

Multiantibiotic-resistant Pattern against Pathogenic Bacteria Isolated from Ready to Eat Food in Ludhiana

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Abstract

Objective: The study was conducted to investigate the bacteriological contamination in the most common street food sold in Ludhiana at different places. 90 samples of street food samples were randomly collected from the selected part of Ludhiana. Samples investigated for the occurrence of bacteria by culturing them on selective and differential culture media. **Methods:** To detect the presence of pathogenic bacteria in food recommended method used like Spread Plate Techniques and identification of bacteria accomplished with the help of differential media and specific media, biochemical testing and molecular characterization also performed to ensure the particular strain of enteropathogenic bacteria. **Results:** The presence of pathogenic bacteria isolated from all street food were quite high, the percentage of *Proteus* sp. was 2%. Predominantly *Staphylococcus* sp were found in street food. This was followed by *Salmonella* (6%) and *Staphylococcus aureus* (14%). *Klebsiella* sp. (5%) and *Enterococcus* sp. (2%) were least predominant in these foods. **Conclusions:** The study exhibited contagion may be due to poor techniques of food preparation adopted by the street food sellers and its big concern community well-being as well as bad for economy state and India, and there is the loss of 5.2% of national gross domestic product. These bacteria are also known for foodborne illnesses and toxic syndrome.

Key words: Coliform count, foodborne illness, pathogenic bacteria, street food

INTRODUCTION

Street foods are the food which sold out in streets and roadside, where the commuters or local persons are coming for business or shopping, enjoyment purpose; for instance, these areas are the court, chopati, bus stand, and banking business area. The food sold in streets or roadside, either already prepared or required mild preparation before serving to the person.^[1] Ingesting of such food may lead to food poisoning and increases the risk of foodborne diseases.^[2,5] A spread of bacteria causes food poisoning and gastroenteritis basically through the unclean water mixed with fecal material such as food, water, nails, and hand or unhygienic way of handling.^[20] Especially in developing countries, it is difficult to safeguard the food from cross contamination.^[6] Street food sellers profited from a direct cash flow and regularly avoid taxation, and second, they can fix their own working hours. Vending snacks, food, and juices or beverages at relatively low prices in comparison of well-maintained hotels and cafeteria, these essential services are easily available for the workers, shoppers, travelers,

and people with low wages.^[5] The individuals who love to have such food are concerned with accessibility instead of its safety, quality, and hygiene. In processing of the street food, clean water to be used but clean water is hardly available specially during the rainy season, sometimes rainy water mixed with municipal corporation water and if this contaminated water used by street vendors may cause health problems.^[13,14] Even the availability of space for utensils washing readily available, sometimes roadside vendors leave utensils in buckets without washing that attracts the flies and rodents leading to food poisoning. Storing the food at low temperature or under refrigeration protects from direct contact of flies and rodent's and bacterial multiplication. Keeping these facilities is very difficult for the people having low income that is why the consumers depending on roadside foods, faces the maximum intestinal problem as well as additional food-borne illness after ingesting unlearned food.^[13]

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These hawkers or street food sellers' play an important role in supply food for travelers and urban dwellers at reasonable and easily accessible.^[8] The demand of ready-to-eat is suitable and growing day by day due to lifestyle change.

METHODS

Location of the study

Ludhiana is situated in central part of the district which found Malwa area of Punjab. The population increases considerably during the harvesting season due to the migration of laborers from the eastern states of Uttar Pradesh, Bihar, Odisha, and Delhi. The resources are occupied mainly by small-scale manufacturing units, which manufacture industrial goods, machinery parts, motor vehicle parts, domestic appliances, woolen garments, and another type of garments. Ludhiana is considered as Asia's largest center for bicycle manufacturing more than 50% of India's bicycle consumption of approximately 10 million every year, so here office employee and labor mostly depend on the street food. City failed in purity test of most food and water, so researcher has collected.

Collection of samples

Ninety food samples were collected from street vendors at Chandigarh Road, Samrala Chowk, Clock Tower, and Jalandhar bypass. The samples were noodles, burger, bhelpuri, samosa chaat, hulcha chole, masala dosa, chole bhature, chicken tandoori, and pote kaleji, these items are prepared either by frying or boiling, and some ingredients are directly used in serving food; samples were aseptically collected and placed in ice pack contain portable thermocol box and processed in the microbiology laboratory.

Isolation and identification of bacteria

Methodology

Ten (10 g) of all food material was taken and made homogenate mixture in mortar and pestle, of that 1 ml of homogenate was added to 9 ml sterile normal saline and prepared the serial dilution of that up to 10^{-6} . The spread plate technique was used in that, diluted sample were poured on *Salmonella-Shigella* agar and eosin methylene blue (EMB) agar, and detected blue-black greenish metallic sheen colony of *Escherichia coli* on EMB, mucoid colonies of *Klebsiella* on MacConkey agar and black-centred colonies of *Salmonella* on SS media after a 24 h incubation at 37°C. *Proteus* sp. was streaked on blood agar to check the swarming growth. All probable isolates were further identified using Kligler Iron Agar.^[9] Biochemical testing was also performed to confirm the type of bacterial strain after isolation it from street food. To validate the report the quality control also done by using specific bacterial strain purchased from MTCC.

Molecular characterization of bacteria

Pure culture was isolated from food samples and was analyzed by following the standard method, the confirmation isolated bacteria, and the result was matched with the bacteria isolated by the conventional method.^[8] In polymerase chain reaction protocol 16S rRNA Universal primers, gene fragment was amplified by means of thermal cycler (MJ Research Peltier make).^[11,12]

Primer details

| Primer name | Sequence details | Number of bases |
|-------------|------------------------|-----------------|
| 27F | AGAGTTTGATCMTGGCTCAG | 20 |
| 1492R | TACGGYTACCTTGTTACGACTT | 22 |

Antibiotic susceptibility testing

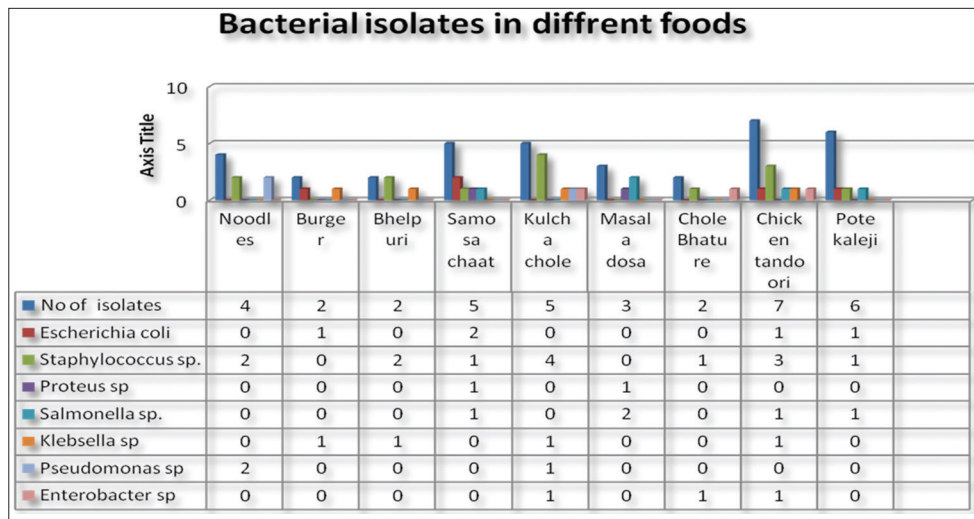
The sensitivity test by the Kirby-Bauer disc diffusion technique was done against the bacteria isolated from the street food at Ludhiana. 10 different antibiotics with different strength viz: Amikacin, ampicillin cefotaxime, ciprofloxacin, cefazolin, ceftazidime, gentamycin, imipenem, levofloxacin and tetracycline [Graph 2] were used. Bacteria stock solution prepared in normal saline and with the help of sterile swab sticks applied on Muller Hinton (MH) agar. The antibiotic discs were aseptically sited on the MH media using sterile forceps and incubated at 37°C for 24 h after incubation report noted in a format of sensitive or resistant.^[12]

RESULTS AND DISCUSSION

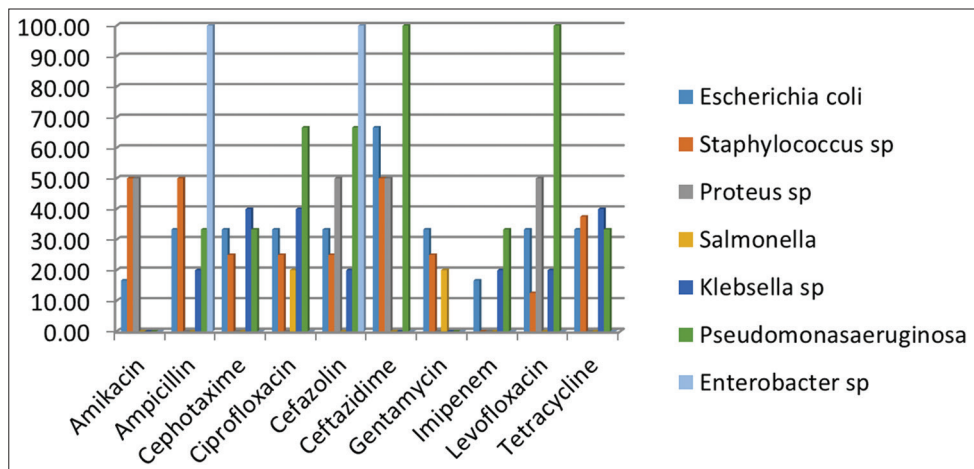
The non-vegetarian food samples noted the highest showing almost 50%, whereas in vegetarian food, there was 32% [Graph 3]. The finding of seven different pathogenic bacteria was isolated from street foods in that *Proteus* sp. were 2% staphylococci was the most predominant. This was followed by *Salmonella* (6%) and *Staphylococcus aureus* (14%). *Klebsiella* sp. (5%) and *Enterococcus* sp. (2%) were the least predominant^[4,10] [Graph 1]. The vendors stay on road or in streets where these foods are contaminated by vehicles passing through. Serving water, direct municipal water, also contributes to several pathogenic bacteria in street food which leads to gastroenteritis or diarrheal diseases, throat infection, etc.^[6] As a result, access to clean water and health edification to sellers on personal hygiene, food protection, and appropriate disposal improves food quality and reduces foodborne diseases.^[7]

Antimicrobial sensitivity test

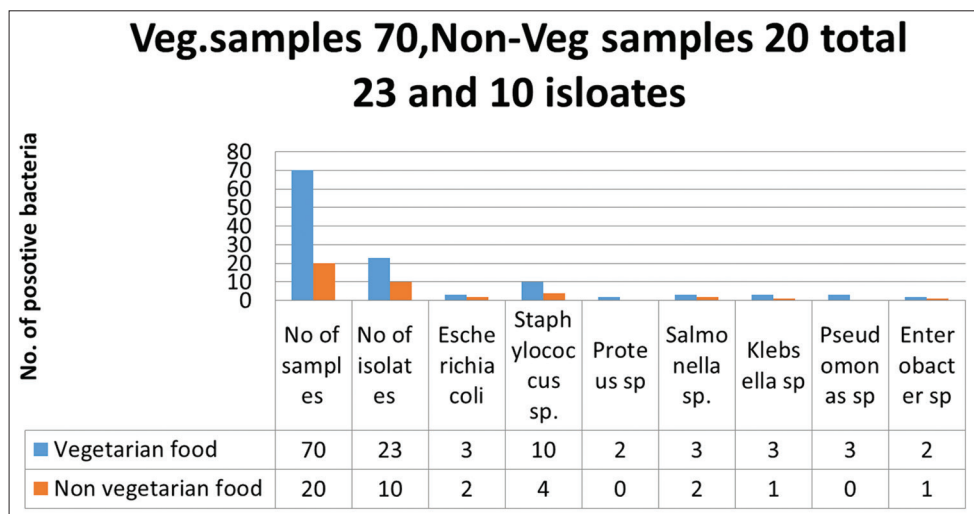
Drug susceptibility against isolated bacteria was performed by disc diffusion; one well-isolated colony was selected from



Graph 1: Number of isolated organisms from food samples



Graph 2: Regarding antibacterial activity against all isolated bacteria



Graph 3: Total bacterial isolates in vegetarian and non-vegetarian foods

the MS agar plates and EMB agar plate. A colony was touched with a sterile loop and streaked over routinely used nutrient agar and incubated overnight at 37°C. Few well-isolated

colonies were inoculated into a tube of sterile physiological saline and mixed well before use.^[15] A sterile cotton swab sticks were dipped into the bacterial suspension.^[19] The

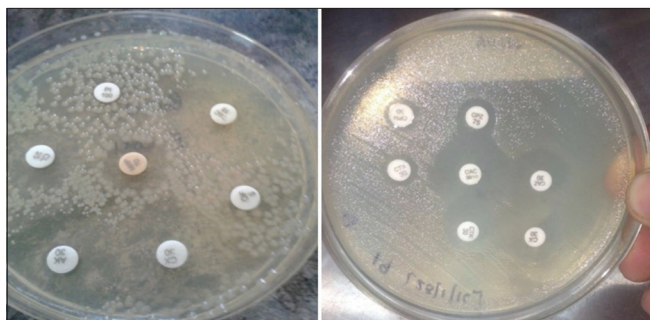


Figure 1: Antimicrobial sensitivity test against isolated organism

swab was streaked over the entire surface of Mueller-Hinton agar medium, 3 times, rotating the plate approximately 60° after each application to ensure an even distribution of the inoculums. After incubation the diameter of the zone of complete inhibition, their results are shown in Figure 1.

CONCLUSION

This study clearly indicated significant levels of contamination in street-vended foods of Ludhiana city. The presence of enteropathogenic or pathogenic bacteria count in all the street samples was high and can be correlated to a number of factors such as inappropriate management and processing, the use of dirty utensils, and rinsing water as probable sources of bacterial contamination in street vendor food.^[17,18] Most street hawker or vendors did not have basic knowledge of hygiene and how to protect the food from bacterial contamination. However, the bacterias isolated from the food are pathogenic and people are catching the infection after consuming it and even the consumer also hardly bother about it. The presence of *S. aureus* is an enterotoxins producer and can cause serious health issues. Excess use of antibiotic also the serious issue for world physicians, because some physicians are prescribing antibiotic without antibiotic sensitivity test or diagnosis and consequences of that bacteria becoming multi-antibiotic resistant^[3,16]

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