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Research Article

Analysis of Bacteriological quality of street-vended ready-to-eat (RTE) foods sold in Selected Towns of Sirajganj, Bangladesh

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ABSTRACT:

Background: ***Ready-to-eat foods are those that are ordinarily consumed in the same state as they are sold, and these do not include nuts, raw fruits, or vegetables that are intended for hulling, shedding, or washing by the consumer.*** *Objective:* ***The specific objectives of microbiological analysis of ready-to-eat foods include determining the susceptibility patterns of the isolates obtained from RTE foods against antibiotics using the disc diffusion method.*** *Method:* ***A prospective cross-sectional study is done on 39 street food samples. A culture of the samples collected was performed, and the organisms isolated were thus identified using standard biochemical methods. Finally, antibiotic sensitivity was tested against conventionally used antibiotics by the Kirby-Bauer disc diffusion method. The data were analyzed using Microsoft Excel 20.*** *Results:* ***Out of 39 street RTE-food samples, 24 (61.5%) samples showed positive growth, and 15 (38.5%) samples showed negative growth. Among samples with positive growth, samples of shingara, vegetable roll, dal-puri, shamocha, and burger were 7 (29.0%), 6 (25.0%), 5 (21.0%), 3 (13.0%), and 3 (13.0%), respectively. Bacterial contaminants were*** **Serratia spp. *8 (33.0%), followed by* E. coli 5 (21.0%), Pseudomonas spp. 5 *(21.0%),* Staphylococcus aureus 3 (13.0%), and Enterobacter spp. 3 (13.0%).** ***Most of the isolates were found to be 100% resistant to amoxicillin, but*** **E. coli *is only 80% resistant.* The bacterial pathogens Pseudomonas spp., Serratia spp., Staphylococcus aureus, and Enterobacter spp. were 100% sensitive to gentamycin and ciprofloxacin.** *Conclusion:* ***The bacterial load of ready-to-eat foods sold within Enayetpur town could pose a substantial risk to its consumers.***

Keywords: Ready-to-eat foods, quality, risk, Enayetpur town, bacterial isolates, antimicrobial patterns

1. INTRODUCTION:

The consumption of ready-to-eat foods is increasing in Bangladesh these days. Consumer desire for easy foods is reflected in ready-to-eat foods, and they seek RTE foods that are fresh, healthy, safe, additive-free, and nutritional (Fang, 2005). Beef kofta, beef burgers, hotdogs, burgers, and other RTE sandwiches are the most popular among street sellers and fast-food establishments. Microorganisms can easily contaminate meat products, resulting in a loss of quality and potential public health issues (Vernozy-Rozand, 2002). Certain spices may cause a significant rise in the bacterial population during manufacturing (Sharaf, 1999).

The presence of *Staphlococcus aureus* in heat-treated food is a sign of poor personal hygiene, incorrect storage, and unsanitary conditions (Achi & Madubuike, 2007). Other research has shown that the *Enterobacteriaceae* family has epidemiological significance since some of its members are dangerous and can cause serious illnesses and food poisoning (Mercuri *et al.*, 1978).

These ready-to-eat products are mainly roasted and fried; they are locally called shingara, shamucha, vegetable rolls, vegetable pakora, puri, burgers, sandwiches, etc. These ready-to-eat products are prepared without further processing or preparation for immediate consumption and are sold on the street. This has raised many concerns because the conditions under which street vendors operate are generally unsuitable for preparation and sale. Ready-to-eat foods are generally not effectively protected from dust and flies that cause foodborne pathogens and find it difficult to maintain safe food storage temperatures (Sabuj *et al.*, 2020).

Thus, there are potential health risks associated with pathogenic bacteria as well as primary contamination of raw foods. Subsequent contamination by vendors through handling and cross-contamination occurs during preparation and after cooking (Khater-Dalia et al.,2013). Food can be contaminated by various types of pathogenic bacteria, e.g., *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Enterobacter cloacae, Acinetobacter baumannii, Salmonella typhimurium, Vibrio cholerae, etc*. The purpose of this study is to investigate the safety and quality of street foods collected from several typical vendors in Sirajganj Sadar and Enayetpur City, Bangladesh.

2. MATERIALS AND METHODS

2.1 Collection of samples

Five locations in Enayetpur town were selected for collecting samples. Based on consumer demand, shingara, puri, shamucha, vegetable roll, and burger were selected for microbial analysis. All these samples were collected in a zipper bag and transported to the laboratory in an ice box. Samples were analyzed within two hours of procurement.

2.2 Sample processing

10 g of the sample was diluted with 90 ml of sterile distilled water and mixed well (up to 10^{-7} dilution). Sterile dilutions were prepared, then the spread plate technique was used for Plate Count Agar (PCA) media and Sabouraud Dextrose Agar (SDA) media, and the streaked plate technique was used on appropriate selective media.


Fig (a): Dal puri


Fig (b): Vegetable


Fig. (c): Singara


Fig (d): Shamucha

Figure 1: Different types of RTE street foods sold in Enayetpur town, Sirajganj district.

2.3 Serial dilutions

Each sample was weighed aseptically in a sterile stomacher bag and homogenized for 2 minutes with 225 mL of buffered peptone water. Unless otherwise specified for specific determinations, the mixture was maintained at room temperature for 1 hour before analysis. Before analysis, the homogenized samples will be serially diluted (from 10^{-1} to 10^{-9}).

2.4 Culturing and Microbiological Analysis

Aerobic plate counts were performed on plate count agar using the spread plate method, then incubated at 37°C for 24 hours; colonies were measured in cfu/g (Mehmet & Aydin, 2008). On violet-red bile agar, total coliforms were counted (Viswanathan &Randhir, 2001). *Staphylococcus aureus* was isolated and counted by pouring serial dilutions onto blood agar plates, followed by a coagulase test to see if any coagulase-positive staphylococci were present (Hasan et al., 2006.). The gram-negative enteric microorganisms that digest lactose were identified and researched. The number of viable cells/g in the original sample was calculated. Chosen the plate containing between 30 and 300 colonies. Multiply the number of colonies on the plate by the final dilution factor. This gives the total viable cells/g in the original sample. Well-isolated colonies were subjected to Gram staining and biochemical characterization. Blood agar was used to identify gram-positive bacteria detected by gram staining as well as catalase (Al-Mohizea, 1996).

Formula of TVC = Total number of bacterial colonies x dilution factor/ amount of samples

2.5 Antibiotic sensitivity of the isolated pathogens

The pathogenic bacterial isolates found in the samples will be subjected to the determination of their sensitivity patterns towards some antibiotic drugs that are commonly used. About 10 antibiotics such as Amoxicillin (30 µg), Gentamicin (10µg), Ceftriaxone (3 µg), Nalidixic acid (30 µg), and Ciprofloxacin (CIP 30 µg) were selected for the antibiotic sensitivity test following the Kirby-Bauer method.

2.6 Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 26.0 software will be used for the statistical analysis of the data.

3. RESULTS AND DISCUSSION

Environmental Analysis of Samples

Five different types of RTE food samples were used in this research, and a total of thirty-nine foods were sampled from twenty-five vendors. A minimum of three samples for each food type were obtained from the vendors. A total of thirty-nine samples were obtained during the day from the roadside street food vendors in Enayetpur town, Sirajganj, between the periods of August 2022 and April 2023. 28 (72.0%) of the food samples, i.e., dal-puri, shingara, and shomucha, were left open to the environment, and only 11 (28.0%) of the samples, namely burgers and vegetable rolls, were sold under hot temperature conditions (**Table 1**).

Table 1: RTE foods samples from different environmental sources.

RTE foods	Individual number	Environment	Total number	Frequency (%)
Puri	8	Open environment	28	72
Shamocha	8			
Shingara	12			
Vegatable roll	6	Hot environment	11	28
Burger	5			
Total	39		39	100%

Enumeration of Bacterial Species

A total of 24 bacteria from five different species were isolated during the research. The five species include *Pseudomonas spp,* (5) *Serratia spp. (*8), *E. coli* (5), *Staphylococcus aureus* (3), and *Enterobacter spp*. (3). (**Table 2**). Another study found the same result (Agbodaze *et al*., 2005) Thus, *Serratia marcescens* was the most predominant species isolated, accounting for 33.0% of the total number of bacteria isolated during the study, followed by *Pseudomonas spp.* and *E. coli as the* second most bacterial isolates, with each constituting 21.0% of the total number of bacteria isolated during the study but Sharma, JA, Mazumdar 2014 found *E. coli* was the most prevalent bacteria.

(a) Different bacterial growth on UTI agar

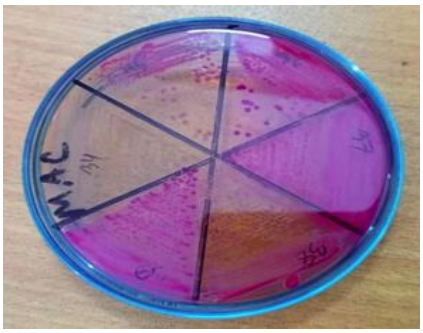

Fig (b): Lactose fermenter and lactose non-fermenter bacterial growth on MacConkey agar

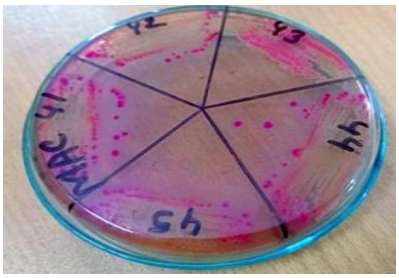

Fig (c): Lactose fermenter bacterial growth on MacConkey agar

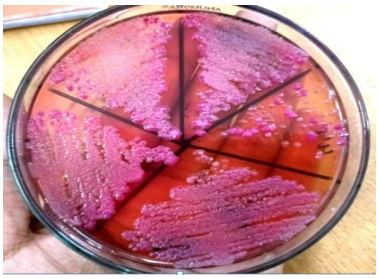

Fig (d): Lactose fermenter bacterial growth on MacConkey agar

Figure 2: Isolation of different pathogenic bacteria on selective medium

Table 2: Distribution of bacterial isolates among the RTE street foods samples

RTE food samples	*E. coli* (n=5)	*S. marcescens (n=8)*	*P. aeruginosa* (n=5)	S. aureus (n=5)	*Enterobacter spp.* (n=3)
Burger	2(40)	1(12.5)	0	0	0
Puri	2(40)	3(37.5)	0	0	0
Shamocha	0	0	2(40)	1(33)	0
Shingara	1(20)	0	3(60)	0	3(100)
Vegetable roll	0	4(50)	0	2(67)	0
Total	5(100)	8(100)	5(100)	3(100)	3(100)

Total Viable Bacterial loads

9 (23.0%) of the samples had Standard Plate Count (SPC) values within the acceptable limits (colonies count: 30-300 cfu/g), while 15 (38.0%) of the samples had counts that were above the acceptable limits (colonies count: > 300 cfu/g). On the other hand, 15 (38.0%) of the samples had too few counts of bacterial colonies within the acceptable limits (<30 cfu/g). No coliform from a fecal source was isolated during the study shown in **Table 3**.

Table 3: Summary of total bacterial counts:

Bacterial colonies	Count	Number	Frequency
<30 cfu /g	TFTC	15	38.0%
>300 cfu /g	TNTC	15	38.0%
30-300 cfu /g mL	Count in limit	9	23.0%
Total		39	100.0%

In this study, the total viable bacterial counts of shingara, shomocha, vegetable roll, and burger were 3×10^7cfu/g, $1.8x10^7$ cfu/g, $1.5x110^7$ cfu/g, $1.8x10^7$ cfu/g, and 1.1×10^7cfu/g, respectively, and the log10 were 7.5, 7.2, 7.1, 7.2, and 7.0, which were found in shingara, shomocha, vegetable roll, and burger in this study respectively. Almost similar result found in another study (Oluwafemi, F., et al, 2014) shown $1.1 \times 10^3 – 2.0 \times 10^5$. According to the 2013 FDA guideline, the acceptable limit is 5×10^4 CFU/g to 5×10^5 CFU/g in cases of total viable bacteria.

Fig (a): TVC plate: TNTC

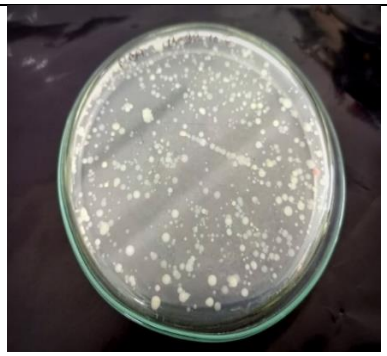
Fig (a): TVC plate: Normal count

Figure 3: Total Viable Count (TVC) Test on PCA Agar Plate

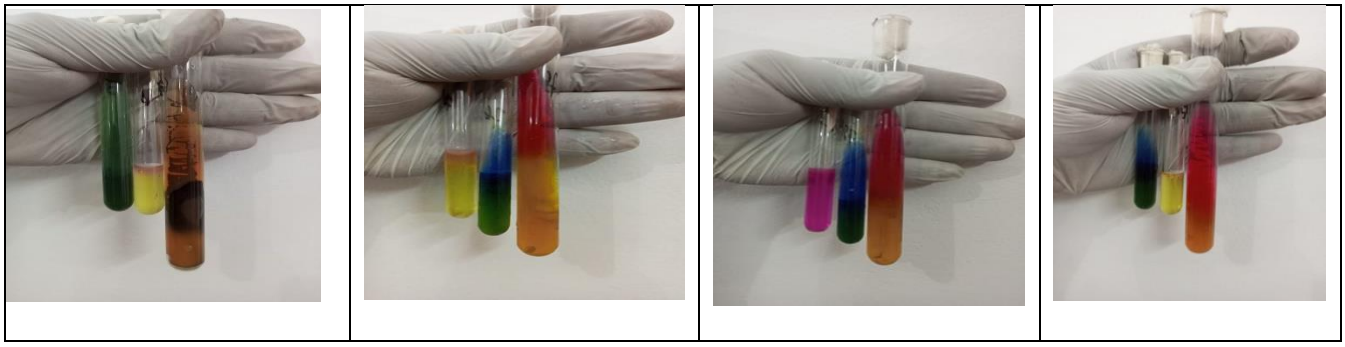

Figure 4: Identification of microorganisms by Biochemical Test

Susceptibility of isolated microorganisms

Antibiotic susceptibility tests for *Pseudomonas spp.*, *E. coli*, *Serratia spp.*, *Staphylococcus aureus*, and *Enterobacter sp.* by the Kirby-Bauer disc diffusion methods shown in **Figure 5**. It shows that all the isolated pathogens are sensitive to Ciprofloxacin and Gentamicin except *S. aureus* and all the isolates showed resistance against Amoxicillin except *E. coli* (**Table 4 and Figure 5**). Another study (Campos, Joana, et al, 2015) found *E. coli* (33.0 %) resistant against one or two more antibiotics.

E. coli was 80% sensitive to Ciprofloxacin and Gentamicin but 80% resistant to Amoxicillin opposite result was found where, *E. coli* was found as a multidrug-resistant (MDR) (Alelign, Dagninet, et al,2023). *Enterobacter cloacae* was most sensitive to Ceftriaxone (100%), Ciprofloxacin (100%), and Gentamycin (100%), and also resistant to Amoxicillin (100%), and Nalidixic acid (66.7%). Tabassum, Anika et.al (2018) also found *Enterobacter* cloacae as MDR. *Pseudomonas spp.* was highly sensitive to Gentamicin (100%) and Ciprofloxacin (100%), which is overwhelming the same result found in another study (Okhuebor, Shadrach Osalumhense et.al, 2018) where *Pseudomonas spp* is sensitive to gentamicin and ciprofloxacin and 100% resistant to amoxicillin. *Serratia spp. was* more effective and sensitive to Ciprofloxacin (100%), Gentamycin (100%), and Amoxicillin (100%), and Nalidixic Acid (60%) but different results were found by Akinyem, Kabiru Olusegun, et al (2013). *Staphylococcus aureus* was most sensitive to Ceftriaxone (100%) and gentamicin (100%), with similar results found where *Staphylococcus aureus* was sensitive to Gentamicin (Bello, Olorunjuwon O, 2013) and resistant to Amoxicillin (100%), and Nalidixic acid (66.7%).

Figure 5: Antibiotic susceptibility test of different pathogenic bacteria

Table 4: In-vitro antibiogram profiles of Gram-negative and Gram-positive bacterial isolates from RTE street food samples

Isolated bacteria	Patterns	AMX	GEN	CTR	NA	CIP
Pseudomonas spp (n=8)	S	0	8(100)	4(50)	7(87.5)	8(100)
	R	8(100)	0	5(50)	1(12.5)	0
E coli (n=5)	S	1(20)	4(80)	4(80)	2(40)	4(80)
	R	4(80)	1(20)	1(20)	3(60)	1(20)
Serratia spp., (n=5)	S	0	5(100)	3(60)	2(40)	5(100)
	R	5(100)	0	2(40)	3(60)	0
Enterobacter spp., (n=3)	S	0	3(100)	3(100)	2(66.7)	3(100)
	R	3(100)	0	0	1(33.3)	0
Staph. aureus (n=3)	S	0	3(100)	3(100)	1(33.3)	2(67.7)
	R	3(100)	0	0	2(66.7)	1(33.3)

Note: AMX= Amoxicillin, GEN=Gentamicin, CTR=Ceftriaxone, NA= Nalidixic acid, CIP=Ciprofloxacin, S=Sensitive, R=Resistant

Foodborne diseases are the foremost global problem, causing considerable morbidity and mortality each year. Pathogenic bacteria are responsible for foodborne diseases. In this study, the goal was to analyze the microbial quality of ready-to-eat food produced by various vendor food shops within Enayetpur town, Bangladesh. Therefore, the current study affected the presence of microorganisms in various ready-to-eat food items in terms of total variable count as well as the finding of different pathogenic organisms such as *E. coli, Psudomonas spp.,* and *Serratia spp*. A similar study was found that relates to the result of this study, which was done at the Bangladesh Agricultural University, Mymensingh 2022, and Bangladesh [Sabuj *et al*., 2020].

4. Conclusion:

The findings of this investigation unequivocally showed that various harmful bacteria were present in the food that was sold on the streets of Enayetpur. The fact that these Foodborne bacteria may cause possible health issues for consumers. The linked risk factors for foodborne illness contamination of foods sold on the street in Enayetpur were inadequate personal hygiene, inappropriate food handling and storage procedures, and inadequate understanding of food sellers on food-borne illnesses. Therefore, to improve the hygienic conditions during the preparation, processing, storing, and serving of food, concerned agencies should provide health education to street food sellers. To improve hygienic conditions, stringent enforcement measures should be implemented after routine sanitary inspections.

5. Acknowledgments:

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