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Microbial Quality of Restaurants Foods in Suburban Area and Antibiotic Resistance

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Abstract

Despite the fact that ready-to-eat foods have gained popularity across the globe. Food safety is frequently overlooked as a major problem in this case. Antibiotic resistance is a long-standing concern regarding microbial diseases. This research was intended to explore the quality of the ready-to-eat foods available in the suburban and rural areas of Bangladesh. In addition, their association with the development of multi-drug resistance was explored. For this purpose, 12 food samples were randomly selected from the restaurants in a suburban area near Dhaka city. Then, the existence of bacteria and their antibiotic resistance capacity were examined through systematic biochemical and microbial experiments. Results showed that the presence of *Staphylococcus epidermitis* in milk was higher and the level of growth was undesirable according to FDA guidelines. The presence of *Klebsiella* in fried rice was also impermissible. Apparently, both Cephradine and Ampicillin revealed that they were not capable of inhibiting microbial growth because of their resistance. From the sensitivity test report, it is clear that people in rural areas are also under the threat of food-borne diseases and the development of antibiotic resistance because of the consumption of these kinds of contaminated ready-to-eat foods.

Keyword: Ready-to-eat food; Microbial contamination; Food borne disease; Antibiotic resistance; Sensitivity test.

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1. Introduction

Human health demands the availability of sufficient safe food. In today's world, ensuring food safety and security is becoming increasingly difficult, particularly in developing nations like Bangladesh. Unfortunately, food safety is frequently overlooked as a major problem for governments, businesses, and individuals in the majority of countries. As a result, the country is paying a high price for its lack of knowledge about food safety. In recent years, ready-to-eat foods have become increasingly popular, particularly in urban areas. Foods that are processed do not require any more processing beyond reheating or the conclusion of a cooking procedure [1-3]. It has been discovered that ready-to-eat takeout meals account for a significant portion of the food service sector's revenues, accounting for more than a third of total volume output [4]. Consumers may rely on ready-to-eat food products to provide them with convenient and nutritious meals [5]. Due to increasing modification and urbanization, the traditional dietary habits of ready-to-eat food are becoming popular day by day in Bangladesh. Foods that people buy from the local market, on the other hand, aren't always edible. Different types of food-borne bacteria have been connected to outbreaks of food-borne illness associated with ready-to-eat meals [3, 6, 7]. Food-borne diseases are becoming more common across the world [8, 9].

Foodborne outbreaks can result in a variety of diseases due to bacterial, viral, protozoal, and chemical contamination. In Bangladesh, the majority of foods are hazardous to eat and are polluted to various degrees (physical, chemical, or biological) of contamination. This issue may be found at every stage of the food supply chain, from production to consumption. Food producers, processors, restaurants, fast food outlets, and customers are all unaware of the need to eat healthy meals [10]. Food-related health hazards are seen in a variety of ways by individuals and society. Nevertheless, factors including handling, processing, storing, and presentation could result in an increase in the microbial load of ready-to-eat meals at the point of sale [11]. The detection of foodborne pathogenic bacteria in food is critical for both quality control and identifying pathogens in the food supply [12].

Apart from that, antibiotic resistance is a long-standing concern and a dynamic issue. The global drug resistance is influenced by variables such as overpopulation, greater international travel, increased use of antibiotics in hospitals and livestock production, selection pressure, poor sanitation, wildlife dispersal, and a subpar sewage disposal system [13, 14]. Antibiotic therapy is one of the most commonly used methods of infection control in modern medicine. During the "golden age" of antibiotic development, which spanned from the 1930s through the 1960s, numerous antibiotics were created [15]. Unfortunately, due to researchers' inability to keep up with the speed of antibiotic discovery in the face of developing resistance bacteria, this period came to an end. Predisposing factors for the establishment of antibiotic resistance include a persistent inability to create or find new antibiotics, as well as indiscriminate antibiotic usage [16].

Antimicrobial resistance (AMR) is a growing global threat to the health of people, animals, and the environment. Multidrug-resistant (MDR) bacteria, also referred to as "superbugs," have developed, spread, and persisted [17, 18]. Animal, human, and environmental triangles or niches may include MDR bacteria, and these diseases are all linked. There are many potential causes of "the global AMR, including overuse of antibiotics in animals (food, pets, aquatic), antibiotics available over-the-counter, increased international travel, poor

sanitation and hygiene, and the release of non-metabolized antibiotics or their residues into the environment through manure or feces. Here in Bangladesh, some studies have been carried out to focus on the microbial quality of ready-to-eat street foods and fruits available on the roadside of capital Dhaka city [19–21]. All these studies have shown that the prevalence of harmful microorganisms, especially bacteria in varieties of street foods and juices, may increase the risk of antibiotic resistance alarmingly among the people living in Dhaka city. With changing lifestyles, food intake behaviors, and urbanization, people living in rural and suburban areas also become accustomed to ready-to-eat foods. Therefore, it is necessary to explore the quality of foods consumed by the people living not only in Dhaka city but other areas of Bangladesh.

In this microbiological investigation, significant quantities of bacteria were sought to be found in ready-to-eat meals available in semiurban areas near Dhaka, Bangladesh. This study also raised the possibility that contaminated ready-to-eat meals may be a community-wide source of multidrug-resistant bacteria. The data obtained from this study will help to focus on our regular food chain and pay more attention to food security in our country.

2. Material and Method

2.1. Material

Nutritional agar powder and MacConkey agar medium were purchased from HIMEDIA Laboratories, India. In addition, Salmonella-Shigella (SS) agar was obtained from TM media, India. Other reagents like 70% ethyl alcohol, or isopropyl alcohol (rubbing alcohol), hydrogen peroxide, and ice boxes (for collecting restaurant food), zip-lock bags (for collecting other samples), hand gloves, and double-distilled water were collected from the local suppliers in Bangladesh.

2.2. Collection of samples

A total of 12 ready-to-eat food samples from 4 different categories were randomly collected from local vendors and shops operating in a suburban area near Dhaka city, Bangladesh. After collection, samples were immediately packed into a zip-lock bag and taken to the lab with proper precautions. All enrichment procedures are briefly described below.

2.3. Microbial Analysis of Food Samples

Spread-plate culture was used to cultivate the microbial load in the food samples. For the culture and isolation of microorganisms, especially for bacteria, three different media were used as nutrient agar (28.0 g/L), MacConkey agar (51.53 g/L), and Salmonella-Shigella (63.0 g/L) agar (SS agar) respectively. The media was prepared through a sequential process. Firstly, a measurement was made with an electrical balance and was taken into a conical flask. Then mix it well with sterile distilled water. Gently heat to boiling (121 C for 15 minutes) with gentle swirling and completely dissolve the medium. Cool to 45-50°C before transferring to sterile petri plates.

To avoid dense growth of bacteria, 1 g of each food sample was appropriately diluted up to 10-1 to 10-6 folds

using a serial dilution technique in sterile normal saline water (0.85% NaCl). 1 mL of each dilution of each sample were spread on selected media using the spread plate method and a sterile glass spreader. Then plates were incubated for 24 to 48 hours at 37C and visible colonies were counted as CFU/g or CFU/mL on a log scale.

2.4. Biochemical test for identification of microorganisms' presence in foods

To identify the existing microorganisms in the food samples, after culture, the growth organisms on each medium were examined by performing the following tests.

2.4.1. Catalase Test

The catalase test is a biochemical test for aerobic organisms that detects catalase enzyme production [22]. This test demonstrates the presence of catalase, an enzyme that breaks down hydrogen peroxide (H_2O_2) and oxygen. It is used to differentiate those bacteria that produce catalase, such as staphylococci, from non-catalase-producing bacteria, such as streptococci.

A small test tube was taken and 3 ml of hydrogen peroxide was poured into it. A small amount was picked up from the petri dish with the wooden tooth peek. The tooth peek was immersed in hydrogen peroxide and examined.

2.4.2. Motility Test

The motility test is used to determine whether an organism is motile or non-motile. Motile organisms contain flagella which help them to travel beyond the point of inoculation. Motile bacteria are generally bacilli, although a few motile cocci do exist. Motile bacteria move with structures called flagella. A small drop of a cultured bacteria colony was taken from the petri dish and placed into the cavity slide with 1 drop of saline. A thin small smear was created and was ready to be observed through the electronic microscope with a 40X objective.

2.4.3. Gram Staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between gram-positive and gram-negative groups by coloring these cells red or violet [23]. For this test, Crystal Violet and Gram's Iodine were used as the primary and moderate stains, respectively. Acetone and alcohol (95%) for decolorization and Safranin as a counter stain were used.

A clean and fresh glass slide was taken. Then prepare the smear on the clean slide with one loop of sample. Allow it to air dry before heating with fix. Crystal Violet was poured and kept for about 1 minute, then washed with water. Flood the gram's iodine for 1 minute and rinse with distilled water. Then, wash with 95% alcohol or acetone for about 10–20 seconds and rinse with water. Add safranin for about 1 minute and wash with water. Observe under a microscope with a 40X objective.

2.4.4. Colony Count

By diluting a sample of microbes and spreading it across a petri plate, one can instead count groups of microbes, called colonies, with the naked eye. Each colony is assumed to have grown from a single colony-forming unit, or CFU. After incubating the culture plate under appropriate conditions (as stated above) for the growth of microorganisms, the colonies are counted. For the spread, pour, or drop methods, the colony counting is self-explanatory: count each colony dot once. A marker can be used to point out each counted colony on the back of the Petri dish [24].

2.5. Antibiotic Susceptibility Test

The Kirby-Bauer method was used to examine the in vitro susceptibility of isolated bacteria to various antimicrobial drugs, utilizing antibiotic disc diffusion on Muller-Hinton agar [25]. It enabled the determination of the antibiotic's effect, which demonstrates the pathogen's inhibition to a degree proportionate to the diameter of the zone of inhibition produced by the antimicrobial's diffusion encircling the disc onto the agar medium. Commercial antibiotic discs containing Ampicillin (10 μg), Cephradine (30 μg), Ciprofloxacin (5 μg), Sulfamethoxazole (23.75 μg), Tetracycline (10 μg), Azithromycin (15 μg), and Cefuroxime (30 μg) were used in this study. In brief, a pure culture of a specific strain was added to 5 mL of Mueller-Hinton broth and incubated at 37° C for an overnight period. The 0.5 McFarland standard was used to account for the turbidity of broth cultures that were actively growing [26].

3. Result

In this study, different kinds of "ready-to-eat" foods available in the local restaurants in the suburban and rural areas of Bangladesh have been used to explore their microbial quality and assess the possible health risk for the consumers (customers). The spread-plate approach was employed to assess the microbial load in samples as listed in **Table 1.** The results of biochemical tests showed a wide variety of microbial organisms were present in all kinds of foods. It was also found that dairy products, including ice cream and packaged milk, had *E. coli*. Some foods, like juice, milk, ice cream, and fried rice, contain more than one bacterium. All of the bacteria are motile and show positive results in the catalase test. In addition, except for *Klebsiella* and *Salmonella* found in fried rice, others are gram-positive.

Table 1: Biochemical profiles of identified bacteria from various ready-to-eat foods available for sale in the resultants in suburban area near by Dhaka, Bangladesh.

Food Samples	Type	Color of Colony	Catalase Test	Motility Test	Gram Staining Test	Identified Bacteria	Colonies per unit (cfu/g)
Fried Chicken	Solid	Golden yellow	+	+	+	***Staphylococcus aureous***	$3.6x10^5$
Lemon juice	Liquid	Golden yellow	+	+	+	***Staphylococcus aureous***	$3.2x10^5$
		White	+	+	+	***Staphylococcus epidermitis***	$1.6x10^5$
Packet Milk	Liquid	White	+	+	+	***Staphylococcus epidermitis***	$7.6x10^5$
		Pink	+	+	+	***E. coli***	$3.2x10^5$
Ice cream	Liquid / Semi solid	White	+	+	+	***Staphylococcus epidermitis***	$1.9\ x10^5$
		Pink	+	+	+	***E. coli***	$3.3\ x10^5$
Bakery Cake	Liquid / Semi solid	Golden Yellow	+	+	+	***Staphylococcus aureous***	$2.4\ x10^5$
Fried rice	Solid	Pink	+	+	_	***Klebsiella***	$8.6x10^5$
		Black	+	+	_	***Salmonella***	$4.4x10^5$
Cooked vegetable	Solid	Pink	+	+	+	***E. coli***	$8.8x10^5$

For more characterization, after isolation and identification of bacteria that grew on multiple culture plates, colonies were counted manually and an average number of colonies was calculated per unit. The colony count for each identified bacteria is also given in **Table 1**. Milk, fried rice, and mixed vegetables were found to be maximally loaded with bacteria ($7.6x10^5$ to $8.4x10^5$ cfu/g). Lemon juice and ice cream contain significantly less bacteria (1.6x105 to 1.9x105 cfu/g).

Glancing at the bar graph in **Figure 1**, it can be concluded that *E. coli* had the highest rate of growth (21%) compared to all other bacteria. *E. coli* growth was noticeably high on a cooked restaurant item (mixed vegetables). On the other hand, *S. epidermitis*, which was discovered in dairy products such as ice cream and packaged milk as well as lemon juice, showed the second greatest frequency (12%) of growth rate. The growth patterns of the remaining bacteria were relatively similar to 11%, indicating some level of contamination in selected food items.

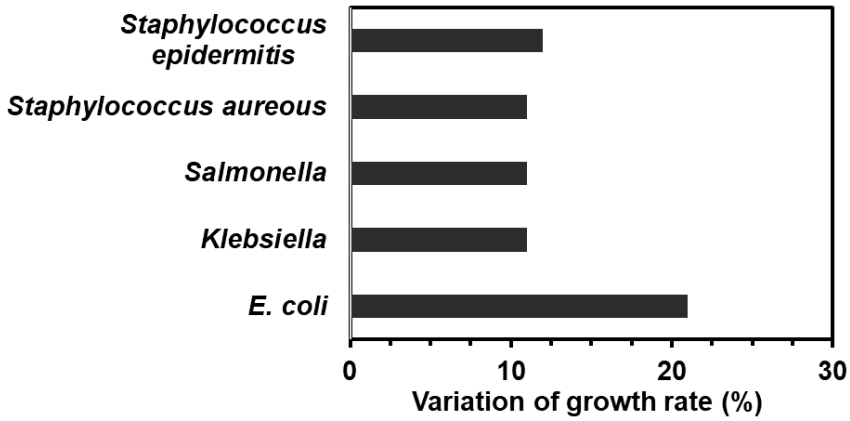

Figure 1: Growth rate of each identified bacteria exist in various food samples.

Utilizing antibiotic disc diffusion on Muller-Hinton agar, the Kirby-Bauer technique was used to assess the sensitivity of isolated bacteria to various antimicrobial medicines in vitro. It is also noteworthy that some of the antibiotics were fully unable to stop the bacteria's growth and some partially succeeded. It was possible to gauge how effective the antibiotic was, with pathogen inhibition proportional to the size of the zone of inhibition caused by antimicrobial diffusion into the agar media around the disc (as shown in **Figure 2**).

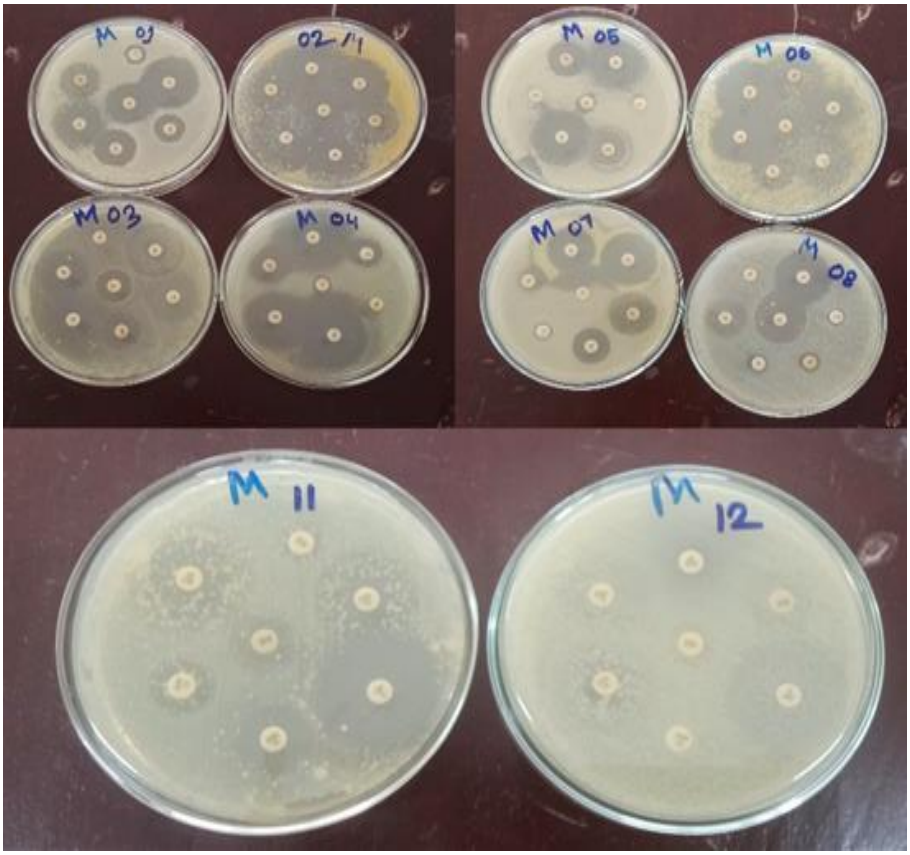

Figure 2: A representative petri dish with an antibiotic sensitivity test showing zone of inhibition. Here, all of the petri dishes were leveled by culture media and sample number (M for Muller-Hinton agar media).

In **Figure 3** and **Table 2,** it has been shown that Ampicillin and Cephradine both yielded a 100% negative result, indicating that neither antibiotic was able to inhibit bacterial growth. On the other hand, bacterial growth has the ability to stop one-fifth portion (only 20%) of the total bacterial growth. Along with that, Sulphamethoxazole has the ability to resist half of total bacterial growth (about 50%).

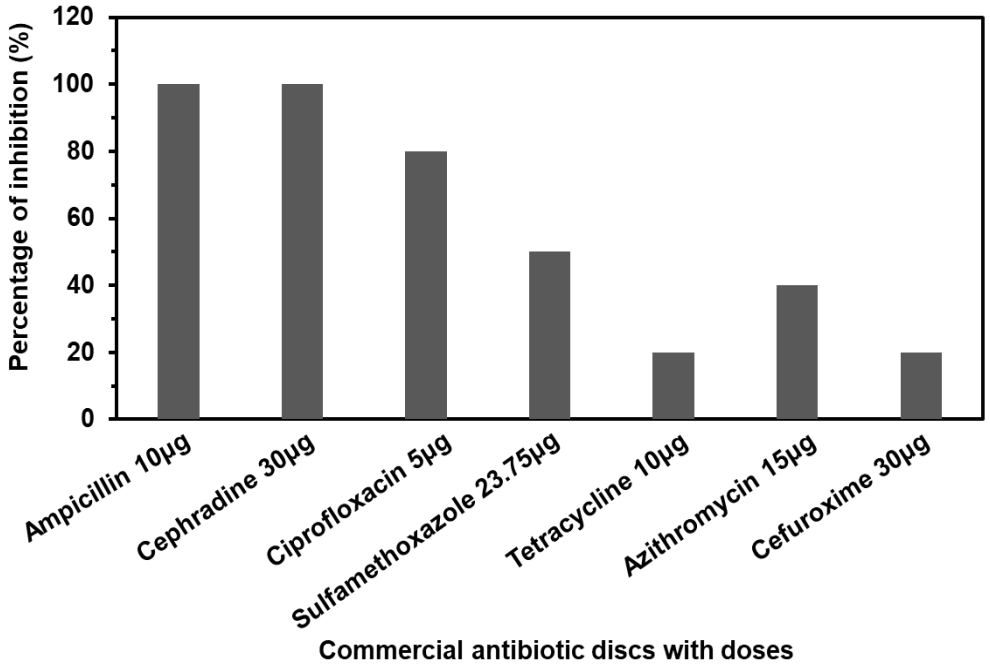

Figure 3: Comparative % of inhibition of various commercial antibiotics against bacteria exist in various ready-to-eat foods.

In a contradiction, Azithromycin has the ability to combat bacterial growth at a modest level with 60% and Tetracycline as well as Cefuroxime hold out against bacterial growth at a significant level with 80%. Only Tetracycline shows a minimum of about 20% of antibiotic resistance. It means it can prevent 80% of food-borne diseases caused by the bacteria in ready-to-eat foods in restaurants.

Table 3: Summary of Antibiotic Resistance Profiles against various bacteria responsible for the contamination of ready-to-eat foods in restaurants This sign (+) is designated for a positive test result, which means that bacteria growth was suppressed due to the presence of antibiotics.

Sample	**Bacteria** **Strain**	**AMP**	**CE**	**CIP**	**SXT**	**TE**	**AZM**	**CXI**
Fried chicken	*Staphylococcus aureous*	-	-	-	+	+	-	+
Lemon juice	*Staphylococcus aureous*	-	-	+	-	+	+	-
	Staphylococcus epidermitis	-	-	-	+	-	-	+
Milk	*E. coli*	-	-	-	+	+	+	-
Ice cream	*E. coli*	-	-	+	-	+	+	+
	Staphylococcus epidermitis	-	-	-	+	+	-	+
Cake	*Staphylococcus aureous*	-	-	-	+	+	+	+
Fried rice	*Salmonella*	-	-	-	-	+	-	+
	Klebsiella	-	-	-	-	+	+	+
Mixed vegetable	*E. coli*	-	-	-	-	+	-	-

Here; APM = Ampiciline, CE=Cephradine, CIP=Ciprofloxacine, SXT=Sulfamithoxazole, TE= Tetracycline, AZM= Azithromycine and CXI= Cfuroxime

4. Discussion

For ease and convenience, demands for ready-to-eat food products are increasing around the world. In comparison to developing countries, food manufacturers and suppliers in developed countries strictly adhere to safety and hygiene standards. According to a survey, the majority of microbiological diseases are caused by a variety of food-borne infections [20, 27]. Some diseases, like bacterial diarrhea, have been directly linked to a high death rate in underdeveloped nations. Moreover, the primary global health issue, which results in significant morbidity and mortality each year, is foodborne infections [28, 29]. The most frequent causes of foodborne diseases are pathogenic microorganisms. The goal of this study was to evaluate the microbiological quality of ready-to-eat and processed foods that are commonly available in rural as well as suburban areas in Bangladesh. In this study, twelve different types of solid and liquid foods, including fried rice, chicken, juice, etc., were collected from local restaurants. Then all of these foods were examined for the investigation of

various pathogenic organisms like *Staphylococcus spp., Salmonella spp., and Shigella spp.,* and so on. Systematic biochemical assays were carried out to identify and characterize the existing microorganisms, mainly bacteria, in these food samples. Surprisingly, there were no food items without bacterial contamination. Some foods, like milk, juice, and fried rice, were contaminated by more than one bacterial species. Moreover, their mortality, growth rate, and CFU/g loading were also very high. As a result, the findings suggest that eating these foods can easily lead to a variety of food-borne diseases. Moreover, people eating these types of ready foods are not only living in metropolitan cities like Dhaka, but also rural areas are at high risk of food-borne illnesses. This kind of unsatisfactory level of hygienic state of ready-to-eat foods in Bangladesh is mostly due to a lack of understanding of food safety and hygiene during washing, preparation, supplies, and storage of foods following standard procedure. In addition, adherence to the country's food safety rules and regulations, as well as the infrequent application of those laws, already in place. Based on a screening of 1,000 people, it was estimated that 3.9 times per year, typhoid affects those living in urban slums. Preschoolers, or kids between the ages of 2 and 5, were predicted to have an 8.9 times higher chance of catching typhoid than other age groups [30, 31]. The main sources of *Salmonella spp.* and *E. coli* are contaminated water, foods, and interpersonal contact. According to FDA guidelines [32], the degree of growth is unacceptable due to the increased prevalence of *Staphylococcus epidermitis, Klebsiella, and E. coli* in milk, fried rice, and cooked vegetables, respectively, compared to other samples in this study.

In Figure 3, the inhibition rate of bacterial growth by commonly used antibiotics shows that it’s tough to treat a food-borne infection. In particular, antibiotics like Ampicillin and Cephradine are quite resistant (100%) against the identified bacteria found in various food samples. The E. coli contamination in prepared and packed foods (both in handmade and commercial packaging) indicated inappropriate handling and local manufacturers' noncompliance with good manufacturing techniques. Because antibiotics have a therapeutic impact, the test results for other bacteria stains were comparable and acceptable. The situation will be more dangerous for small children and the general public.

An increase in awareness among food manufacturers, suppliers, retailers, and inspection authorities may lead to an improvement in food safety. The results of the current study showed that consumers in suburban and rural areas in Bangladesh who purchase ready-to-eat meals have a significant risk of contracting food-borne illnesses as well as the development of multi-drug resistant bacteria pathogens in their daily consumption of ready-to-eat restaurant foods.

5. Conclusion

This study revealed that most ready-to-eat foods in suburban and rural areas of Bangladesh are highly contaminated with varieties of pathogenic bacteria. Therefore, along with the people living in mega-cities here, rural and suburban people who are getting used to the consumption of these types of ready-to-eat foods in restaurants are under a high risk of food-borne diseases. Moreover, antibiotic susceptibility tests demonstrated that multi-drug resistant organisms may also develop due to the resistance of growth inhibition for most of the antibiotics against these bacterial pathogens in foods. The study's findings could be attributed to the increased social awareness of improper processing, poor handling techniques, and contaminated foods, which can

endanger consumers' health.

6. Recommendation

It is highly necessary to collect raw foods materials from contamination free sources especially for fruits, vegetable and raw meat. Freshly prepared food should be supplied to consumers to avoid risk of possible growth of microorganisms. After certain time of cooking, leftover foods in restaurants should destroy properly. Good hygiene measures, such as the adoption of Hazard Analysis Critical Control Point (HACCP) in the chain of food production (cooking) area, processing, and storage, can reduce the incidence of foodborne illness.

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7. Conflict of interest

Author has affirmed that there is no conflict of interest.

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