate knowledge of hygiene practices followed by food handlers, leading food borne illness due to transmission of microorganisms by fecal oral route and this remains a continuous problem. It is found that the level of bacterial contamination of hands and equipments is related to the cleaning methods and the type of food establishment.

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