**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. The National Policy for Metropolitan Street Vendors stated that they constitute approximately 2% of the residents of a metropolis in India. 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.

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. 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. 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 H₂S 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 AgNO₃ (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 as IMViC test, starch hydrolysis test, catalase test, and H₂S 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.

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Gram staining</th>
<th>Indole test</th>
<th>Methyl red test</th>
<th>Voges–Proskauer test</th>
<th>Citrate utilization test</th>
<th>Urease test</th>
<th>Starch hydrolysis test</th>
<th>Catalase test</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E. coli</td>
<td>–</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>


**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/
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. pneumonia* can be attributed to mishandling of food by the sellers. The present study indicated the prevalence of *K. pneumonia*, 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. pneumonia* 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.[19] 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>
<thead>
<tr>
<th>Name of microbial species isolated from test samples included in the study</th>
<th>AgNPs (mm)</th>
<th>Reference drug streptomycin (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vulgaris</em></td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>13</td>
<td>15</td>
</tr>
</tbody>
</table>

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