Isolation and identification of common food borne bacteria from spicy puffed rice and determination of their antibiotic resistance profile

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ABSTRACT

The aim of this study was to determine bacterial load in street vended spicy puffed rice (jhalmuri), to isolate and identify the common food borne bacteria, and to know the antibiogram profile of the isolated bacteria. Samples were collected from different vendors at Sadar Upozila, Dinajpur, Bangladesh. Total viable bacteria were counted, and the samples were inoculated into various selective media. The bacteria were identified through cultural properties, staining characteristics and biochemical tests. Antibiotic sensitivity of the bacteria were conducted using disc diffusion method. Among 20 samples, all (100\%) had bacterial contamination. The total viable count (TVC) in the samples were ranged from $8.4 \times 10^3$ CFU/g to $3.24 \times 10^7$ CFU/g. From the samples \textit{Pseudomonas aeruginosa} (37.5\%), \textit{Staphylococcus aureus} (25\%), \textit{E. coli} (19.5\%) \textit{Bacillus cereus} (13\%) and, \textit{Bacillus subtilis} (5\%) were isolated and identified. Antibiotic sensitivity test showed that \textit{Bacillus subtilis}, \textit{Staphylococcus aureus}, and \textit{Pseudomonas aeruginosa} were sensitive to Chloramphenicol (60\%) and Ciprofloxacin (60\%). \textit{Bacillus cereus} was moderately sensitive to Chloramphenicol, Ciprofloxacin and Kanamycin; \textit{E. coli} was moderately sensitive to Chloramphenicol and Ciprofloxacin; \textit{Staphylococcus aureus} and \textit{E. coli} were found resistant to Kanamycin (40\%). All isolates were found 100\% resistant to Amoxycillin, Cefixime, Cephalexin, Neomycin and Ceftriaxone. Presence of coliforms in the sample might be due to poor quality of water, unhygienic vendor places and poor personal hygiene of vendors. The results of this study suggested that street vended ready to eat (RTE) foods should be manufactured under good hygienic practices.

Keywords: Antibiotic resistance, Bacterial load, Identification, Spicy puffed rice

INTRODUCTION

Foods from street-vendors are usually ready-to-eat (RTE) foods, prepared and sold on streets and other public places (Dawson et al., 1991). The types of street-vended food vary significantly on countries and cultures (Moy et al., 1997). According to a study from the Food and Agriculture Organization (FAO), 2.5 billion people eat street food every day.

The street foods play an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income groups and are appreciated for their unique flavors and convenience (Ackah et al., 2011; Cross et al., 2007; Muzaffar et al., 2009). Street foods also assure food security for low income urban population and livelihood for a significant proportion of
the population in many developing countries (Rane, 2011). The consumption of street foods is also common in countries where unemployment is high, salaries and work opportunities are low and social programs are limited (Oladipo and Adejumobi, 2010). There are about 128 varieties of street foods in Bangladesh. Among them Phuchka, Chotpothi, Belpuri, Samucha, Jhalmuri, Daalpuri, Lassi, Pakura, Hallim are most popular (Rahman et al., 2014). Spicy puffed rice or jhalmuri is a popular street food in Bangladesh as well as West Bengal, India. Spicy puffed rice or jhalmuri is widely available and a favorite among children and female. People of all ages and all classes like students, laborers, informal sectors, rickshaw puller, and children all eat spicy puffed rice. In contrast to these potential benefits, street food vending has perceived to be a major public health risk due to lack of basic infrastructure and services and also difficulty in controlling the large numbers of street food vending operations. The food vendors’ diversity, mobility and temporary nature; low socioeconomic and educational status, lack of knowledge of safe food handling also contribute to a public health risk (WHO 1996; Artemis et al., 2000).

It is also recognized that street food vendors are often poor, uneducated, and lack knowledge in safe food handling, environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials, and use of potable water. Consequently, street foods are perceived to be a major public health risk (Bhowmik, 2010). The vendors in Bangladesh lack education regarding the basic food safety issues. Vendors generally use carts and stands, where they do not have easy access to running water. Furthermore, dish and hand washing is done using the same bucket, sometimes even without soap. Garbage and waste water is typically discarded in the streets nearby and thus attracting and providing food for rodents and insects. Toilets are not available nearby in several cases thus forcing the vendors to eliminate their body wastes in nearby areas and return to their vending sites without washing their hands (Bryan et al., 1988). Environmental condition and practices like this often lead to contamination of cooked food. Vendors may purchase raw materials from doubtful sources which may either be contaminated with food borne pathogens or be unfit for consumption due to other reasons (Dawson et al., 1991). The traditional processing methods that are used in the preparation, inappropriate holding temperature, and poor personal hygiene of food handlers are some of the main causes of contamination of street foods (Barro et al., 2006; Mensah et al., 2002).

In Bangladesh, street foods are mostly prepared and processed manually and sold to the public at various lorry terminals, by the roadside or by itinerant vendors (Mamun et al., 2013). As food is biological in nature, it is capable of supporting the growth of microorganisms and foodborne diseases result from the ingestion of contaminated foods and food products (Sheth et al., 2005). More than 250 different types of viruses, bacteria, parasites, toxins, metals, and prions are associated with foodborne diseases in humans (Tambekar et al., 2008). Although viruses are more responsible for more than 50% of all foodborne illnesses; generally hospitalizations and deaths associated with foodborne infections are due to bacterial agents. The infections range from mild gastroenteritis to life threatening neurologic, hepatic, and renal syndromes caused by either toxin from the disease-causing microbe or by the human body’s reaction to the microbe itself (Schelin et al., 2011). Foodborne bacterial agents are the leading cause of severe and fatal foodborne illnesses. Of the many thousands different bacterial species, more than 90% of food-poisoning illnesses are caused by species of Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus, and Enteropathogenic Escherichia coli (Schmidt et al., 2003).

Microbiological study of different food items, drinking water, and hand swab samples showed the prevalence of overwhelmingly high numbers of aerobic bacteria, coliform bacteria, and pathogens (Food and Agricultural Organization, 2010). Foodborne illnesses of microbial origin are a major health problem associated with street foods (Biswas et al., 2010; Tabashsum et al., 2013; Mamun et al., 2013). In addition, resistance of foodborne microorganisms in multi-drug made the food safety situation more vulnerable in public health (Ali et al., 2011). Approximately, 30 million people in Bangladesh are suffering from foodborne illnesses each year (Food and Agriculture Organization, 2012). Diarrheal diseases are the most common food poisoning cases in Bangladesh and in some cases, these can cause death. The diseases are caused by either toxin from the microbe or by the human body’s reactions to the microbe (Barro et al., 2006; Mensah et al., 2002).
Although there is a growing demand for RTE food products, no recent information is available regarding the microbiological quality of this product in Sadar Upozila, Dinajpur, Bangladesh. The present study was hence undertaken to determine the microbiological quality and safety of a street-vended RTE food product collected from several typical vendors surrounding the street of different areas of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. It is obvious that few assessment of street vended spicy puffed rice has yet been performed in Bangladesh. The objectives of this research work were therefore to determine bacterial load in street vended spicy puffed rice, to isolate and identify the food borne bacteria from those samples and to know the antibiogram profile of isolated bacteria.

MATERIALS AND METHODS

The present study was conducted during the period from January to July 2016, in the Bacteriology Laboratory of the Department of Microbiology, Faculty of Veterinary and Animal Science (VAS), Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

The samples were collected from different vendors located in Sadar Upozila, Dinajpur. A total number of 20 spicy puffed rice (jhalmuri) samples collected from different spicy puffed rice vendors. The bacteriological quality of samples was analyzed. The bacteriological analysis comprise enumeration of TVC. Isolation of the samples was performed onto Mannitol salt (MS) agar, Eosin Methylene Blue (EMB) agar, MacConkey (MC) agar, Salmonella-Shigella (SS) agar. Identification of bacteria was performed by morphological, cultural and biochemical tests. The pattern of antibiotic sensitivity of bacterial isolates obtained from spicy puffed rice (jhalmuri) was studied.

Materials

The materials used in the study comprise sample materials and laboratory materials. Sample materials include spicy puffed rice and associated instruments; those are clean rubber hand gloves, eppendorff tubes, and ice box containing ice. Different bacteriological culture media and reagents were for isolation and identification and also propagation of bacteria from spicy puffed rice. The culture media and reagents that were used in this experiment are Nutrient Broth (NB) was used as liquid culture media. Nutrient agar (NA), MacConkey (MC) agar medium. Eosin methylene blue (EMB) agar medium, Salmonella-Shigella (SS) agar medium and Manitol Salt (MS) agar medium were used as solid culture media. All of them were purchased from Hi Media, Mumbai, India.

In order to identify bacteria tetramethyl-p-phenylenediamine, 3% H₂O₂, triple sugar iron agar slant, MIU agar base media, buffered peptone water, methyl red-Voges Proskauer (MR-VP) medium base and Simmon’s citrate were used for biochemical tests. All of them were purchased from Hi Media, Mumbai, India.

For conducting the various bacteriological tests crystal violet, Gram’s iodine, acetone alcohol, safranin, methyl red solution, alpha-naphthol, potassium hydroxide solution, Kovac's reagent, phenol red solution, phosphate buffered saline solution and distilled water were used. The different kinds of glass wares and appliances used during the course of the experiment were test tubes (with or without Durham’s fermentation tube and stopper), conical flask, petri dishes, pipette, slides, cover slips, hanging drop slide, stop watch, test tube stand, inoculating loop (straight and coiled), water bath, detergent power, gas burner, ice box, electronic compact balance, aluminium foil, sterile cotton, bacteriological incubator, autoclave, refrigerator, hot air oven, compound microscope. Muller Hinton Agar (Hi Media, Mumbai, India) plates were specially used for the antibiotic sensitivity test (Hi media, India). Commercially available antibiotic disc (Oxoid, UK) were used for the test to determine the drug sensitivity pattern.

The spicy puffed rice samples were collected from different vendors located in, Sadar Upozila, Dinajpur, for the isolation and identification of bacterial pathogens by morphology, staining and cultural and biochemical reactions and antibiogram feature of the isolated bacteria.
Samples collection:

A total of 20 samples were collected for bacteriological examination. The street vendors on the busiest street areas were chosen for sample collection. The samples were collected from Basher hat, Gopalgonj, Ranigonj, HSTU High School, Dosmile. The samples were congregated during the months of January to July 2016. The time interval between sampling from each vendor was approximately 15 minutes. Two samples were collected on every sampling day. Food samples included cereal based (jhal-muri). Approximately 300 g of each food sample was collected using the vendors serving utensils, take parcel and placed into sterile plastic bags. All the collected samples were kept on an ice-box during transportation to the laboratory and stored at 4°C until testing. They were analyzed within 24 hours of sampling.

Preparation of samples:

Adequate amount of spicy puffed rice (jhal-muri) samples were uniformly homogenized in mortar and pastel using a sterile diluent as per recommendation of ISO (1995). A homogenized suspension was made with the help of mortar and pastel. A quantity of 10 gm homogenate sample of each spicy puffed rice (jhal-muri) was taken aseptically with a sterile spoon and transferred carefully into a sterile pastle containing 90 ml of PBS. Thus 1:10 dilution of the samples was obtained. The samples were then serially diluted at 10 fold dilution.

Total viable count (TVC):

50 μl of each fivefold dilution was transferred and spread onto Nutrient agar using a micropipette for each dilution for the determination of total bacterial count. The diluted samples were spread as quickly as possible on the surface of the plate. The plates were kept in an incubator at 37°C for 24 hrs. After incubation, plates exhibiting 25-250 colonies were counted. The average number of colonies in particular dilution was multiplied by the dilution to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of colony forming units (CFU) per gram of food samples.

Isolation of bacteria from spicy puffed rice:

Homogenized samples were enriched into nutrient broth by overnight incubation at 37°C. Overnight enriched culture was streaked duplicate onto MS agar, MC agar, EMB agar, SS agar and incubated at 37°C for 24 hrs.

Identification of bacteria from spicy puffe rice:

The cultural examination of spicy puffed rice (jhalmuri) samples for bacteriological analysis was done according to the standard method (ICMSF, 1985). Identification of bacteria was performed on the basis of colony morphology; Gram’s staining reaction and biochemical test. Colony characteristics such as shape, size, surface texture, edge and elevation, color and opacity developed on various selective media after 24 hrs of incubation at 37°C were recorded.

Gram stain

On a clean glass slide smear of bacteria was prepared. The smear was air dried and fixed with heat. Crystal violet was added on the smear, treated for 2 minutes and then gently washed with tap water. Gram's iodine was added on the smear, treated for 1 minute and then gently washed with tap water. Acetone alcohol was added on the smear, treated for 15 seconds and then gently washed with tap water. Finally safranin was added on the smear, treated for 1 minute and then gently washed with tap water. The smear was observed under microscope using 10x immersion objective.
Endospore staining:

Some bacteria are capable of changing into dormant structures that are metabolically inactive and do not grow or reproduce. These structures are called as endospores. An endospore develops in a characteristic position with a cell i.e. central, sub-terminal or terminal. Thin smears of bacterial isolates were prepared on clean glass slides which were then flooded with 5% malachite green solution. The slides were heated to steaming for about 5 minutes then slides were washed with distilled water then counter stained with 0.5% safranin for 30 seconds and again washed with distilled and observed under microscope.

Biochemical tests:

Oxidase Test:

This test was used to help in the identification of the organism which produces the enzyme oxidase. A portion of the test organism was picked up from agar plate by means of a sterile wooden-pick. Streaked on to the filter paper soaked with the oxidase reagent. Formation of a dark purple color developed within 5-10 seconds indicate positive for oxidase.

Catalase Test:

The presence of catalase is determined by its ability to break down peroxide into water and oxygen, releasing bubbles of oxygen. A colony of the bacteria was picked up from a plate and transferred the colony on a glass slide in a drop of water. A few drops of 3% H₂O₂ was placed over the culture. Production of gas bubbles (released oxygen) indicate a positive reaction.

Methyl red (MR) Test:

This test was performed to differentiate enterobacteria. Some enterobacteria when cultured in buffered glucose peptone water, ferment glucose to produce sufficient acidity, which gives red color with methyl red indicator (pH range: 4.4-6.2, color change: red yellow). Sterile MR-VP broth was inoculated with the test organism and following incubation at 37° C for 24 hours. Few drops of methyl red solution was added. A distinct red color indicated MR positive test while yellow or orange color indicated a negative result.

Voges proskau (VP) Test:

The test organisms were cultured in glucose phosphate peptone water for 24 hours. Acetone formed was converted to diacetyl. It was converted to a pink compound by the action of creatine. This test was used to assist in the differentiation of enterobacteria such as Vibrio cholerae, K. pneumonia and some strains of enterobacter. Sterile MR-VP broth was inoculated with the test organism and following incubation at 37° C for 24 hours. After incubation, 5 rops of napthol solution and 5 drops of KOH solution was added. The development of a bright red or pink-red color was recorded as a positive result.

Triple Sugar Iron (TSI) agar Test:

TSI slants are useful in the identification of enterobacteria by their specific reaction on the slants. Alkaline reaction (red color) was shown by the organisms, who fail to ferment any one of the sugars. Fermentation of the sugars was indicated by yellow color since pH range of phenol red is 6.8 and color change from yellow to red. Since the glucose (dextrose) present on the surface of the medium was used up and since the surface of the slant was exposed to atmosphere, under aerobic conditions, the acid reaction on the surface reverts to alkaline (red color) in 18 to 24 hours.. In the butt, since anaerobic condition exists, the color of the butt remains yellow. Gas production (carbon dioxide) was indicated by splitting of the agar. Production of hydrogen sulfide imparts black shade to slant by reacting with ferrous ions. It was an indication of H₂S producing organisms. TSI slants are useful in the identification of enterobacteria by
their specific reaction on the slants. A loop of bacteria was spread across the surface of the agar. A needle of bacteria was inserted (stabbed) into the bottom (butt) of the tube. The tube was kept at 37º C for 24 hours for incubation and then the tube was examined for result.

**Citrate Utilization Test:**

The test organism was cultured in a medium containing sodium citrate, an ammonium salt and bromothymol blue indicator. The organisms use citrate (the only source of nitrogen). The citrate utilization is followed by alkaline reaction (change of the color form light to blue) and growth in the medium was indicated by appearance of turbidity. This test was performed in the identification of enterobacteria. A loop of bacteria was spread across the surface of the agar. The tubes were kept at 37º C for 24 hours for incubation. The tube was examined for the result (blue color indicate positive result; no color change indicate negative result).

**Indole test:**

The test organism was cultured in a medium containing tryptophan. The organisms break down tryptophan and indole is released. It was detected by the action of Kovac’s reagent or Ehrlich reagent (formation of red colored compound). This test was important for the identification of enterobacteria such as *E. coli*, *P. rettgeri* etc. Tryptophan containing broth was inoculated with bacteria. The tube was incubated at 37º C for 24 hours. 0.5 ml of the Kovac’s reagent was added after the bacterial growth. In positive case after 2 minutes a red color ring appeared at the junction of medium and reagent in the tube.

**Characterization of bacteria**

Individually isolated colonies of the same morphology were selected from appropriate agar plates, cloned and checked for purity of growth prior to characterization of the respective genera and species. Characterization into respective genera and species were done on the basis of morphological, cultural and biochemical reactions. The classification and specification of organisms was based on the scheme presented in Bergey’s Manual of Systematic Bacteriology (Holt, 1985). Stock culture was prepared and maintained for subsequent studies. Strict aseptic measures were maintained throughout the period of study.

**Antibiotic sensitivity assay of isolated organisms**

Bacterial susceptibility to anti microbial agent was determined in vitro by using the standardized agar disc-diffusion method known as the Kirby Bauer (Barry and Thomsberry, 1985). The covers of each of the agar plates were labeled with name of the test organisms was inoculated. Using sterile technique all agar plates were inoculated with their respective test organisms. A sterile cotton swab was dipped into a well mixed saline test culture and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube. Using the swab the entire agar surface was streaked horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface. All culture plates were allowed to dry for about 5 minutes. Individual antibiotic discs were distributed at equal distance with forceps dipped in alcohol and flamed. Each disc was gently pressed down with the wooden end of the cotton swab or sterile forceps to ensure that the discs adhered to the surface of the agar. The plates were then inverted and incubated at 37º C for 24 hours. After incubation, the plates were examined and the diameter of the zones of complete inhibition was measured in mm. The zone diameter for individual antimicrobial agents was used to determine susceptible, intermediate, and resistant categories by referring to an interpreting table (Barry and Thomsberry, 1985).
Recording and interpreting results

The zones of growth inhibition were compared with the zone-size interpretative table standard for *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *E. coli* (table 1) provided by Clinical and Laboratory Standards Institute (CLSI, 2007).

Maintenance of stock culture

**Agar slant method**

The stock culture was maintained following the procedures of Chowdhury et al. (1987). Isolated and identified bacteria were inoculated into nutrient agar slants and incubated at 37ºC for 24 hours and then examine for growth. One slant was used for an individual isolate. Then the sterile mineral oil was poured into the tube until the colonies were covered completely. The tube were sealed off with paraffin and kept at room temperature for future use as seed. By this method bacteria can be preserved with no deviation of their original characters for few months (Buxton and Fraser, 1977).

**Sterile buffered glycerine method**

Sterile buffered glycerin (20%) was prepared by mixing 20 parts of pure glycerin and 80 parts of PBS. Then a loopful of thick bacterial culture was mixed with 20%, sterile buffered glycerin in small vials and was preserved at -20ºC. This method is more appropriate for preserving bacteria with no deviation of their original characters for several years (Buxton and Fraser, 1977).

**RESULTS AND DISCUSSION**

The results describe the total viable count, isolation, identification and characterization of bacteria from spicy puffed rice (*jhal-muri*).

**Total viable count (TVC) of spicy puffed rice:** The TVC of spicy puffed rice samples collected from different vendors are presented in Table 1.

<table>
<thead>
<tr>
<th>Place of Vendor</th>
<th>Dilution</th>
<th>Number of colony</th>
<th>Total viable count (TVC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basher hat</td>
<td>-</td>
<td>23</td>
<td>TFTC</td>
</tr>
<tr>
<td>Gopalgonj</td>
<td>-</td>
<td>42</td>
<td>8.2x10³ CFU/g</td>
</tr>
<tr>
<td>Ranigonj</td>
<td>-</td>
<td>17</td>
<td>TFTC</td>
</tr>
<tr>
<td>HSTU School</td>
<td>-</td>
<td>22</td>
<td>TFTC</td>
</tr>
<tr>
<td>Dosmule</td>
<td>10⁻¹</td>
<td>150</td>
<td>1.5x10⁵ CFU/g</td>
</tr>
<tr>
<td></td>
<td>10⁻²</td>
<td>81</td>
<td>8.1x10⁴ CFU/g</td>
</tr>
<tr>
<td></td>
<td>10⁻³</td>
<td>45</td>
<td>4.5x10³ CFU/g</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>32</td>
<td>3.2x10² CFU/g</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>14</td>
<td>TFTC</td>
</tr>
</tbody>
</table>

Legend: TVC= Total viable count, TFTC= Too few to count, CFU= Colony forming unit, HSTU School = Hajee Mohammad Danesh Science and Technology University School.

The bacterial load was the highest in Dosmule (3.2x10⁷ CFU/g) followed by Gopalgonj, Basher hat, Ranigonj and HSTU School (Table 1).

**Bacteriological investigation**

A total of 20 spicy puffed rice (*jhal-muri*) samples were collected from Sadar Upozila, Dinajpur for this study. From the samples *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *E. coli* bacteria were isolated and identified. Result of morphological, staining, cultural, biochemical, antibiotic sensitivity pattern and percentage of incidence of isolated bacteria are presented in different table and described below.
Isolation and identification of bacteria from spicy puffed rice (jhal-muri)

Five genera of bacteria such as Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and E. coli were isolated from twenty spicy puffed rice samples.

Identification of bacteria

Identification of bacteria was performed by determining staining reactions, cultural characteristics and different biochemical properties.

Cultural Characteristics

Cultural characteristics of each type of bacteria isolated from spicy puffed rice (jhal-muri) were studied for the determination of size, shape and colony characteristics in various bacteriological media. The staining property of primary culture of each of the spicy puffed rice (jhal-muri) samples indicated the presence of more than one type of bacteria in the same smear. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different simple and selective solid media for study. The individual cultural characteristics of bacterial isolates are presented in Table 2. The cultural characteristics of Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and E. coli exhibited on the media are presented in Table 2 and Figure 1.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Suspected case of Bacteria</th>
<th>Name of Media</th>
<th>Cultural Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Bacillus subtilis</td>
<td>Nutrient Agar</td>
<td>Large, White, regular, Undulate, Translucent, Circular colony</td>
</tr>
<tr>
<td>02</td>
<td>Staphylococcus aureus</td>
<td>MS agar</td>
<td>Small whitish colony or yellowish colony</td>
</tr>
<tr>
<td>03</td>
<td>Pseudomonas aeruginosa</td>
<td>Nutrient Agar</td>
<td>Smooth, Raised, irregular and semi-translucent colony.</td>
</tr>
<tr>
<td>04</td>
<td>Bacillus cereus</td>
<td>Nutrient Agar</td>
<td>Small, White, Entire, Circular, Opaque and smooth circular colony</td>
</tr>
<tr>
<td>05</td>
<td>E. coli</td>
<td>EMB agar, MacConkey agar</td>
<td>Metallic sheen (greenish black) colony, bright pink lactose fermenter colony.</td>
</tr>
</tbody>
</table>

Legend: MS = Manitol salt, EMB = Eosin methylene blue.

Figure 1. Cultural properties of the isolated bacteria. (A) Staphylococcus sp. colonies on nutrient agar, (B) Staphylococcus sp. colonies on manitol salt agar, (C) Escherichia coli colonies on EMB agar, (D) Bacillus sp. colonies on nutrient agar.
**Staining characteristics**

The staining characteristics of the isolated organisms were determined according to Gram’s staining technique. Morphological and staining characteristics of bacteria recorded from spicy puffed rice by Gram’s staining are presented in Table 3 and Figure 2.

**Table 3.** Morphological and staining properties of the bacterial isolates of spicy puffed rice by Gram’s staining

<table>
<thead>
<tr>
<th>Shape</th>
<th>Staining Characteristics</th>
<th>Gram’s Staining character</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod in shape</td>
<td>Single or short chain</td>
<td>(+) ve</td>
<td><em>Bacillus subtilis</em></td>
</tr>
<tr>
<td>Cocci in Shape</td>
<td>Arranged in grapes like cluster</td>
<td>(+) ve</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Rod in shape</td>
<td>Single</td>
<td>(-) ve</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>Rod in shape</td>
<td>Single or short chain</td>
<td>(+) ve</td>
<td><em>Bacillus cereus</em></td>
</tr>
<tr>
<td>Short plump rods</td>
<td>Single, paired or in short chain</td>
<td>(-) ve</td>
<td><em>E. coli</em></td>
</tr>
</tbody>
</table>

Legend: (+)Ve = Positive, (-)Ve = Negative

**Figure 2.** Light microscopic images of the isolated bacteria. (A) *Staphylococcus* sp., (B) *Bacillus* sp., (C) *Escherichia coli*, (D) *Pseudomonas* sp. Magnification 1000x.
Biochemical Properties

Table 4. A total of 5 bacterial species were identified from 37 isolates and following are the results of biochemical tests presented in Table 4 and Figure 3.

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Cata</th>
<th>Oxi</th>
<th>Ind</th>
<th>Cit</th>
<th>MR</th>
<th>VP</th>
<th>MIU</th>
<th>Spore</th>
<th>TSI</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Slant alkaline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Butt acidic</td>
</tr>
<tr>
<td>2.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Slant and Butt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>both acidic</td>
</tr>
<tr>
<td>3.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Slant and Butt</td>
</tr>
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<td>both alkaline</td>
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Legend: SL No. = Serial Number, Cata=Catalase test, Oxi=Oxidase test, Ind=Indole test, Cit= Citrate Utilization test, MR= Methyl red, VP=Voges-Proskauer, TSI=Triple Sugar Iron, MIU=Motility, Indole and Urease test, (+) = positive, (-) = negative.

Figure 3. Biochemical properties of the isolated bacteria. (A) *Escherichia coli* showing MR positive result (right) with control (left), (B) *E. coli* showing negative result (left) on citrate utilization test with control (right), (C) *E. coli* showing TSI positive result (left) with control (right), (D) *E. coli* showing VP negative result (right) with control (left), (E) *E. coli* showing indole positive result (right) with control (left), (F) *Pseudomonas aeruginosa* showing MR Negative result (right) with control (left), (G) *Pseudomonas aeruginosa* showing TSI negative result (left) with control (right), (H) *Bacillus subtilis* showing MIU positive result (right) with control (left), (I) *Bacillus subtilis* showing positive result (right) on citrate utilization test with control (left), (J) *Bacillus subtilis* showing VP positive result (right) with control (left), (K) *Staphylococcus aureus* showing indole negative result (right) with control (left), (L) *Staphylococcus aureus* showing MIU negative result (right) with control (left)
Results of antibiotic sensitivity tests

A total of five isolates such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *E. coli* were subjected to antibiotic sensitivity assay. The results of antibiotic sensitivity assay are presented in Table 5 and Figure 5.

Table 5. Antimicrobial profile of isolated bacteria

<table>
<thead>
<tr>
<th>Name of the antibiotics</th>
<th><em>Bacillus subtilis</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Bacillus cereus</em></th>
<th><em>Escherichia coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZOI (mm)</td>
<td>Inter</td>
<td>ZOI (mm)</td>
<td>Interpretation</td>
<td>ZOI (mm)</td>
</tr>
<tr>
<td>Chloramphenicol (C)</td>
<td>19</td>
<td>S</td>
<td>23</td>
<td>S</td>
<td>22</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>21</td>
<td>S</td>
<td>29</td>
<td>S</td>
<td>27</td>
</tr>
<tr>
<td>Cefixime (CFM)</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Cephalexin (CL)</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Kanamycin (K)</td>
<td>14</td>
<td>I</td>
<td>18</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Neomycin (N)</td>
<td>09</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic Acid (AMC)</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriaxone(CRO)</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>R</td>
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</table>

Legend: ZOI= Zone of inhibition, Inter=Interpretation, R=Resistant, S=Sensitive, I=Intermediate
DISCUSSION

Street foods are very much popular in the recent days. Consumption of ready-to-eat street foods is increasing significantly due to its cheap price and taste. Street food vending is a prevailing and distinctive part of a large informal sector in Bangladesh. It has a major economic impact in many countries and is a major source of employment (Mahon, 1999). Street foods are usually handled unhygienically as a result they can get contaminated with food borne and antibiotic resistant bacteria that may cause public health hazard. Present research work was conducted to measure TVC and common bacteria in spicy puffed rice (jhalmuri) sold at different Sader Upozila in Dinajpur.

This study was carried out to investigate the microbiological quality of spicy puffed rice (jhalmuri) sold by various street vendor at Sader Upozila in Dinajpur, Bangladesh. A total of twenty spicy puffed rice samples were collected from street vendors at Basher hat, Gopalgonj, Ranigonj, HSTU High School, Dosmile, area and tested for the presence of microorganisms following conventional microbiological processes. Samples were inoculated into various selective media such as EMB agar, MacConkey agar, SS agar, and MS agar. Among 20 spicy puffed rice (jhalmuri) samples, all (100%) had bacterial contamination. The TVC in spicy puffed rice sample was ranged from 8.4x10³ CFU/g to 3.2x10⁷ CFU/g. A total of 5 bacterial species were identified from 37 isolates. From spicy puffed rice (jhalmuri) five bacterial genera were isolated such as Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and E. coli. Pseudomonas aeruginosa were 37.5%; Staphylococcus aureus were 25%; E. coli were 19.5% Bacillus cereus were 13%, Bacillus subtilis were 5% of total isolates.

Findings of our research work are in agreement with findings of Berbicz et al. (2010) in which they collected of 42 food samples in the marketing points in Maringa (PR) city for Microbiological analysis. The microbiological analysis found contamination by Coliforms in 14.3% of the samples and by Staphylococcus positive coagulase in 2.4% of the samples (RDC 12/2001). No contamination by Salmonella spp. and B. cereus was found in any of the samples. In the first checklist, a 33.4% non-conformity was detected. Similarly Sharma et al. (2016) were examined total 37 street vended food samples for bacterial and the colony forming units counts ranged from 4.5×10⁵ to 1.12×10⁶. The isolates were identified as Escherichia coli (37.5%), Pseudomonas aeruginosa (3.57%), Staphylococcus aureus (14.20%), Salmonella sp. (5.36%), Klebsiella sp. (10.71%), Shigella sp. (19.64%) and Enterobacter sp. (8.93%) respectively. Eromo et al. (2016) also collected 72 samples from six food items such as local bread (‘ambasha’ and ‘kita’), raw fish, chilli (‘awaze’), avocado and cooked potato. About 31% of the food samples showed total colony counts ranging from 1.7×10⁵ to 6.7×10⁶ colony-forming unit per gram (CFU/g) which is beyond the acceptable limits set for microbiological quality of ready-to-eat foods. E.coli was the most frequent isolate (29.6%) followed by Salmonella species (12.7% and S.aureus (9.9%). All isolates were 100% sensitive to ciprofloxacin.

Antimicrobial sensitivity test was performed according to the procedure Kirby-bauer disk diffusion susceptibility test protocol suggested by Jan Hudzicki (2009). Antibiotic sensitivity test showed that Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa were sensitive to Chloramphenicol (60%) and
Ciprofloxacin (60%) but the Bacillus cereus and E. coli were moderately sensitive. Bacillus subtilis, Bacillus cereus were moderately sensitive to Kanamycin (40%) but the Staphylococcus aureus and E. coli were resistant. All isolates were found 100% resistant to Cefixime, Cephalexin, Neomycin, Amoxycillin and Ceftriaxone.

Data of this study indicated that spicy puffed rice sold at sadar upozila, Dinajpur showed wide range of TVC which exceed accepted level. According to the microbiological standard of foods in Bangladesh, aerobic plate counts ranges from 10 to 10² CFU/g can be said to be safe, 10² to <10⁴ CFU/g acceptable, 10⁴ to <10⁵ CFU/g not acceptable and 10⁵ CFU/g can be said to be extremely hazardous for public health. Antimicrobial sensitivity test result showed that all of the sample harbor multidrug resistant food borne bacteria which might cause public health hazards if these antibiotic resistant transfer to human. Hence, it is recommended that a more close supervision of such food type should be carried out by relevant authorities to avoid any future pathogen outbreaks.

CONCLUSION

The recent study was conducted for the isolation and identification of food borne bacteria from spicy puffed rice study of their antibiotic sensitivity pattern. Among 20 spicy puffed rice (jhalmuri) samples, all (100%) had bacterial contamination. TVC in spicy puffed rice sample was ranged from 8.4x10³ CFU/g to 3.24x10⁷ CFU/g. From the spicy puffed rice Pseudomonas aeruginosa (37.5%), Staphylococcus aureus (25%), E. coli (19.5%) Bacillus cereus (13%) and, Bacillus subtilis (5%) were isolated and identified. Presence of coliforms in the sample might be due to poor quality of water, unhygienic vendor places and poor personal hygiene of vendors. Antibiotic sensitivity test showed that Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa were sensitive to Chloramphenicol (60%) and Ciprofloxacin (60%) but the Bacillus cereus and E. coli were moderately sensitive. Bacillus subtilis, Bacillus cereus were moderately sensitive to Kanamycin (40%) but the Staphylococcus aureus and E. coli were resistant. All isolates were found 100% resistant to Cefixime, Cephalexin, Neomycin, Amoxycillin and Ceftriaxone.

The results of this study suggested that street vended RTE foods should be manufactured under good hygienic practices and conservation practices should be developed in order to minimize the microbial contamination of food. Moreover, it is recommended to develop an appropriate hazard analysis critical control point (HACCP) system, and a more close supervision of RTE food should be carried out by relevant authorities to enhance food safety.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

No conflict of interest.

AUTHORS’ CONTRIBUTION

Please clearly mention the contribution of each author. Check the style here-
MIR designed the study, conducted the experiments and analyzed data. MKH designed the study, analyzed data and prepared the manuscript. MDB, NAR, MF and MRI prepared the manuscript.

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