

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

NARESH KUMAR\*, JANGIR OP

Department of Biotechnology, Maharaj Vinayak Global University Jaipur, Rajasthan, India. Email: naresh1.kumar@outlook.com

Received: 04 January 2017, Revised and Accepted: 09 February 2017

### 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  $2.0 \times 10^3$  to  $1.8 \times 10^6$  CFU/g. The nonvegetarian food samples recorded the  $1.8 \times 10^6$  CFU/g; however, other street foods also have highest total viable microbial count  $1.1 \times 10^6$  CFU/g. The total coliform count ranged from  $1.9 \times 10^3$  to  $9.4 \times 10^5$  CFU/g, and pani-puri has significant count. The range of *Staphylococci* count was  $1.9 \times 10^3$  to  $5.3 \times 10^3$  CFU/g, chicken tandoori sample also has 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.

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i5.16818>

### 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 thermocol box and processed in the microbiology laboratory.

#### Isolation and identification of bacteria

##### Conventional method

About 10 g of each food sample was taken and made homogenate mixture in mortar 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 Cheesebrough (2006) and WHO (2003) manual [9,10].

#### Molecular characterization of bacteria

Pure culture was isolated from food samples and sent to reference laboratory YAAZXENOMICS 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	AGAGTTTGATCMTGGCTCAG	20
1492R	TACGGYTACCTGTACGACTT	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, cefotaxime, ciprofloxacin, 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  $1.8 \times 10^6$  CFU/g bacterial growth; however, other street foods also have highest total viable bacterial count ( $1.1 \times 10^6$ ). The total coliform count ranged from  $1.9 \times 10^3$  to  $9.4 \times 10^5$ , and pani-puri also has significant count. The range for *Staphylococci* count was  $1.9 \times 10^3$  to  $5.3 \times 10^3$ , 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 Parbhani city, three different restaurants were selected to check the microbial contamination in chicken curry, it was screened and found significant aerobic count, i.e.  $2.06 \times 10^6$  to  $2.80 \times 10^6$  CFU/g, *S. aureus* count were  $1.1 \times 10^6$  to  $1.47 \times 10^6$  CFU/g; in tandoori chicken, the total count were  $3.54 \times 10^6$  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 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 shown 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  $2.1 \pm 0.70 \times 10^2$  CFU/g to  $4.0 \pm 0.15 \times 10^4$  CFU/g, coliform count ranges from  $0.7 \pm 0.36 \times 10^1$  CFU/g to  $2.6 \pm 0.45 \times 10^2$  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  $1.0 \times 10^2$  to  $8.7 \times 10^4$  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* spp. (22.7%) was the most predominant. This was followed by *E. coli* (13.6%), *Bacillus* spp. (13.6%) and *S. aureus* (13.6%). *Enterobacter aerogens* (9.1%), *Klebsiella* spp. (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 bacteria count in all the samples varied between  $2.0 \times 10^3$  and  $1.8 \times 10^6$  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.

#### ACKNOWLEDGMENT

The authors would like to acknowledge the support of Microbiology Laboratory in LPU allow me to conduct my research work and Yaazh Xenomics, Chennai, India, helped me in microbial gene sequencing.



19. Estrada-Garcia T, Lopez-Saucedo C, Zamarripa-Ayala B, Thompson MR, Gutierrez-Cogco L, Mancera-Martinez A, *et al.* Prevalence of *Escherichia coli* and *Salmonella* spp. In street-vended food of open markets (tianguis) and general hygienic and trading practices in Mexico City. *Epidemiol Infect* 2004;132(6):1181-4.
20. Ates M, Ozkizilcik A, Tabakoglu C. Microbiological analysis of stuffed mussels sold in the streets. *Indian J Microbiol* 2011;51(3):350-4.
21. Sethi S, Dutta A. Antimicrobial activity of spices against isolated food borne pathogens. *Int J Pharm Pharm Sci* 2013;5(1):260-2.
22. Ike CC, Emeka-Ike PC, Nwokorie CC, Anochie CC. Microbiological quality evaluation of locally prepared snacks sold in Aba Metropolis, Abia State, Nigeria. *Int J Sci Eng Appl Sci IJSEAS* 2015;1(7):46-59.
23. Monday IE, Francis JI, Mohammad SU. Microbiological quality of ready-to-eat foods (Rice and Moimoi) sold by food vendors in federal polytechnic Bali, Taraba State Nigeria. *IOSR J Environ Sci Toxicol Food Technol IOSR JESTFT* 2014;8(2):145-9.
24. Odu NN, Akano UM. The microbiological assessment of ready-to-eat-food (Shawarma) in Port Harcourt city, Nigeria. *Nat Sci* 2012;10(8):1-8.