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DETERMINATION OF MICROBIOLOGICAL (Staphylococci) QUALITY OF FAST FOOD SOLD IN THE DIFFERENT RESTAURANT IN SYLHET SADAR

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ABSTRACT

Fast food monger and restaurants premises are more rapidly growing in Sylhet city. This may cause serious health concern due to unhygienic environment are noticeable almost all types of fast food vendors and restaurants. Hence, the study was undertaken to assess a total viable bacterial count and especially emphasis given on total staphylococcal count of fast food items from various restaurants in and around Sylhet town. From January to February 2016, a total of 45 samples were collected and assessed of five fast food items (Singara, Shamucha, Chicken burger, Chicken roll and cake) belongs to three types of restaurants (Street, mid-level and high level). Assessment revealed, the highest mean value of TVC (Total Variable Count) was found in Shamucha (89.6×10^9 CFU/g.) nearly all types of restaurants whereas lowest was observed in Chicken burger (58.8×10^9 CFU/g.). However, Staphylococcal load (mean value of TSC) was seen highest in both Shamucha and Chicken burger (26×10^3 CFU/g.) and lowest were in cake (16×10^3 CFU/g.) of all types of restaurants. Street level restaurants were observed highly risk for microbial as well as Staphylococcal load when comparison with mid-level and high level restaurants. Based on International Microbiological Criteria, the total viable count and total staphylococcal count found in fast food samples were unsatisfactory of all types of restaurants. Therefore, this study recommends that further analysis is needed regarding this issue. Besides, necessary steps should be taken by the Government for maintaining hygienic standard for preparing, processing and handling cooked food in various restaurants.

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INTRODUCTION

The term "fast food" was appeared in a dictionary by Merriam–Webster in 1951 (Harun et al., 2013). Fast food is ready made in nature and easy to eat (Tabassum and Rahman, 2012). People eat away from home whiles travelling, studying, at work. Basically, the main consumers of street foods were fellow hawkers and hustlers and casual wage laborers, importantly children and students, office workers, and housewives (Amissah and Owusu, 2012). Besides, fast-food has become a growing trend amongst the upper class society, teenagers and adults alike (Harun et al., 2013). Thus, fast food industry is a high growing sector of Bangladesh ((Islam and Ullah, 2010) . Usually non-homemade foods sold by street vendors and restaurant premises are unhygienic for human consumption has become globally health problem (Afzal, 2014, Khater et al., 2013).People may suffer a lot due to consumption of this type of unhygienic foods. Microbial food borne illness is major health problem associated with street foods. Most studies on street foods concluded that it could be harmful to health due to presence of harmful pathogenic microorganisms with their toxins in foods. The common reason for the unacceptable microbiological quality was unhygienic food cooking and handling practices. Handling of foods with bare handed, use of contaminated water for hand and dish washing by food handlers and under lighting preservation of food for long time in display glass boxes with elevated temperatures (up to 55-60°c) and storage of uncooked and half cooked meat items side by side in the refrigerator were possible microbiological contamination of the fast food items (Faruk and Akhter, 2011). Considering the aforementioned facts, this study is divided the restaurants on (basis of economic status of the consumer) street, mid-level and upper level and has conducted to observe the microbial load (total viable count and also total *Staplylococcal* count) of non-home foods from street and restaurants. Food items were selected on the basis of daily consumption of people and their availability. Five food items were selected such as Singara, shamucha, burger, Chicken roll and Cake. These food items were randomly collected from street, mid and high level restaurants of Sylhet sadar upazila.

MATERIALS AND METHODS

A total of 45 fast food samples (9 samples each of Chicken burger, Chicken roll, cake, Singara and Shamucha) were collected that were sold in street level, mid-level and high-level restaurants in Sylhet sadar during January to February, 2016. All samples were collected aseptically and kept in secure box. Due aseptic care was taken during transportation and the samples were kept cool and labeled with proper identification no. and then transported in an icebox to laboratory of the Department of Microbiology and Immunology, Sylhet Agricultural University, Sylhet for bacteriological analysis.

A quantity of 10 g sample of each fast food sample was aseptically transferred into a sterile pestle containing 90 ml 0.1% peptone water. A homogenized suspension (1:10) was made. Later on using whirl mixture machine 10 fold serial dilutions ranging from 10^{-1} to 10^{-5} were prepared (ISO, 1995) and enriched for 24 hours at 37^0C.

Enumeration of Total Viable Count (TVC)

One ml of each ten-fold dilution was transferred and spread duplicate onto PCA (Plate Count Agar) using micropipette. The inoculated samples were spread on to the entire surface of the agar plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were kept in an incubator at 37°C to for 24-48 hrs. After incubation, plates exhibiting 30-300 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 the method of ISO (1995). The results of the total bacterial count- expressed as mean log Colony Forming Unit (CFU) per gram of sample.

Enumeration of Total Staphylococcal Count (TSC)

In case of Staphylococcal count, Mannitol salt (MS) agar was used. Protocols used in this method were similar to that of TVC.

Table 1. Bacterial status in various fast foods in different restaurants

Name of the sample	Types of restaurant	No. of the examined sample	TVC range (CFU/g.), Mean	TSC range (CFU/g.), Mean
Singara	Street Level	3	51×10^9 -96×10^9,77.3×10^9	11×10^3-23×10^3, 18.3×10^3
	Mid-Level	3	45×10^9 -80×10^9, 64.2×10^9	13×10^3-29×10^3, 20.6×10^3
	High Level	3	30×10^9 -57×10^9,45.3×10^9	8×10^3-20×10^3, 15.3×10^3
Total		9	30×10^9 -96×10^9, 62.4×10^9	8×10^3-29×10^3, 18.1×10^3
Shamucha	Street Level	3	95×10^9 - 111×10^9,103.6×10^9	30×10^3-38×10^3, 33×10^3
	Mid-Level	3	92×10^9 - 107×10^9,97.3×10^9	18×10^3-35×10^3, 27×10^3
	High Level	3	57×10^9 - 79×10^9,68×10^9	13×10^3-24×10^3, 18×10^3
Total		9	57×10^9 - 111×10^9,89.6×10^9	13×10^3-38×10^3, 26×10^3
Chicken burger	Street Level	3	55×10^9 - 75×10^9, 66.6×10^9	14×10^3-29×10^3, 21.6×10^3
	Mid-Level	3	45×10^9 - 70×10^9, 61.3×10^9	7×10^3-22×10^3, 14.6×10^3
	High Level	3	40×10^9 - 57×10^9, 48.6×10^9	11×10^3-17×10^3, 13.6×10^3
Total		9	40×10^9 - 75×10^9, 58.8×10^9	7×10^3-29×10^3, 26×10^3
Chicken Roll	Street Level	3	76×10^9 - 102×10^9, 91×10^9	23×10^3-48×10^3, 36.6×10^3
	Mid-Level	3	45×10^9 - 80×10^9, 64.6×10^9	15×10^3-27×10^3, 21.3×10^3
	High Level	3	55×10^9 - 65×10^9, 60.3×10^9	11×10^3-25×10^3, 19.6×10^3
Total		9	45×10^9 - 102×10^9, 72×10^9	11×10^3-48×10^3, 25.8×10^3
Cake	Street Level	3	54×10^9 - 96×10^9, 71.6×10^9	12×10^3-25×10^3, 18.6×10^3
	Mid-Level	3	55×10^9 - 70×10^9, 64.6×10^9	17×10^3-22×10^3, 19×10^3
	High Level	3	30×10^9 - 55×10^9, 41.3×10^9	5×10^3-14×10^3, 10.3×10^3
Total		9	30×10^9 - 96×10^9, 59.2×10^9	5×10^3-25×10^3, 16×10^3

Isolation and Identification of Staphyloococus:

The collected samples were separately inoculated for enrichment in the Nutrient broth with aseptically at least 24 hours of incubation at 37° C, revealed bacterial growth as turbidity. One or two loop of broth culture was streaked in the Nutrient agar plate aseptically with 24 hours of incubation at 37° C. Characteristics colonies were considered by the growth of circular, smooth, opaque, translucent colonies. All samples were inoculated into Mannitol salt (MS) agar for *Staphylococcus* and incubated at 37 °C for 24 hrs. Bacterial growths were recovered from samples inoculated onto MS agar where small golden yellow Mannitol fermenting colonies were considered characteristics colonies. Grape-like clusters when viewed under microscope and has large, round, golden-yellow colonies, with beta hemolysis were considered in blood agar for *Staphylococcus.* The pure culture were streaked on nutrient agar and incubated at 37ºC for 24 hours were further characterized for biochemical tests.

Morphological Characteristics

On clean grease free microscopic glass slide, a thin smear was prepared from the isolate culture and carried out Gram's Staining method. The stained slide was observed under microscope with emulsion oil revealed gram positive, spherical, grape like clustered cells.

Biochemical examination

Biochemical tests were performed to confirm *Staphylococcus* by using sugar fermentation (Dextrose, maltose, lactose, sucrose and mannitol), catalase and coagulase tests.

RESULTS

Analysis of this study depicts that the highest mean value of TVC (Total Variable Count) (CFC/g) was found in Shamucha (89.6×10^9 CFU/g.) nearly all types of restaurants whereas lowest was observed in Chicken burger (58.8×10^9 CFU/g.). But staphylococcal load (mean value of TSC) was seen highest in both Shamucha and Chicken burger (26×10^3 CFU/g.) and lowest were in cake (16×10^3 CFU/g.) of all types of restaurants. Street level restaurants were observed highly risk for microbial as well as Staphylococcal load when comparison with mid-level and high level restaurants. Morphological characteristics, culture on media and biochemical tests were used for detection of *Staphylococcal* contamination in fast food items. Results found from morphological characteristic, cultural characteristics and biochemical tests were interpreted in table 2 and table 3.

Table 2. Morphological and Cultural characteristics of *Staphylococcus*

Bacterial isolate	Gram's staining Method	Culture characteristics on selective medium
Staphylococcus	Gram positive, grape like clustered, spherical cells	**MS agar:** Small golden yellow mannitol fermenting colonies. **Blood Agar**: Beta-hemolysis, lightened (yellow) and transparent. **Nutrient Agar**: Growth of circular, smooth, opaque, translucent colonies.

Table 3. Biochemical characteristics of *Staphylococcus*

Biochemical tests		Reaction
Sugar Fermentation tests	Dextrose	Positive
	Maltose	Positive
	Lactose	Positive
	Sucrose	Positive
	Mannitol	Positive
Catalase		Positive
Coagulase		Positive

DISCUSSION

A total of 45 fast food sample were collected from 3 different types of restaurants (street level, mid-level and high-level) in Sylhet metropolitan area and carried aseptically to the laboratory. The result was expressed in CFU/gm of sample. The range and mean value of TVC and TSC of examined fast food sample were showed in Table 8. From that table we can find that, the lowest mean of TVC was found in chicken burger (58.8×10^9) and highest mean is found in samucha (89.6×10^9). And highest mean value of TSC was found in Shamucha and Chicken burger (26×10^3). Lowest mean value of TSC was found in cake (16×10^3). Based on International Microbiological Criteria (Food, 2003), the total variable count and total staphylococcal count found in fast food samples are unsatisfactory. Street level restaurants were highly contributed with microbial load and staphylococcal load than mid- level and high level restaurants.

Street foods contribution to dietary intake are very little, surveyed by (FAO, 2006) . Basically, the main consumers of street foods were fellow hawkers and hustlers and casual wage laborers, importantly children and students, office workers, and housewives (Khairuzzaman et al., 2014 and FAO, 2010). Most of the street level restaurants are situated nearby contaminated areas. The workers are not conscious and care enough to maintain the hygienic condition. Uncovering ready to eat food stuffs, spoiled and back dated materials use to preparation fast food may be important cause for contamination.

In Bangladesh, processing system of food stuffs is unsafe for consumption. The unhygienic and unsafe treatment of food is seriously impacting public health by causing numerous chronic and non-chronic diseases (Ali, 2013). The considerable numbers of illnesses are transmitted by food worker (Ross and Guzewich, 1999). Hand-contact is considered the most important vehicle for transfer of micro-organisms. In developing country, therefore, public health risks are associated with the consumption of street food (Ackah et al., 2011). The primary limitation of this study was the relatively small sample size. A larger sample size would provide a more complete representation of the bacteriological quality of fast foods in local food establishments. There are undoubtedly differences in the chemical properties of examined food items that inhibit or promote bacterial growth; however, this research study did not address these differences. Alternatively, the use of culture methods in this study may have resulted in an underestimation of the number of CFU/ml enumerated. Culture methods provide valuable information on the microbial quality of the samples collected; however, unlike Polymerase Chain Reaction (PCR) methods, this method is limited to the enumeration of healthy, non-injured viable microorganisms. Injured organisms are likely not included in the counts of the enumerated colonies by culture. In addition, the filtration method used in this study could cause additional stress and injury to the microorganisms within the samples, which could result in an underestimation of the bacterial contamination. This is an important factor because although injured pathogenic microorganisms exhibit a temporary decrease or loss in virulence, they can recover from injury in which virulence can be completely restored.

CONCLUSION

From the assessment of total variable bacterial count and staphylococcal count from collected sample interprets that fast food samples sold at various types restaurants are unhygienic and contaminated. These findings concern serious potential health problems for the people who take mentioned types of fast food from street vendors and restaurants. The study also suggests the Government for implementation of rules and regulationto the food maker and restaurants premises to maintain hygienic procedure strictly.

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