MOLECULAR CHARACTERIZATION AND DRUG RESISTANCE OF ENTEROPATHOGENIC BACTERIA ISOLATED FROM READY-TO-EAT FOOD IN LUDHIANA

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ABSTRACT

Objective: The study was conducted to investigate the enteropathogenic bacteria from the most common street food sold in Ludhiana at different places.

Methods: A total of 90 samples of street food samples were randomly collected from four locations of Ludhiana. Samples investigated for the presence of bacteria using appropriate selective and differential culture media. The spread plate technique was used to analyze the samples.

Results: A total of 90 samples were analyzed, and the total viable counts of the bacterial population in all food samples were in the range of 2.0 × 10^3 to 1.8 × 10^6 CFU/g. The nonvegetarian food samples recorded the 1.8 × 10^6 CFU/g; however, other street foods also have the highest total viable microbial count 1.1 × 10^6 CFU/g. The total coliform count ranged from 1.9 × 10^2 to 9.4 × 10^6 CFU/g, and pani puri has significant count. The range of Staphylococci count was 1.9 × 10^2 to 5.3 × 10^6 CFU/g, chicken tandoori sample also had high microbial count. The incidence of manifestations of the seven pathogenic bacteria isolated from all street food Proteus sp. (2%), Staphylococci were the most predominant. This was followed by Salmonella (6%), Staphylococcus aureus (14%), Klebsiella sp. (5%), and Enterococcus sp. (2%) were least predominant.

Conclusion: The study exhibited contagion may be due to poor techniques of food preparation adopted by the street food vendors. This is of community health worry as these bacteria are known as food-borne illnesses and toxic syndrome.

Keywords: Pathogenic organisms, Ready-to-eat, Food, Toxic syndrome, Ludhiana.

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 or for banking business. Vending snacks, food, and juices or beverages at relatively low price in comparison to well-maintained hotels and cafeteria, these essential services are easily available for the workers, shoppers, travelers, and people with low wages [1]. The individuals who love to have such food are concerned with accessibility instead of its safety, quality, and hygiene. Street food already prepared and required mild preparation before serving to the person, and frequently served in public places in India. Ingesting of such food may lead to food poisoning and increases the risk of foodborne diseases [2]. The entry of enteropathogenic bacteria in food basically through the unclean water mixed with fecal material, or unhygienic way of handling [3,4]. Particularly in developing countries, it is difficult to safeguard the food from cross-contamination [5]. The availability space for utensils washing is the big problem. Sometimes they are leaving utensils in buckets without washing that attracts the flies and rodents leading to food poisoning [6].

Street food sellers profited from a direct cash flow and regularly avoided taxation; second, they can fix their own working hours. These hawkers or street food sellers play an important role in supply food for travelers and urban dwellers at reasonable price [7]. The demand of ready-to-eat food is well suitable for nuclear and working family.

METHODS

Study location

Ludhiana is the most centrally located district which located in the Malwa area of the State of Punjab. For administrative purposes, it has been placed in the Patiala Division. It lies amid north latitude 30-34’ and 31-01’ and east longitude 75-18’ and 76-20’. It is the major city in the state, with a projected population of 3,487,882 as of the Census. The population increases considerably during the harvesting season due to the migration of workers from the eastern states of Uttar Pradesh, Bihar, Odisha, and Delhi. The resources are taken mostly by small-scale industrial units, which produce industrial goods, machine parts, auto parts, household appliances, hosiery, apparel, and garments. Ludhiana is Asia’s largest center for bicycle work and produces more than 50% of India’s bicycle consumption of more than 10 million each 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 [8].

Collection of samples

A total of 90 selected street 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, kulchachole, masala dosa, chole bhature, chicken tandoori, potekaleji; 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 thermocoll box and processed in the microbiology laboratory.

Isolation and identification of bacteria

Conventional method

About 10g of each food sample was taken and made homogenate mixture in mortor and pestle, out 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. By using the spread plate technique, 1 ml of the dilution was poured on the eosin methylene blue (EMB) agar. Salmonella-Shigella agar and detected blue-black greenish metallic sheen colony of Escherichia coli
on EMB, mucoid colonies of *Klebsiella* on MacConkey agar and black
centered colonies of *Salmonella* after a 24 hrs incubation at 37°C.
Other doubtful growths also detected on plates such as presumptive
*Proteus* sp. were streaked on blood agar to check the swarming growth.
All probable isolates were further identified using Kigler iron agar
media and tested for motility, indole and urea formation in tubes
incubated for 24 hrs at 37°C. The identification of isolated bacteria
was done by the method mentioned in Chessbrough (2006) and WHO
(2003) manual[9,10].

**Molecular characterization of bacteria**

Pure culture was isolated from food samples and sent to reference
laboratory YAAH XENOMICS for the confirmation isolated
bacteria [11,12]. The result was matched with the bacteria isolated
by the conventional method. In polymerase chain reaction protocol
16S rRNA universal primers gene fragment was amplified using MJ
Research Peltier Thermal Cycler [13].

**Primer details**

| Primer name | Sequence details | Number of bases |
|-------------|------------------|-----------------|
| 27F         | AGACCTTGTACMTGGGTCAG | 20              |
| 1492R       | TAGGYATACCTGTTGAGACTT | 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 [14,15].
The antibiotics were viz: Amikacin, ampicillin, cefotaxim, ciprofloxacin,
cefazolin, ceftazidime, gentamycin, imipenem, levofloxacin and
tetracycline. Bacteria stock solution prepared in normal saline and
with the help of sterile swab sticks applied on Muller-Hinton agar. The
antibiotic discs were aseptically placed on the agar with a sterile forceps
and incubated at 37°C for 24 hrs. Next day the sensitivity checked and
given the report in the format of sensitive or resistant.

**RESULTS**

The nonvegetarian food samples noted the highest number
1.8 × 10^8 CFU/g bacterial growth; however, other street foods also
have highest total viable bacterial count (1 × 10^8). The total coliform
count ranged from 1.9 × 10^9 to 9.4 × 10^8, and pani-puri also has
significant count. The range for *Staphylococci* count was 1.9 × 10^8
to 5.3 × 10^7, chicken tandoori recorded the highest. The incidence of
manifestations of the seven pathogenic bacteria isolated from all street
food *Proteus* sp. (2%) *Staphylococci* was the most predominant. This
was followed by *Salmonella* (6%) and *Staphylococcus aureus* (14%).
*Klebsiella* sp. (5%) and *Enterococcus* sp. (2%) were least predominant.
The study showed that contamination may be as a result of poor
preparation employed by the food vendors. This is of public health
concern as these organisms are known causes of foodborne illnesses
and toxic syndrome (Table 1) [16].

**DISCUSSION**

Street food is very popular food and most of the people love to have it
for almost all age group of persons. Street food is readily available at
the minimal distance in a market. However, these street food vendors
stay on the 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 and throat infection, etc.
Therefore, access to clean water and health education to sellers on
personal hygiene, food protection, and appropriate disposal improve
food quality can reduce foodborne diseases (Table 2).

At Parbani city, three different restaurants were selected to check the
microbial contamination in chicken curry, it was screened and found
significant aerobic count, i.e., 2.06 × 10^8 to 2.80 × 10^7 CFU/g. *S. aureus*
count were 1.1 × 10^8 to 1.47 × 10^8 CFU/g; in tandoori chicken, the total
count were 3.54 × 10^6 CFU/g; the organisms which were isolated from
tandoori chicken were *Salmonella, Proteus, Shigella, S. aureus, Klebsiella*,
and *Lactobacillus* sp. Tandoori chicken was highly contaminated and not
good for public health. The basic problem is unhygienic way of
processing [17].

Bacteriological examination of street food in Pune city, it was done and
found the presence of pathogens associated with possible health
risk approximately 88% street food were contaminated. The basic
problem was that street hawkers were unaware with good sanitary
practices [18,19].

The traditional food sold in the streets of Ankara was analyzed for
microbial contamination; total 600 samples were collected, after
the analysis approximately 50% sample showed the presence of bacteria.
That is why public facing stomach disturbances after consuming it.
Common bacteria isolated from food samples *S. aureus, E. coli,
Salmonella sp., and Clostridium sp. in terms of Salmonella sp. [20,21].
In another study of street food sold in Aba Metropolis, Nigeria was
conducted; these samples were collected from four different busy
locations locally prepared snacks such as meat pies, fish pies, plantain
chips, and potato chips. The total samples 120 and analyzed, microbial
load was different at different locations, total viable counts ranges from
2.1 ± 2.07 × 10^8 CFU/g to 4.0 ± 0.15 × 10^8 CFU/g, coliform count ranges from
0.7 ± 0.36 × 10^8 CFU/g to 2.6 ± 0.45 × 10^8 CFU/g, seven different type
of bacteria were isolated such as *S. aureus, E. coli, Bacillus species,
Pseudomonas species, Clostridium species, Enterococcus species*, and
*Klebsiella species* [22].

Federal Polytechnic, Bali Campus, Taraba State, samples were analyzed
for bacteriological examination of ready-to-eat food. The outcome
attained shown that total bacterial count ranged from 1.0 × 10^7 to
8.7 × 10^8 CFU/g, the most pathogenic bacteria were *S. aureus, E. coli,
Klebsiella spp., and Salmonella spp.* [23].

The incidences of pathogenic bacteria isolated from all shawarma
samples showed that *Proteus* sp. (22.7%) was the most predominant.
This was followed by *E. coli* (13.6%), *Bacillus* spp. (13.6%) and
*S. aureus* (13.6%). *Enterobacter aerogenes* (9.1%), *Klebsiella* sp.
(9.1%), *Serratia marcescens* (9.1%), and *Micrococcus* spp. (9.1%)
were least predominant. These all bacteria were isolated from
ready-to-eat food or food prepared at home and then sold in streets
the contamination may be as a result of poor manufacturing practices
done by the food vendors. This is of community health concern as these
organisms are known causes of foodborne diseases and food
intoxications [24].

**CONCLUSION**

This study clearly indicated significant levels of contamination in street
vended foods of Ludhiana city. The presence of enteropathogenic or
pathogenic bacterial count in all the samples varied between 2.0 × 10^7
and 1.8 × 10^8 CFU/g and can be linked to a number of factors such as
improper handling and processing, use of contaminated water, cross
contamination from flies and rodents, the use of dirty utensils and
rinsing water as possible sources of contamination of street vended
food. Most street hawkers or vendors did not have basic knowledge
of hygiene and how to protect the food from bacterial contamination.
However, the bacteria’s isolated from the food is pathogenic and people
are catching the infection after consuming it and even the consumer
also hardly bother about it. The presence of *S. aureus* an enterotoxin
produced and can cause serious health issues. Excess the use of
antibiotic also serious issue for the world physicians are prescribing
the medicine without antibiotic sensitivity test, resultant most of the
bacteria becoming multi-drug resistant.

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Table 1: Number of isolated organisms from food samples

| Source      | Number of samples | Number of isolates |
|-------------|-------------------|--------------------|
| Noodles     | 10                | 1                  |
| Burger      | 10                | 2                  |
| Bhelpuri    | 10                | 2                  |
| Samosa      | 10                | 2                  |
| chaat       | 10                | 4                  |
| Kulcha      | 10                | 5                  |
| chole       | 10                | 3                  |
| Masala      | 10                | 1                  |
| Chole       | 10                | 2                  |
| Bature      | 10                | 4                  |
| Chicken     | 10                | 3                  |
| tandori     | 10                | 7                  |
| Pote        | 10                | 6                  |
| kafeji      | 10                | 1                  |
| Total       | 90                | 37                 |

Table 2: Antibiotic sensitivity pattern of bacteria isolated from food samples

| Antibiotics | E. coli | Staphylococcus sp. | Proteus sp. | S. typhi | Klebsilla sp. | P. aeruginosa | Enterobacter sp. |
|-------------|---------|--------------------|-------------|----------|---------------|--------------|-----------------|
|             | 1 2 3   | 4 5 6 7 8         | 1 2         | 1 2 3 4 5 6 7 | 1 2         | 1 2 3         | 1 2 3 4 5 6 7 8 |
| Amikacin    | S S S   | R S S             | R S R R S   | S S S S   | S S S S       | S S S S S       | S S S S S S S |
| Ampicillin  | S R S S  | S S S S           | S S S R S   | S S S S   | S S R R S     | S S S R S S     | S R R R R R R |
| Cephalotaxine | S S R S | S S S S           | S S S R S   | S S S S   | S S S S R R   | S S R R R S     | S S S S S S |
| Ciprofloxacin| S S S S | S S S S           | S S S S R   | S S S S   | S S S S R R   | S S S S S R     | S S S S S S |
| Cefazolin   | R S R S R | S S S S           | S S S S S   | S S S S   | S S S S S R   | S S S S S S     | S S S S S S |
| Cefazidime  | S R R S S | S R S S           | S R S S S   | S S S S   | S S S S S R   | S S S S S S     | S S S S S S |
| Gentamycin  | R S R S S | S R S S           | S S S S S   | S S S S   | S S S S S R   | S S S S S S     | S S S S S S |
| Imipenem    | R S R S S | S S R S           | S S S S S   | S S S S   | S S S S S R   | S S S S S S     | S S S S S S |
| Levofloxacin| R R S S S | R S S S           | S S S S S   | S S S S   | S S S S S R   | S S S S S S     | S S S S S S |
| Tetracycline| S S S R S | S R R R           | S S S S S   | S S S S   | S S S S S R   | S S S S S S     | S S S S S S |

S: typhi: Salmonella typhi, P: aeruginosa: Pseudomonas aeruginosa

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