ORIGINAL ARTICLE

Bacteriological Quality of Panipuri in Historical Gwalior City (MP), India

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Abstract

Introduction: The food safety is becoming a serious threat to public health, particularly in developing countries. In common street foods, vendors are using normal water without any treatment. The consumption of street foods is very popular in India and all over the world. **Materials and Methods:** In food samples, the microbiological analysis was done to determine the bacteria, including pathogenic bacteria. **Results:** Different bacterial cultures were isolated from 20 samples of panipuri collected from different locations of Gwalior, Madhya Pradesh, India. The dominant contaminant bacteria reported in the study were from *Enterobacteriaceae* family including *Proteus vulgaris* and *Klebsiella pneumoniae*. The antibacterial efficacy of silver nanoparticles (AgNPs) was tested against isolated bacteria. Antibacterial activity showed that AgNPs synthesized had a good antibacterial activity to kill foodborne pathogens. Although it is a preliminary data, it will increase our understanding of the presence of bacteria in street food as well as the use of AgNPs as antibacterial agents. **Conclusion:** The risks associated with street foods may be controlled by clean water and maintaining proper hygienic conditions.

Key words: *Escherichia coli, Klebsiella pneumoniae,* microbial content, *Proteus vulgaris,* street food

INTRODUCTION

All over the world, street foods have very important role in economy of numerous urban communities and towns of developing countries, especially for providing the alternatives of regular foods. During the past 10 years, the street food has expanded quickly in urban zones of low- and middle-income groups, providing a diversity of inexpensive foods for low-pay families and offering job opportunities for many local vendors.[1,2] The National Policy for Metropolitan Street Vendors stated that they constitute approximately 2% of the residents of a metropolis in India.[3]

In developing countries like India, several food products such as panipuri, snacks, and drinks sold by vendors are widely consumed by millions of people in all the major cities as well as in small cities. Among all the street food, panipuri or gol gappas are more popular. Selling of roadside foods without any protection against dust, smoke, uses of normal water without any treatment, poor handling as well as unhygienic conditions put together the street food one of the main resources of foodborne illnesses. Previously several cases were reported in India and abroad regarding foodborne illness due to consumption of street foods.[4,5]

The street food is not very much protected from flies, which may possibly get contaminated with foodborne pathogens. Other possible physical factors are also responsible for contamination of foods. Several bacteria are directly related to contamination of food such as *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Proteus* sp., and *Pseudomonas* sp.[4]

Previously in food industry, several chemical or physical methods were used at large scale. These agents are inexpensive and easily available, but they are harmful for human body. Natural biological compounds are always in demand as these are non-toxic in nature, easily soluble, eco-friendly, and cheaper than chemical or physical preservatives.[6-8] Recently, the application of nanoparticle as food preservative has revolutionized the food industry. Due to their nano size, they have very effective antimicrobial activity and it can be used into the food packaging materials or in food to kill the pathogenic microorganisms.[9] Considering the previous history of microorganisms present in street food, we defined

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the objectives of the present studies: Bacteriology of street food "*panipuri*" in different areas of Gwalior city and antibacterial efficiency of nanoparticles against them.

MATERIALS AND METHODS

Materials

Nutrient broth and nutrient agar, as well as selective media, MacConkey agar, Tryptic Soy Agar, Simmons citrate agar, Starch agar, Urea broth, Peptone water, and methyl red Voges–Proskauer (MR-VP) broth have been procured from HiMedia, Mumbai, and prepared as per the manufacturer's instructions. Antibiotic discs were prepared by Whatman filter paper no.1 and silver nitrate was purchased from Fisher Scientific.

Sample collection

A total of 20 panipuri samples were collected during the month of March (temperature 30–35°C) from the best possible places in the Gwalior region. These samples were collected separately, kept in sterile tubes, and placed in a cool box with ice packs until it gets to the laboratory. Within 2–3 h, the samples were transported from the field to the laboratory of Amity Institute of Biotechnology, situated in Amity University, Madhya Pradesh, Gwalior, where the samples were stored at 2–4°C temperature till the sample processing started at the laboratory. The types of panipuri samples collected are summarized in Table 1.

Isolation of bacteria from panipuri samples

For the isolation of pure culture of bacteria, serial dilution method was preferred and it was applied to all of the 20 samples collected from various areas of Gwalior region. In this method, a total of 10 sterilized tubes were dispensed with 9 ml of sterilized distilled water. Then, 1 ml of the panipuri sample was added into the 9 ml sterilized distilled water (10^{-1} dilution) and mix well. After that, 1 ml of solution was transferred into the second tube containing 9 ml of sterilized distilled water (10^{-2} dilution). This procedure was repeated for the dilution up to 10^{-8}. After completion of the serial dilutions process, 1 ml of the diluted sample from the last tube (10^{-8}) was spread into a sterile Petri dish containing media. After the incubation of these plates, a number of colony-forming units was counted.

Identification of microorganisms by biochemical tests

For the identification of bacteria, the culture was confirmed using Gram's staining and biochemical examinations as reported previously.[10] The culture was streaked on MacConkey Agar and incubated at 37°C overnight. The isolated colony was picked for the Gram's stain examination. Biochemical tests such as indole test, MR test, VP test, citrate utilization test, starch hydrolysis test, catalase test, and H_2S production test were performed.[11]

Synthesis of nanoparticles using *catharanthus roseus*

Leaf sample of *C. roseus* was collected from Amity University, Gwalior campus, during the month of March. Leaf powder of *C. roseus* was dissolved in 100 ml of distilled water overnight, and then, leaf extract was filtered using Whatman paper. Centrifuged the filtrate at 4000 rpm for 30 min and the supernatant was collected. The filtrate was used for the synthesis of silver nanoparticles (AgNPs). For the synthesis of AgNPs, 10 ml of aqueous leaf extract were added to 90 ml of $AgNO_3$ (1 mM). The mixture was kept in the dark overnight at room temperature. The color of solution changed and synthesized AgNPs were purified by centrifugation at 10000 rpm for 30 min, supernatant was discarded again, and the pellet was resuspended in autoclaved water for washing and then completely dried it.

Table 1: Details of the bacterial isolates obtained from different places in Gwalior region

Area	Collection centers	Number of samples	Number of contaminated samples
Gola ka Mandir zone	Hawkers	2	2
	Panipuri shop	2	2
Mela ground zone	Hawkers	2	1
	Panipuri shop	2	1
Kampoo zone	Hawkers	2	2
	Panipuri shop	2	2
Govindpuri zone	Hawkers	2	1
	Panipuri shop	2	1
Railway station zone	Hawkers	2	2
	Panipuri shop	2	2
Total		20	16

The formation of nanoparticles was confirmed by ultraviolet (UV) visible spectra analysis.

Drug resistance testing on various panipuri samples

Agar disk diffusion method (Kirby–Bauer method) was used to identify drug-resistant bacteria and to analyze nanoparticles activity against those bacteria.[12] After overnight incubation on Mueller Hinton agar plates containing antibiotic discs, isolates were examined and the diameters of the zones of inhibition were measured using millimeter scale. The zone diameter of inhibition was analyzed (susceptible or resistant) with the help of interpretation table of Becton Dickinson Microbiology Company, USA.

RESULTS AND DISCUSSION

Isolation and identification of bacteria from collected panipuri sample

A total of 20 *Panipuri* samples were collected from different Hawkers, panipuri shop [Table 1]. After culturing these samples, 16 samples were found to be contaminated with different bacteria. However, only 3 bacterial isolates were identified on the basis of different biochemical analysis and selective media. The remaining samples of bacterial isolates were not identified because the results were not reproducible. On the basis of previous reports, following biochemical tests such sa IMViC test, starch hydrolysis test, catalase test, and H_2S production tests were used to demonstrate the presence of *Proteus vulgaris*, *Klebsiella pneumoniae,* and *E. coli* in different panipuri samples [Table 2]. The same results were obtained when the growth pattern of bacteria on selective media such as MacConkey agar was analyzed.

Synthesis of nanoparticles

In the present study, nanoparticles were synthesized using the biological method. *C. roseus* leaf extract contains a number of secondary metabolites such as vincristine, vinblastine, and serpentine which act as stabilizer, reducing agent, and capping agent respectively. The reaction mixture is containing leaf extract of *C. roseus* and silver nitrate solution. The color change from yellowish-brown to reddish-brown was indicated the formation of AgNP with the reduction of silver ion. The characteristic surface plasmon absorption bands were observed at 440 nm by UV spectra.

Antibiotic susceptibility

Antibiotic resistance of bacteria has been increased worldwide due to excessive consumption of antibiotics without sufficient knowledge, which is leading to failures in the treatment of human infectious diseases. The Kirby–Bauer disk diffusion test[12] was a very simple and effective method, to determine whether the isolated organisms are susceptible or resistant to the prescribed antibiotics. In the present study, to find the susceptibility pattern of isolates toward antibiotics, all the isolated bacterial samples were subjected to antibiotic susceptibility test. Four antibiotics, namely, ciprofloxacin (5 µg/ml), ampicillin (10 µg/ml), co-trimoxazole (25 µg/ml), and chloramphenicol (30 µg/ml) were used to detect drug-resistant bacteria. A number of bacterial isolates, isolated from vendors and shops, were found to be resistant against these antibiotics [Table 3]. The zones of inhibition were measured and analyzed by scale.

The antimicrobial activities of AgNPs synthesized from *C. roseus* were determined by disc diffusion method against bacteria isolated from panipuri. Disc was prepared using Whatman No.1 filter paper soaked in the AgNPs (250 µg/

Table 2: Biochemical tests performed to identify the test isolates in the study

Name	Gram staining	Indole test	Methyl red test	Voges–Proskauer test	Citrate utilization test	Urease test	Starch hydrolysis test	Catalase test
K. pneumoniae	–	+	+	–	+	+	+	–
P. vulgaris	–	+	+	–	+	+	+	–
E. coli	–	+	+	–	+	+	+	–

–: Negative, +: Positive. K. pneumoniae: Klebsiella pneumoniae, P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli

Table 3: Result of antibiotic susceptibility test of test isolates against commonly prescribed antibiotics

Microbes	Antibiotic resistance			
	Chloramphenicol (30 µg/ml)	Co-trimoxazole (25 µg/ml)	Ampicillin (10 µg/ml)	Ciprofloxacin (5 µg/ml)
K. pneumoniae	–	–	+	+
P. vulgaris	–	–	+	+
E. coli	–	+	+	+

–: Resistant, +: Sensitive. K. pneumoniae: Klebsiella pneumoniae, P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli

ml). The disc was placed on the medium of Mueller-Hinton Agar plates along with synthesized AgNPs. The Petri dishes were incubated at 37°C for 24 h. Streptomycin (10 µg/ml) disc was utilized as reference drug. It was reported that the efficiency of nanoparticles against bacteria isolated from panipuri was better than the antibiotic against bacteria, namely, *K. pneumonia* and *P. vulgaris* [Table 4].

DISCUSSION

Gwalior is a well-known historical city and famous for its tourist attraction in Madhya Pradesh, India. Street food consumption is relatively high in almost all parts of the city. Improper handling of food in the pre-cooking, during, and post-cooking processes is responsible for contamination of food. Street food vendors are often uneducated regarding proper hygienic conditions, unlicensed, and untrained and they work in under crude unclean situations.[13]

Cleanliness is very important in handling street food and cooking. According to the FAO, the food handler must have the necessary knowledge and skills to clean the food.[14] From this study, it has been found that 80% of vendors have prepared food in unhygienic conditions. The same type of results was obtained by a group of researches in Nairobi where 85% of vendors prepared their food in unhealthy conditions.[15,16] Muinde and Kuria[14] have confirmed in their study that roadside sites do not provide proper safety of food from the dust and smoke from vehicles. Food security also depends primarily on personal hygiene. Handling food with uncovered hands may result in cross-contamination and hence the introduction of microbes in food. It has been found in one of our survey studies related to unhygienic conditions prevalent among street food vendors and in their shops that 95% of food vendors did not use aprons, while 98% handled with bare hands. Almost 99% of the food vendors did not wear hair caps (unpublished data).

In this study, 80% of food samples confirmed the presence of different bacteria. The high count of *K. pneumoniae* can be attributed to mishandling of food by the sellers. The present study indicated the prevalence of *K. pneumoniae*, which is able to grow at a wide range of pH (4.5–6.5) and temperatures (7–43°C). A high number of *K. pneumoniae* may result in the production of enterotoxins causing foodborne diseases. The presence of *P. vulgaris* in street-vended foods can be linked to contamination resulting from inappropriate processing, incomplete heating, and use of contaminated water during preparation and washing or secondary contamination through contact with contaminated materials such as chopping boards, knives, and serving wares as mentioned in some other related study also.[17]

Enterobacteriaceae were detected in drinking water samples. This detection rate was consistent with a previous study carried out in Gwalior city, which reported the finding of 66.6% of all food samples.[18] In this study, the presence of *Enterobacteriaceae* can be attributed to post-processing contamination, contamination of sewage, and poor hygienic practice of food handlers. One of the major sources of contamination of foods sold by street vendors is that the water used for washing and processing.[19]

CONCLUSION

The results of this study clearly show that the street-vended food is contaminated with different pathogenic bacteria and this is due to not following proper hygienic practices while handling the food material or utensils used for food preparation or serving. The existence of these bacteria in food can induce potential health problems for consumers. Poor personal hygiene, improper handling, and storage practice of foods and unawareness of food vendors about foodborne diseases are the responsible factors which are associated with contamination of street-vended foods in Gwalior. As street food consumption provides employment to a large group of population and street food consumption is a habit of a number of food loving people, ways should be developed by which healthy and hygienic practices can be encouraged or the risk of microbial food contamination can be minimized. The present study results are promising in this aspect as the synthesized nanoparticles give a solution for combating commonly found food contaminants and thus pave the way for safer, more hygienic street food. The street food vendors may be sensitized with the fact of the importance of hygiene and the synthesized nanoparticles may be analyzed and processed further for making their use in daily life such as in soaps, dish washes, or hand sanitizers.

Table 4: Antimicrobial activity of synthesized nanoparticles using leaves of *C. roseus*

Name of microbial species isolated from test samples included in the study	Zone diameter (in mm)	
	AgNPs (mm)	Reference drug streptomycin (mm)
P. vulgaris	15	25
K. pneumoniae	13	17
E. coli	13	15

AgNPs: Silver nanoparticles, *K. pneumoniae: Klebsiella pneumoniae, P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli, C. roseus: Catharanthus roseus*

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REFERENCES

1. WHO. Global Strategy for Food Safety: Safer Food for Better Health. Geneva, Switzerland: World Health Organization; 2002.
2. Maxwell DG, Levin CE, Klemesu MA, Ruel MT, Morris SS, Ahiadekeet C. Urban livelihood and food and nutrition security in greater Accra, Ghana. International Food Policy Research Institute, Research Report; 2000. p. 112.
3. Bhowmik SK. Street vendors in Asia: A review. Econ Polit Wkly 2005;40:2256-2264.
4. Hanoshiro A, Morita M, Matte GR, Matte MH, Torres EA. Microbiological quality of selected foods from a restricted area of Sao Paulo city, Brazil. Food Control 2004;16:439-44.
5. Bryan FL. Risks associated with practices, procedures, and processes that lead to outbreaks of food borne diseases. J Food Prot 1998;51:663-73.
6. Manavalan AR, Manian K. Medicinal and Aromatic Plants–Diversity and Utility. New Delhi: Allied Publisher Ltd.; 2001.
7. Mohammad AS, Asgari S. Effects of plants bioactive compounds on foods microbial spoilage and lipid oxidation. Food Sci Technol 2013;1:52-61.
8. Rath CC. Prospects and challenges of essential oils as natural food preservatives—a review. Food 2007;1:172-80.
9. Pradhan N, Singh S, Ojha N, Shrivastava A, Barla A, Rai V, *et al*. Facets of nanotechnology as seen in food processing, packaging, and preservation industry. Biomed Res Int 2015;2015:365672.
10. Sherman N, Cappuccino JG. Microbiology: A laboratory Manual. Dunfermline, United Kingdom: Benjamin/ Cummings Publishing Company, Inc.; 2005. p. 265-7.
11. Holt JG, Kreig NR, Sneath PH, Steley JT, Williams ST. Bergey's Manual of Determinative Bacteriology. Philadelphia, PA: Williams and Wilkins; 2000.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966;45:493-6.
13. FAO. Street Foods. Report of an FAO expert Consultation. Jogjakarta, Indonesia, 5-9 December, 1998. FAO Nutr. 1990;46:3-30.
14. Muinde AM, Kuria E. Hygienic and Sanitary Practices of Vendors of Street Foods in Nairobi. Kenya. Ajfand. 2005;5:1-13.
15. Martins JH. Socio-economic and hygiene features of street food vending in Gauteng. South Afr J Clin Nutr 2006;19:18-25.
16. Von HA, Makhoane FM. Improving street food vending in South Africa: Achievements and lessons learned. Int J Food Microbiol 2006;111:89-92.
17. Oranusi US, Braide WA. Study of microbial safety of ready-to-eat foods vended on highways. Int Res J Microbiol 2012;3:66-71.
18. Chumber SK, Kaushik K, Savy S. Bacteriological analysis of street foods in Pune. Indian J Public Health 2007;51:470-6.
19. Seth M, Gurudasani M, Mudbidri R. Screening for pathogenic microorganisms in street vended Bhelpuri in urban Vadodra: A HACCP approach. J Food Sci Technol 2005;42:395-9.

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