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Evaluation of bacterial load in traditional fast-foods of Rajshahi City and Characterization of isolated bacteria

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Abstract: Load of viable bacteria in traditional fast foods and characterization of bacterial isolates were studied. A total of five fast food samples were randomly collected from different restaurants of Rajshahi City. Food samples were examined for bacterial viable count and cultured in nutrient agar media at 37^0 C for 24 hours and then the bacteria were isolated. The bacteria were examined microscopically for their morphological, physiological and biochemical characteristics. Results showed that the isolated bacterial specimens belonged to *Escherichia coli*, *Campylobacter* sp. and *Bacilli* sp. Colonies of all the samples were then counted separately on nutrient agar media. Experiments for total viable counts (TVC) of bacteria were replicated three times per food. Load of viable bacteria in chicken sandwiches, beef burger, vegetables roll, chicken hotdog and viscera were 96×10^9 , 162×10^9, 369×10^9, 158×10^9, 450×10^9 cfu/ml (colony forming units per millilitre), respectively. The highest number of bacterial count was recorded in the fast food viscera (450×10^9 cfu/ml). In beef burger, vegetable roll and viscera, *E. coli*, in chicken hotdog, *Campylobacter* sp. and in chicken sandwiches, *Bacilli* sp. were identified. The presence of the high load of viable bacteria in the fast-foods was indicative of contamination which reinforces the need for effective control measures, hygiene in processing and handling of the foods.

Key words: Bacterial load, viable count, fast-foods

Introduction

Food is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth, several factors encourage, prevent, or limit the growth of microorganisms in foods, and the most important are water availability, pH and temperature (Smith & Fratamico, 1995).

The busy and neetic life schedule has opened the way for the fast food industry in most parts of the world. The traditional or conventional way of cooking is over and the fast food joints are eligible everywhere. Fast food does not only include the traditional fast it also includes Chinese as well as Indian. Consumption of contaminated ready to eat foods including red meat, eggs, cheese and vegetables have been documented to serve as vehicles for transmission of several bacterial pathogens and food-borne outbreaks (Borch & Arinder, 2002).

Hot foods have been the source of outbreaks of *Staphylococcus aureus, Clostridium perfringens and Salmonella enteritidis* (Hatakka, 1998).

The main sources of pathogenic bacteria in food are contaminated raw food, food handlers, dust, water, utensils and insects (Ray, 1996). Microorganisms in fast and traditional fast foods are responsible for many human diseases, e.g. *Salmonella* bacteria is a common cause of food borne illness, particularly in undercooked chicken and chicken eggs (Kaneko *et al*., 1999; Angelillo *et. al*., 2000).

Pathogenic microorganisms on raw vegetables and fruits suggested that the use of poor quality water for irrigation could increase the incidence of enteric pathogens (*E. coli*), *Aeromonas, Serratia spp.* and some gram negative bacteria, *Pseudomonas, Clostridium* and *Xanthomonas* and also *Lactobacillus* Spp., *Streptococcus, Micrococcus* spp., as gram positive bacteria. The consumption of fast foods, raw milk and raw milk products have been reported to be associated with serious health problems (Food and Drug Administration-FDA), 2000 and Pelezar *et al*., 2006).

Unsafe food represents a major threat to public health in Bangladesh. Each year millions of citizens suffer bouts of illness following the consumption of unsafe food. Aside from acute effects arising from food contaminated by microbial Pathogens, long term adverse health impacts may occur following consumption of food tainted by

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chemicals substances and toxins (Mahoney, 2010).

Each year millions of Bangladesh citizens experience bouts of diarrhoea, and the prevailing view is that the majority of cases are food and water borne. A significant proportion of the burden of diarrhoea is caused by the consumption of contaminated food or though poor hygienic practices employed by food handlers. Diseases such as cholera and typhoid fever are frequently transmitted via food, along with illnesses due to nontyphoidal *Salmonella* sp., *Campylobacter* sp. and enterotoxigenic *E. coli* etc. The objectives of the study were to isolate the bacteria and bacterial viable count from fast foods of Rajshahi city and also were to determine the presence of pathogenic bacteria in traditional fast foods causing human disease. This study carried out to give information about the methods of preventions of disease due to food borne pathogen.

Materials and Methods

Collection of samples

A total of five fast food samples *viz*., chicken sandwiches, beef barger, vegetables roll, chicken hotdog and viscera were randomly collected from different restaurants of Rajshahi City and taken immediately to the laboratory of Genetics and Molecular Biology for bacteriological study.

Isolation of bacteria from food samples

One gm each food samples were taken separately into nutrient broths which were incubated at 37^0C for 2 days with shaking at 120rpm. Control flasks without inoculates were incubated at 37^0C with shaking an orbital shaker. After 2 days cultures were found to be turbid and then it was used as inocula for further experiments.

Microscopic examination and identification of bacterial cells

For the identification of the organisms, morphological characterization, microscopic observation, growth characteristics, biochemical tests were performed. The microorganisms were identified according to Bergey's Manual of Systematic Bacteriology.

Bacterial viable count of food samples

The viable count was done according to (Quinn *et al*., 2000) using method of Miles-Misra. One gm food samples for each were taken and 10 ml distilled water was poured into the test tube and mixed with the samples. The mixed food samples were serially diluted 9 times (10^{-1}-10^{-9}). Sterile distilled water was added to each test tube to make the volume of each preparation 10ml. Preparations were homogenized. One ml food sample was taken from each of the above homogenized preparations and added separately in different test tubes containing 9ml sterile distilled water to make 9-fold serial dilutions of each preparation. Preparations were homogenized in every steps of serial dilution. Serial dilutions were made of up to 10^9 dilutions. A 0.1ml aliquot of the seventh (10^7), eight (10^8), and nine (10^9) dilutions were each inoculated in triplicates by the spread plate technique on nutrient agar plate. Then, inoculated petriplate were incubated at 37°C for 24 hours. Thus, serial dilutions of other samples were done by the same way and incubated overnight at 37°C. After 24 hours of incubation the bacteria of different samples were grown and formed many colonies to the NA media. Then these colonies were counted and recorded respectively.

The plates were labeled by the number of the dilution. The number of bacteria per ml of original sample was calculated by multiplying the number of colonies counted by dilution factor. The formula used for counting was (*The total number of bacteria per ml= numbers of colonies count* × *dilution factor*).

Results

Isolation of bacteria

Bacteria were isolated separately from five types of traditional fast foods *viz*., chicken sandwiches, beef burger, vegetables roll, chicken hotdog and viscera by plating of previous broth cultured onto an agar solidified nutrient medium. The plates were incubated at 37°C for 24 hours and bacterial colonies were found to grow on the medium. The bacterial specimens belonged to *Escherichia coli*, *Campylobacter* sp. and *Bacilli* sp. are presented in Table 1.

Microscopic observation of the bacterial strain

Microscopic observation of the bacterial strain was done after simple and gram staining and the results are given in the Table 2. However, sugar utilization and biochemical tests of the bacteria are presented in table 3 respectively.

Table 1. Presence or absence of bacterial species isolated from different poultry food samples.

Fast food samples	*Bacilli*	*E. coli*	*Campylobacter* sp.
Sample 1 (Chicken sandwiches)	+	-	-
Sample 2 (Beef burger)	-	+	-
Sample 3 (Vegetable roll)	-	+	-
Sample 4 (Chicken hotdog)	-	-	+
Sample 5 (Viscera)	-	+	-

Table 2. Microscopic observation of the isolated bacterial strain.

Characters	*Bacilli*	*E. coli*	*Campylobacter* sp.
Microscopic observation	Gram positive	Gram negative	Gram negative
Shape	Rod shaped	Rod shaped	Spiral shaped
Motility	Motile	Motile	Motile

Table 3. Sugar utilization and biochemical activities of different isolates from different fast foods

Test parameters	*Bacillus*	*E. coli*	*Campylobacter sp.*
Indole production	-	+	+
Methyl red	+	+	-
Voges-proskaure(VP)	+	-	-
Simon citrate	+	-	-
Motility	+	+	+
Oxidase	+	-	+
Catalase	-	-	-
TSI	-	-	-
Nitrate	-	-	-
Mac Conkey agar	+	+	+
Sugar utilization	*Bacillus*	*E. coli*	*Campylobacter sp.*
Glucose	+	+	+
Maltose	+	+	-
Lactose	+	+	-
Sucrose	-	+	-
Galactose	+	-	-
Xylose	+	+	-
Fructose	+	+	-

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The '+' signs indicate the growth of the microorganisms

Load of viable bacteria in fast foods

The bacterial viable count of traditional fast foods from different restaurants were 96×10^9, 162×10^9, 369×10^9, 158×10^9, 450×10^9 (cfu/ml) for chicken sandwiches, beef barger, vegetables roll, chicken hotdog and viscera respectively (Table 4). The highest number of bacterial counts was recorded in the fast food viscera (450×10^9 cfu/ml) (Fig. 1).

Table 4. Bacterial viable count of fast foods

Name of the traditional fast foods	Dilution no.	Colony forming unit (cfu)/ml	No. of bacteria
Chicken sandwiches	10^7	130	130×10^7
	10^8	115	115×10^8
	10^9	96	96×10^9
Beef barger	10^7	198	198×10^7
	10^8	185	185×10^8
	10^9	162	162×10^9
Vegetable roll	10^7	405	405×10^7
	10^8	389	389×10^8
	10^9	369	369×10^9
Hot dog	10^7	198	198×10^7
	10^8	180	180×10^8
	10^9	158	158×10^9
Viscera	10^7	589	589×10^7
	10^8	559	559×10^8
	10^9	450	450×10^9

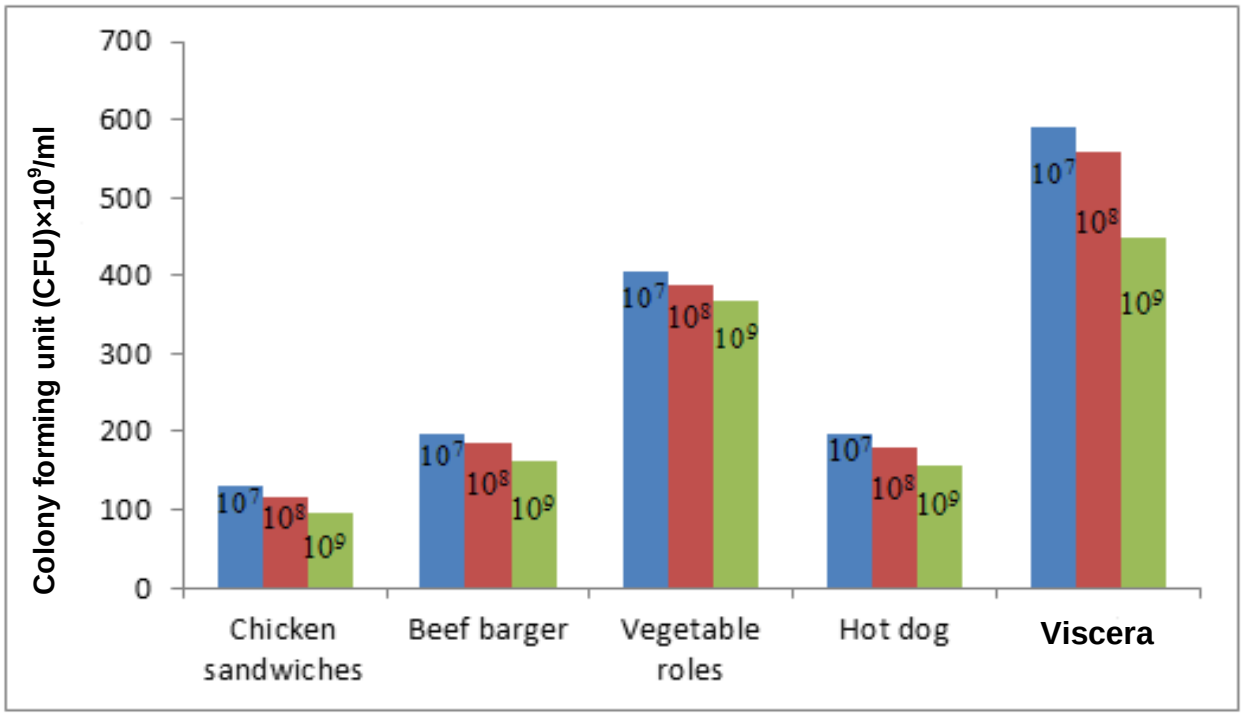

Fig. 1. Bacterial viable count of fast foods

Discussion

The busy nature of people all over the world has increased the patronage of restaurants, cafeteria and fast food centers. In Bangladesh, beef burger, vegetable roll, chicken hotdog, chicken sand wiches, chicken sharma are the prevalent food and soup respectively in most confectionary and restaurants and are present in the menu in various forms.

Food borne diseases are important causes of morbidity and mortality worldwide. It is estimated that food borne diseases cause approximately 76 million illness, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead *et al*., 1999). People's increased traveling and free movement of food stuffs has increased the risk of contracting food poisoning (Korkeala & Lindstrom, 2009).

In Bangladesh, diarrhea is responsible for one third of childhood deaths. It is estimated that about 230,000 children die from diarrhea each year (Piechulek *et al*., 2003).

The present study was designed to describe the microbiological quality of traditional fast foods based on bacteriological isolation and total viable count. Nutrient agar media were used for the isolation of bacteria. The isolated bacteria were *Escherichia coli*, *Campylobacter* sp. and *Bacilli* species. The viable counts found were 96×10^9, 162×10^9, 369×10^9, 158×10^9, 450×10^9 (cfu/ml) for chicken sandwiches, beef burger, vegetables roll, chicken hotdog and viscera respectively. Harakeh *et al*. (2005) reported the isolation of *E. coli* and *Salmonella* isolates from meat-based fast food in Lebanon. Shahram *et al*. (2012) showed that *E. coli* (40.3%) was the most prevalent food-borne pathogen isolated from sandwich in Kerman City, Iran. Abdalhamid *et al.* (2013) reported that the *Enterobacter aerogenes*, *E. coli*, *Ent. cloacae*, *Klebsiella freundii*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* were isolated from Shawerma sandwiches in Misurata City, Libya.

Salmonella spp. was isolated from sandwich and these sandwich fast foods in Iran could be a public health hazard, as they may act as a potential vehicle for many antimicrobial-resistant pathogenic organisms (Saha *et al.,* 2009). Improper hygienic standards and indiscriminate use of antimicrobials are two of the main causes for the prevalence of these pathogenic resistance strains in Iran (Meldrum & Smith, 2007).

E. coli is the most common cause of food and water-borne human diarrhea worldwide. In developing countries causing it is 800000 deaths out of 650 million cases per year primarily in children under the age of five years (Turner *et al*., 2006).

Pathogens of fecal, nose or throat, and skin origin are most likely to be transmitted by the hands, highlighting the need for effective hand hygiene and other barriers to pathogen contamination, such as no bare hand contact with ready-to-eat food. The pathogens most likely to be transmitted by food workers are norovirus, hepatitis A virus, *Salmonella*, *Shigella*, and *S. aureus* (Todd *et al*., 2008).

E. coli and *Salmonella* species have marked importance in foodborne diseases and the worldwide emergence of resistant or multi-drug resistant strains of these two bacteria (Korkeala and Lindstrom, 2009).

Bichai *et al*. (2008) showed that, the presence of *E. coli* can be related to use of polluted irrigation waters during growth. Contamination is occurred through human handling, the use of contaminated containers, or washing after harvest with polluted water. It was suggested that it could increase the incidence of enteric pathogens (Angelillo *et al*., 2000). Thus products fresh or processed vegetables are the chapped salad ingredients (cabbage, carrots, tomato, cucumber etc.) sold in the grocery store and to the institutional trade (Kaneko *et al*., 1999).

Richard *et al*. (2007) reported that some pathogenic bacteria cause sick for human when eat fast foods these bacteria such as: *Listeria monocytogenes* has been associated with such foods as raw milk, pasteurize fluid milk, raw vegetables, fermented raw meat sausages, raw and cooked poultry, raw meat and raw and smoked fish. Its ability to grow at temperature as low as 0^0 C permits multiplication in refrigerated foods. In refrigeration temperature such as 4^0C the amount of ferric iron promotes the growth of *L. monocytogenes* (Dharmarha & Vaishali, 2009).

Richard *et al*. (2007) also showed that gastrointestinal disease has been reported by eating raw or inadequately cooked meat containing bacillus spores. *B. cereus* causes food poisoning by means of enterotoxins, reported that the prevention, because of the resistance of endospores to chemical disinfectants, autoclaving is the only reliable means of decontamination. *B. subtilis*, *B. coagulans* were isolated from traditional fast food samples in Qassium, Saudi Arabia.

Campylobacter jejuni is widely distributed in nature, its infects the intestine, where it can cause ulcerative, inflammatory lesions in the jejunum, ileum, or colon. It is the leading cause of food borne disease. Prevention by good hygiene avoiding contaminated water, pasteurizing milk and milk products and thoroughly cooking potentially contaminated food (eg. poultry). Saadia (2010) isolated *C. jejuni* from fast foods samples of chicken shawarmas.

Obeta & Abriba (2004) isolated *Bacillus* sp., *Kiebsiella aerogenes*, *K. pneumonia, Lactobacillus* sp. and *Micrococcus* from egusi soup. Bess *et al.* (2009) reported that food handlers in ready-to-eat centers had no formal training on food safety practices and prevention of food borne diseases. Due to minimal or no adherence and the indiscriminate establishment of restaurants in most developing and countries, there is need to examine the microbiological quality of some of the foods in the Restaurants from time to time. Unsafe practices, in food processing can lead to foodborne diseases such as typhoid fever when contaminated food is consumed.

Conclusion

The results of this investigation indicate that *Escherichia coli* was the most prevalent food-borne pathogen isolated from ready to eat fast food samples *viz*. beef burger, vegetable roll and viscera collected from different restaurants of Rajshahi City were of poor sanitation quality and may pose a considerable risk to human health. Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing and handling or preparation. Good hygienic practices, proper handlings, storage and clean environment will be applied to reduce the levels of food contamination. Food poisoning also can be controlled by the adjustment of P^H, water activity, temperature control. In conclusion, using of high quality raw materials, efficient heat treatment, adequate cleaning and sanitization of utensils day-by-day observance of proper personal, food handling of cooked food and lastly adequate education of food hygiene should be done. Also, strict hygienic measures should be applied during preparation of ready to fast food to improve the quality of the product and to safeguard the consumers.

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