Forensic Identification of Drug Resistance Pathogens from Roadside Nonvegetarian Food in Ootacamund, The Nilgiris District, Tamilnadu, India

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Abstract

The Nonvegetarian foods market has long-drawn-out rapidly in incipient nations such as India. The sellers cook and sell food for direct human consumption on streets and in public areas, eliminating the need for additional preparation. In present study Nonvegetarian food samples were taken from different street food, restaurants and hotels in Ootacamund, town, Tamilnadu, India. Microbiological analysis was performed in all the samples. Bacterial growing status was observed. Bacteria isolated include Salmonella typhi , Escherichia coli, Klebshiella pneumonia, Serratia marcescens, Staphylococcus aureus, E. coli, K. pneumoniae, S. typhi, Serratia, Lactobacillus, S. aureus were predominantly identified. Operating tainted raw food materials, dirty water, unhygienic preparation methods, and infected containers all contributed to these microbial contamination. Investigating the microbial quality of samples of street food is the study's principal goal. A total of 50 samples of street cuisine from ten different vendors were randomly selected for the investigation. The usual microbiological procedure for isolating, counting, and identifying bacteria was employed to analyze the collected samples for the predominant presence of bacteria. Salmonella spp. (10%), E. coli (30%), Staphylococcus aureus (12%), Shigella spp. (10%), Klebsiella spp. (18%), and *Clostridium* spp.(20%), were discovered in the samples that were collected. As a result, it is encouraged to apply sound risk analysis policies to give a scientific foundation to a variety of risk management options that food safety department of Nilgiris district may need to consider in order to ensure the health and safety of the general public.

Keywords: Street foods, Microbial contamination, Staphylococcus aureus, Shigella spp., Klebsiella spp.
How to cite this article: Thampy A, Devi PM, Sathish DN, Kalyani G, Gunasheela N, Thamizharasan S. Forensic identification of drug resistance pathogens from roadside nonvegetarian food in Ootacamund, The Nilgiris District, Tamilnadu, India. Int J Drug Deliv Technol. 2026;16(74s): 469-478. DOI: 10.25258/ijddt.16.74s.55

1.Intoduction

Street foods consist of ready-to-eat items and drinks prepared and sold by vendors in urban and public settings. They are a vital part of modern eating habits and a significant contributor to the informal urban sector in developing countries. Approximately 2.5 billion people eat street food every day globally and India is no exception with an estimated 37 lakh vendors serving street foods[1]. However, there are many concerns about its safety. People consuming street-vended foods including Nonvegetarian food products are known to experience foodborne diseases like diarrhea, typhoid fever, and food poisoning and the majority of them go unreported.

A recent report indicated that foodborne disease cases in India are projected to increase from 100 million to between 150 and 177 million by 2030. This implies that, on average, one in nine people is expected to fall ill from foodborne illnesses by 2030. Diarrheal disease agents were the leading cause of foodborne disease burden, and non-typhoidal *Salmonella enterica* is an important burden globally[2-6]. Other main organisms include enteropathogenic Escherichia coli, enterotoxigenic Escherichia coli and Vibrio cholerae, and Campylobacter spp. These disease-causing agents

have been reported in street foods especially in Nonvegetarian food varieties[7-8]. Most type of street food is generally prepared in the open. There is a chance of contamination of the food with dirty water. It is very common to see street food stalls located near open drains especially in developing countries like India. So there is a high chance of contamination with drain water containing fecal matter, in which diarrhea causing coliform bacteria can be present[9-11].

Also street vendors are not washing fishes, meats and utensils properly. The water used for washing fishes, meats and their utensils is contaminated, it also increase the chance of infection. The raw material used for preparation is placed in open, it also increases chances of food infection through flies and other insects[12-15]. Nonvegetarian food products, including fish, shellfish, and crustaceans, chickens, mutton etc., are widely appreciated for their nutritional advantages and are consumed by a millions of people globally.

However, the existence of harmful bacteria and viruses in seafood can present notable health hazards and result in foodborne diseases. Therefore, the aim of the study was to identify pathogenic microorganisms in Nonvegetarian food samples. This paper will work towards bridging gaps in knowledge, policy, and to tackle the issue comprehensively. This research paper aimed to estimate the prevalence of pathogenic microorganisms in roadside seafood samples.

2. Materials and Methods

The study will be conducted in selected roadside Nonvegetarian foods vending locations across Ootacamund town. Laboratory investigations will be carried out at collaborating microbiology and molecular biology laboratories. Ootacamund town divided into zones: 1.Central Ooty town, 2.The Eastern zone (Doddabetta),3.The Western zone(Pykara route), and 4.The Southern zone /Avalanche areas.

(a)Collection of samples:

A total 40 different samples were randomly collected from all 4 zones each consists of ten Nonvegetarian foods street vendors from different places in Ootacamund, The Nilgiris District, Tamilnadu (India). The food samples taken in a cup with 100 gm x 10 place of each zone finally a total of 4kg were collected in sterilized container and taken to laboratory(Figure 1and 2). The food samples were mixed uniformly in a mixing vessel of the capacity 10 kg capacity (AELAB Rotator AE-RM-10 | Precision Lab Tube Mixer 0–80 rpm). This Rotational mixing container cum equipment (Figure.3) for food testing, typically involves tumbler blenders or rotating test-tube rotators operating at slow speeds (4 to 10 RPM up to 80 RPM) to gently homogenize food samples without damaging physiological nature and bio chemical characteristics.

Figure 1. A Nonvegetarian food sample selection

Figure 2. The zone wise collection of Nonvegetarian food sample

Figure 3. Mixing process of all collected food samples

(b).Isolation of pathogens

The selected, collected and mixed nonvegetarian food sample 400 gram was diluted in normal saline in 500 ml conical flask. From which, 1 ml sample was taken and spread on nutrient agar medium. The nutrient agar plates were incubated at 37°c for 48 hours. The obtained bacterial colonies were isolated on the basis of their morphological parameters and then sub cultured in nutrient agar media by continuous quadrant streaking. All the samples were inoculated in the triplicate. After inoculation all the plates were kept for incubation aerobically at 37^0c for 24 hours and observation were recorded.

(c).Identification of foodborne pathogens

The sub cultured bacterial colonies were used again as per the procedure adopted for forensic Identification of foodborne pathogens . For the forensic identification of food born pathogens different selective media, different trials, along with different incubation period and temperature were used.

(d).Statistical analysis

To calculate the p- value of percentage, descriptive statistics, and other appropriate inferential statistics were used. Significance level $p<0.05$ is obtained. A p-value ≤ 0.05 means the difference in pathogen percentage is statistically significant.

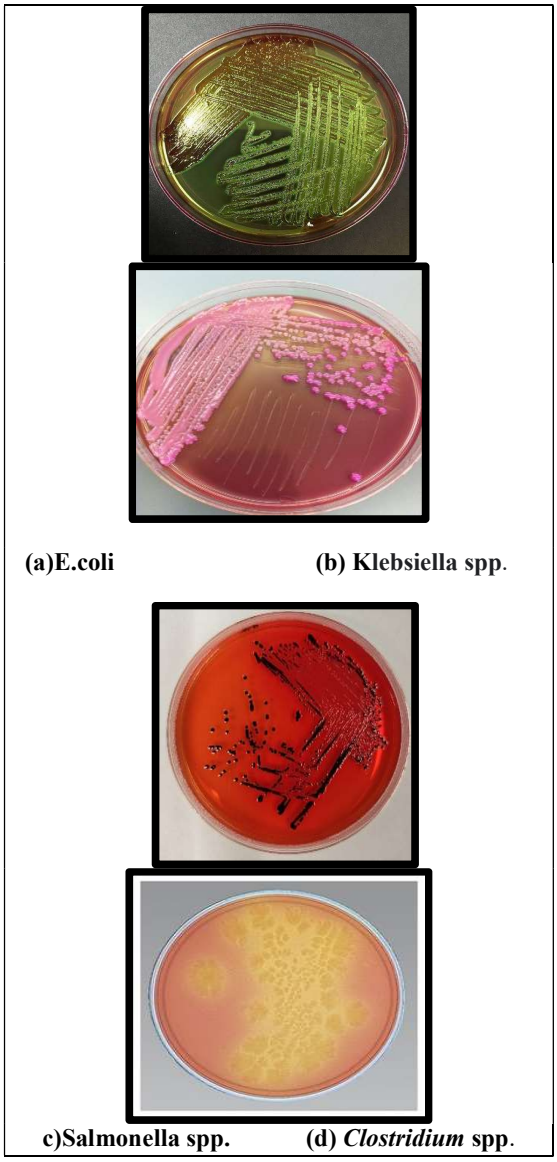
3.Results and Discussion

The results showed that all street Nonvegetarian food samples in Ootacamund were contaminated with mutable level of bacterial entireties. More than 11 species were forensically identified, of which E. coli (30%), Klebsiella spp. (10%), Salmonella spp. (18%), Staphylococcus aureus (12%), Shigella spp.(10%), and *Clostridium* spp. (20%) predominantly found. They were isolated and identified by using the specialized selective media. The isolation and identification process carried out three times and also three times samples used and culture plates were produced (Table 1; Figure 4).

Table 1

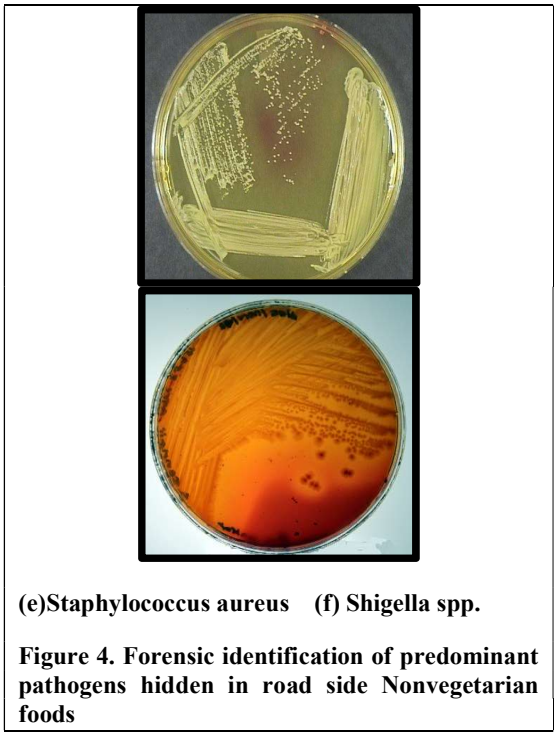
Forensic identification of bacterial pathogens

Types of pathogens	Type of medium used	Incubation period	Results
E. coli	Eosin methylene blue agar (EMB).	35–37 °C/18–48 hr	Positive
Klebsiella spp.	Klebsiella EMB Agar: *Klebsiella* colonies as a distinct purple-magenta color.	37 °C/24 hr	Positive
Salmonella spp.	XLD Agar, Inhibits non-target flora with sodium deoxycholate. *Salmonella* forms red colonies with black centers from hydrogen sulfide (H_2S) production.	37 °C/24 hr	Positive
Clostridium spp.	Cycloserine-cefoxitin-fructose-egg yolk agar (CCFA),	37 °C/24 hr	Positive
Staphylococcus aureus	Here, the primary specific media used for the isolation and identification of *Staphylococcus aureus* are Mannitol Salt Agar (MSA),	37 °C/24 hr	Positive
Shigella spp.	Isolation of *Shigella* species requires selective and differential media such as Hektoen Enteric (HE) agar.	37 °C/24 hr	Positive



(a)E.coli (b) Klebsiella spp.

c)Salmonella spp. (d) *Clostridium* spp.



(e)Staphylococcus aureus (f) Shigella spp.

Figure 4. Forensic identification of predominant pathogens hidden in road side Nonvegetarian foods

Calculating a p-value for foodborne pathogen percentages involves comparing proportions using the Chi-Square test of independence or Fisher's exact test. These tests determine if the difference between contamination percentages (e.g., 15% in chicken vs. 5% in mutton) is statistically significant ($p < 0.05$) or due to random chance.

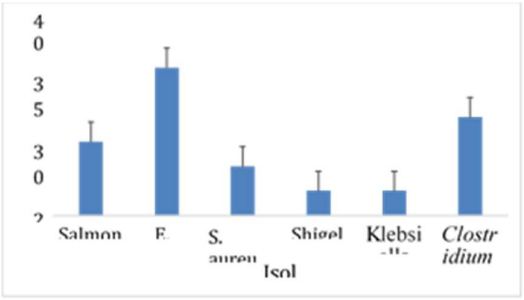


Figure-5: Prevalence percentage of pathogens from street foods

The isolation of bacterial pathogens in all the Nonvegetarian foods from different places in Ootacamund, The Nilgiris District, Tamilnadu (India), indicated that the frequency of *E.coli* and *Clostridium* spp. was more significant in street food. The unacceptable total bacterial count was screened from food samples implies extreme contamination and potential health risk of these street food samples. The high incidence of bacterial contamination encountered in this study were mainly due to the

largely unhygienic nature of the food preparations and services areas of foods are good indicators of the state of environment in which they are prepared or served [16-18]. Majority of the street food centers are located beside waste disposal points and duty roads. Furthermore lack of running water, sewage disposal infrastructure, inappropriate storage conditions and the presentation of these food in the open encouraged multiple contaminations. Results showed that isolates gotten from hotel samples shows less pathogenic bacterial count than street food. *Salmonella* spp, isolated in Chicken biryani are major causes of food borne gastroenteritis and typhoid fever{19]. *S. aureus* isolated from curry, is a pointer to largely poor personal hygiene, improper storage facilities, use of low quality raw materials and unhygienic environment[20].

In another study, collected total of 52 samples of street vended Nonvegetarian food items[21], the dominant bacterial pathogen recorded by them was *E.coli* (40%), followed by *P. aeruginosa* (25%), *Salmonella spp.* (16%), *Proteus spp.* (9%), *S. aureus* (6%), *Klebsiella spp.* (3%) and *Enterobacter spp.* (1%). The study also revealed that *E. coli* is major food borne pathogen in Ootacamund, The Nilgiris District, Tamilnadu (India). Poor personal hygiene can result in food contamination for example when a food personnel, fails to wash hands properly after using the restroom, toilet, is a serious risk of faucal contamination[22]. Food service personnel can contaminate food and cause food-borne illness. Food workers may transmit pathogens to food from a contaminated surface, from one food to another food or from hands contaminated with organisms from the gastrointestinal tracts.

13. Conclusion

Our study results, also indicated that factors such as the vendors itself (e.g personal cleanness etc), the type of food, have an effect on the bacterial contamination present in foods. Therefore, it is very important and necessary for food vendors to always clean and sanitize food contact surfaces, cook and store food properly, so as to reduce the level of food contamination and also to reduce bacterial load to the lowest level, thereby preventing cases of food borne infections. The findings of the present forensic investigation unequivocally showed that *Staphylococcus spp., Salmonella spp., Clostridium* spp. and *E. coli* are highly present in street Nonvegetarian meals marketed in Ootacamund, The Nilgiris District, Tamilnadu (India). The presence of these microorganisms in food can have negative effects on consumers' overall health. Poor personal hygiene, careless handling techniques, prolonged storage of food at room temperature, and street vendors' ignorance of food safety and food borne illnesses are the factors that contribute to the contamination of street meals. Food safety departments must take urgent action on street food by enforcing vendor registration, banning toxic chemical additives, and launching hygienic model zones.

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