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RESEARCH ARTICLE

Microbial contamination and antibiotic resistance of ready-to-eat bakery products in Jashore, Bangladesh

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ABSTRACT

Background: Bakery products play an important part in the diet of daily life and provide vital nutrients to human. Ready-to-eat foods made from wheat and flour are rich in essential nutrients such as proteins, fats, minerals, and carbohydrates which may increase exponential microbial proliferation in these products when kept under improper storage conditions or through unhygienic handling and as a result cause mild to-severe illnesses when consumed. **Objective:** This study aims to assess the bacteriological value of bread, doughnut, cake, and snacks sold in Jashore town. **Materials and Methods**: This study was conducted to determine the bacteriological value of bakery products sold in Jashore, Bangladesh. A total of 20 samples were collected from different bakeries and hawkers and examined using the conventional bacterial isolation, identification, biochemical tests, and enumeration. **Results:** The total viable bacterial number of the samples demonstrated the highest (32×10^6) count in sample (cake), while the lowest (2×10^6) count was observed in bread factory. On the other hand, the total yeast & mold number of the samples demonstrated the highest (33×10^4) count in sample (bread), while the lowest (2×10^4) count was observed in doughnut factory. Methicillin resistant *Staphylococcus aureus* was detected. On the other hand, *Escherichia coli* was totally absent in all the samples. **Conclusion:** This result indicated that bakery products prepared under unhygienic environments and which may serve as a reservoir of various pathogenic bacteria and most of them were showed resistance against common antibiotics. To ensure the health safety of consumer's government should take necessary actions to educated food handlers.

Keywords: Bakery; Microbial; Staphylococcus aureus

1. Introduction

Bakery products are a popular food source worldwide, providing essential nutrients like carbohydrates, proteins, lipids, vitamins, and minerals. They are generally considered safe due to their low water activity

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and high-temperature cooking. However, improper storage conditions and unhygienic handling can lead to the proliferation of microorganisms in cereal grains and bakery products. Common bacteria like Bacillus sp. and mold can cause spoilage, which depends on seasons, product type, and processing methods[1]. Contaminated flour can cause food poisoning, while wheat and flour contain pathogenic microorganisms. Factors like slicing equipment, post-baking contamination, and high moisture can also influence spoilage[2].

Bakery products often come into contact with moulds, yeasts, and bacteria, such as *Bacillus subtilis*, which can cause spoilage problems[4]. Penicillium is a common fungal contaminant, and some bacterial spores can survive high temperatures and germinate during baking[3]. Contaminated food causes millions of people to suffer from communicable and non-communicable diseases, causing social, cultural, and economic burdens. Food safety is a World Health Organization strategic objective, and *Staphylococcus aureus* is considered the third most economically significant foodborne disease. Understanding the etiological role of *S. aureus* contamination is crucial for effective control of illness outbreaks. Foodborne diseases are global public health problems affecting health and economy, causing various illnesses and even mortality[4].

Staphylococcus aureus are Gram-positive cocci that produce catalase and coagulase enzymes. They are non-motile and non-spore forming facultative anaerobes that can cause millions of gastrointestinal and acute respiratory infections (ARIs) and skin infections. They are opportunistic and can spread through direct contact, contaminated objects, or inhalation of infected droplets. They can travel through the bloodstream, infect heart valves and bones, and cause skin infections, often called abscesses. Certain staphylococcal infections are more likely in certain situations, such as bloodstream infections, endocarditis, osteomyelitis, and lung infections. *Staphylococci* can multiply readily in food, with ready meat and dairy products being the most implicated[5]. They thrive in environments free of competition from other bacteria, such as high-salt and sugar-containing foods. Diarrheagenic *E. coli* are classified into six different pathotypes based on epidemiological, clinical, and pathogenic characteristics[6].

E. coli outbreaks have been linked to various types of food, including water, and contaminated well water[14]. Water supply was suspected to be contaminated in a large outbreak in Crater Lake National Park, Oregon, in 1975[7]. Contaminated well water and drinking water reservoirs of cruise ships have also been linked to ETEC outbreaks. Cheese, turkey mayonnaise, crab meat, and scallops have also been linked to ETEC outbreaks[8]. Salads containing raw vegetables have also been suspected to cause ETEC-diarrhea. Bottle-feeding of milk has been a risk factor for classical ETEC summer epidemics. This study aims to assess the bacteriological value of bread, doughnut, cake, and snacks sold in Jashore town.

2. Materials and methods

2.1. Sampling

The study was conducted in the Bosundia, Rupdia, Ramnagar, Muroli, and Chanchra areas within the Jashore region, as depicted in **Figure 1**. A total of 20 food samples were collected, including biscuits, bread, doughnuts, and cakes. These samples were placed in sterile polybags and handled with sterile gloves to prevent skin contamination. They were then transported to the Department of Microbiology in food-grade polybags and analyzed within 2 hours of collection. To minimize microbial changes, analyses were performed promptly. If there was any delay, the samples were stored at 4°C. Each food sample (10 g) was mixed with 90 ml of sterile buffered peptone water. Following this, a 10-fold serial dilution was performed using buffered peptone water, with 9 ml of buffered peptone water added to each of five test tubes per sample for the dilution process[9].

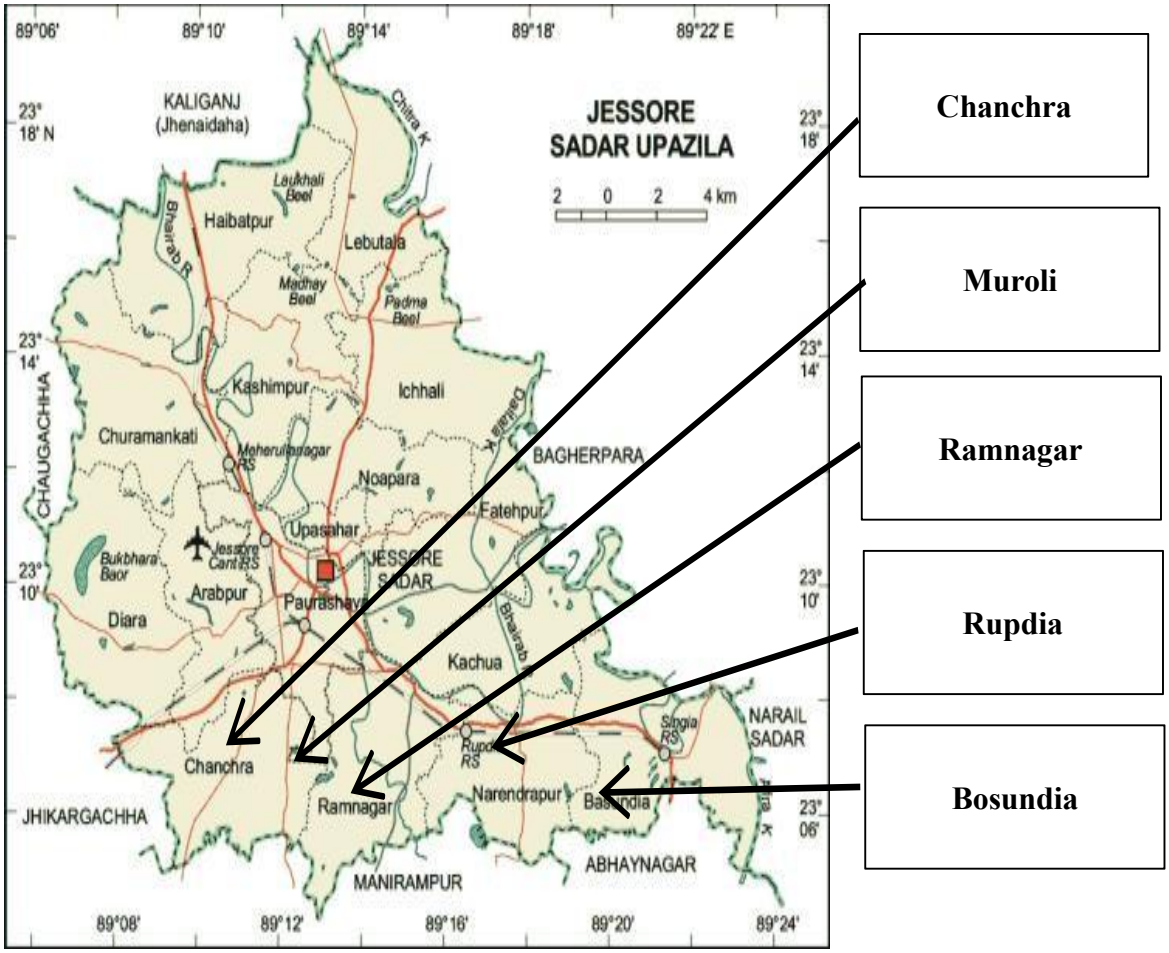

Figure 1. Study area.

2.2. Isolation, Identification and Biochemical Characterization of S. aureus

The diagnosis of *Staphylococcus aureus* was confirmed through a series of identification and isolation techniques, including serological tests, coagulase and catalase tests, microscopy, and culture methods. The isolate was stored using Mueller Hinton agar slants for susceptibility testing and characterization. After subculturing on Mannitol Salt Agar (MSA), the samples were incubated at 37°C for 24 to 48 hours. Identification of S. aureus was based on several criteria: colony morphology, which showed golden-yellow colonies due to mannitol fermentation and a resulting pH drop in the medium; Gram stain reaction, which revealed Gram-positive cocci in clusters or chains; and biochemical characteristics, including deoxyribonuclease (DNA) enzyme production, and positive results for catalase and plasma coagulase tests. Cheesbrough's methods were used for distinguishing S. aureus from other related organisms.

2.3. Antibiotic sensitivity test of S. aureus

Antibiotic susceptibility profiles of the isolates were determined using the agar disc diffusion method[18], as described in reference, with three commonly used antibiotics: penicillin, methicillin, and vancomycin. Colonies from an overnight culture of *S. aureus* isolates were suspended in 5 mL of normal saline to achieve a 0.5 McFarland standard. S. aureus ATCC 25923 was utilized as the control strain.

2.4. Ethical statement

No ethical approval was needed and verbal permission was taken from restaurant owners during sample collection.

3. Results

The samples were subjected for culture isolation to detect total viable count, total yeast & mold, *E. coli*, *S. aureus* isolates or their virulence associated characteristics.

Table 1. List of total aerobic count and yeast & mold count.

Isolated ID	Total Aerobic Count		Total Yeast & Mold Count	
	Dilution	Count	Dilution	Count
Biscuit1	10^{-6}	23×10^{6}	10^{-4}	22×10^{4}
Biscuit2	10^{-6}	28×10^{6}	10^{-4}	21×10^{4}
Biscuit3	10^{-6}	18×10^{6}	10^{-4}	12×10^{4}
Biscuit4	10^{-6}	4×10^{6}	10^{-4}	3×10^{4}
Biscuit5	10^{-6}	5×10^{6}	10^{-4}	19×10^{4}
Bread1	10^{-6}	16×10^{6}	10^{-4}	21×10^{4}
Bread2	10^{-6}	19×10^{6}	10^{-4}	15×10^{4}
Bread3	10^{-6}	16×10^{6}	10^{-4}	33×10^{4}
Bread4	10^{-6}	18×10^{6}	10^{-4}	17×10^{4}
Bread5	10^{-6}	13×10^{6}	10^{-4}	12×10^{4}
Cake1	10^{-6}	25×10^{6}	10^{-4}	14×10^{4}
Cake2	10^{-6}	23×10^{6}	10^{-4}	3×10^{4}
Cake3	10^{-6}	22×10^{6}	10^{-4}	21×10^{4}
Cake4	10^{-6}	32×10^{6}	10^{-4}	19×10^{4}
Cake5	10^{-6}	12×10^{6}	10^{-4}	22×10^{4}
Doughnut1	10^{-6}	26×10^{6}	10^{-4}	17×10^{4}
Doughnut2	10^{-6}	23×10^{6}	10^{-4}	2×10^{4}
Doughnut3	10^{-6}	2×10^{6}	10^{-4}	12×10^{4}
Doughnut4	10^{-6}	28×10^{6}	10^{-4}	25×10^{4}
Doughnut5	10^{-6}	3×10^{6}	10^{-4}	23×10^{4}

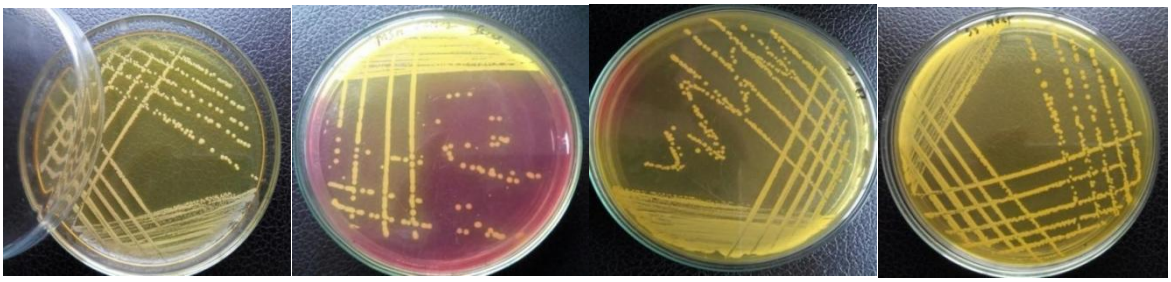

Figure 2. Mannitol salt agar plate.

Table 2. Description of the culture characteristics of Staphylococcus aureus on mannitol salt agar.

Selective media	Name of the organism	Shape & rearrangement	Nature of the colony	Appearance
Mannitol salt agar	*Staphylococcus aureus*	circular	Smooth, shiny surface, golden yellow	Grey to deep golden yellow

Gram staining can have performed to differentiate gram positive and gram negative bacteria. The stained smear was observed under microscope. Smear reveal Gram positive, spherical cell arranged in irregular clusters resembling to bunch of grapes were considered to be Staphylococci. (**Figure 2**)

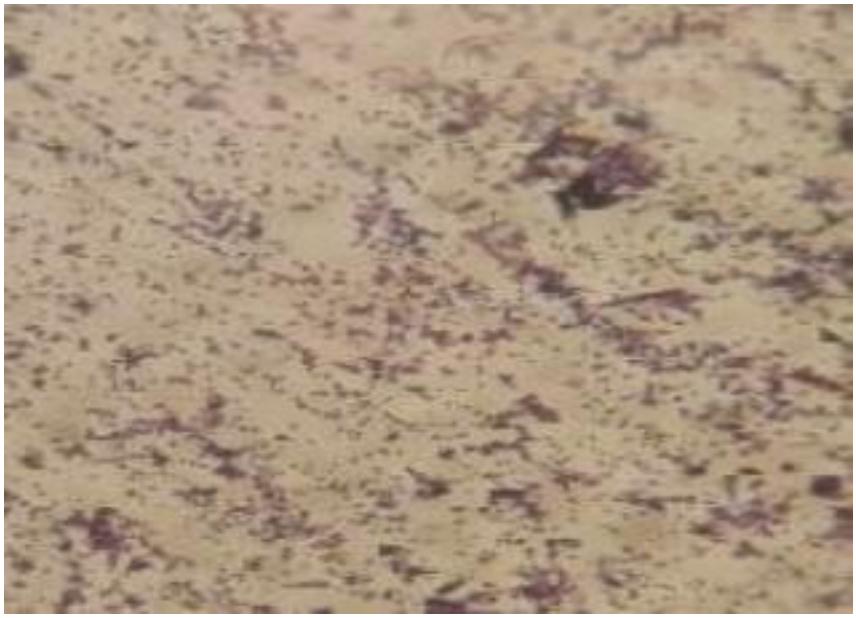

Figure 3. Gram staining.

The color change in a colony on Hofman filter paper indicates oxidase positive or oxidase negative organisms. *Staphylococcus aureus* shows negative results. All samples are catalase positive. Inoculating MR-VP broth with Barritt's A and B reagents converts crimson to crimson. Methyl red indicator changes color to red, indicating methyl red positive. Motility test medium shows movement from inoculated line, while S. aureus

Table 3. Result list of total biochemical test.

Isolated ID	Catalase	Oxidase	VP Test	MR Test	Motility	Gram Stain	Manitol Fermentation Test
Biscuit1	+	_	+	+	_	+	+
Biscuit2	+	_	+	+	_	+	+
Biscuit3	+	_	+	+	_	+	+
Biscuit4	+	_	+	+	_	+	+
Biscuit5	+	_	+	+	_	+	+
Bread1	+	_	+	+	_	+	+

Isolated ID	Catalase	Oxidase	VP Test	MR Test	Motility	Gram Stain	Manitol Fermentation Test
Bread2	+	_	+	+	_	+	+
Bread3	+	_	+	+	_	+	+
Bread4	+	_	+	+	_	+	+
Bread5	+	_	+	+	_	+	+
Cake1	+	_	+	+	_	+	+
Cake2	+	_	+	+	_	+	+
Cake3	+	_	+	+	_	+	+
Cake4	+	_	+	+	_	+	+
Cake5	+	_	+	+	_	+	+
Doughnut1	+	_	+	+	_	+	+
Doughnut2	+	_	+	+	_	+	+
Doughnut3	+	_	+	+	_	+	+
Doughnut4	+	_	+	+	_	+	+
Doughnut5	+	_	+	+	_	+	+

Table 3. (*Continued*)

Table 4. Antibiotic sensitivity pattern of Staphylococcus aureus.

Isolated ID	Methicillin (5µg)/ZOI	Penicillin (10 µg)/ZOI	Vancomycin (30 µg)/ZOI
Biscuit1	R /0mm	I /24mm	S /17mm
Biscuit2	R /0mm	I /23mm	I /15mm
Biscuit3	R /0mm	R /22mm	I /14mm
Biscuit4	R /0mm	R /21mm	I /15mm
Biscuit5	R /0mm	I /23mm	S /18mm
Bread1	R /0mm	R /19mm	I /16mm
Bread2	R /0mm	I /24mm	S /17mm
Bread3	R /0mm	R /22mm	S /17mm
Bread4	R /0mm	I /22mm	I /15mm
Bread5	R /0mm	I /24mm	I /14mm
Cake1	R /0mm	R /21mm	I /15mm
Cake2	R /0mm	R /19mm	S /18mm
Cake3	R /0mm	I /23mm	I /16mm
Cake4	R /0mm	I /23mm	S /17mm
Cake5	R /0mm	R /21mm	S /19mm
Doughnut1	R /0mm	R /21mm	S /15mm
Doughnut2	R /0mm	R /21mm	I /16mm
Doughnut3	R /0mm	R /19mm	I /14mm
Doughnut4	R /0mm	I /23mm	S /16mm

Doughnut5	R /0mm	R /0mm	I /15mm

ZOI=Zone of inhibition

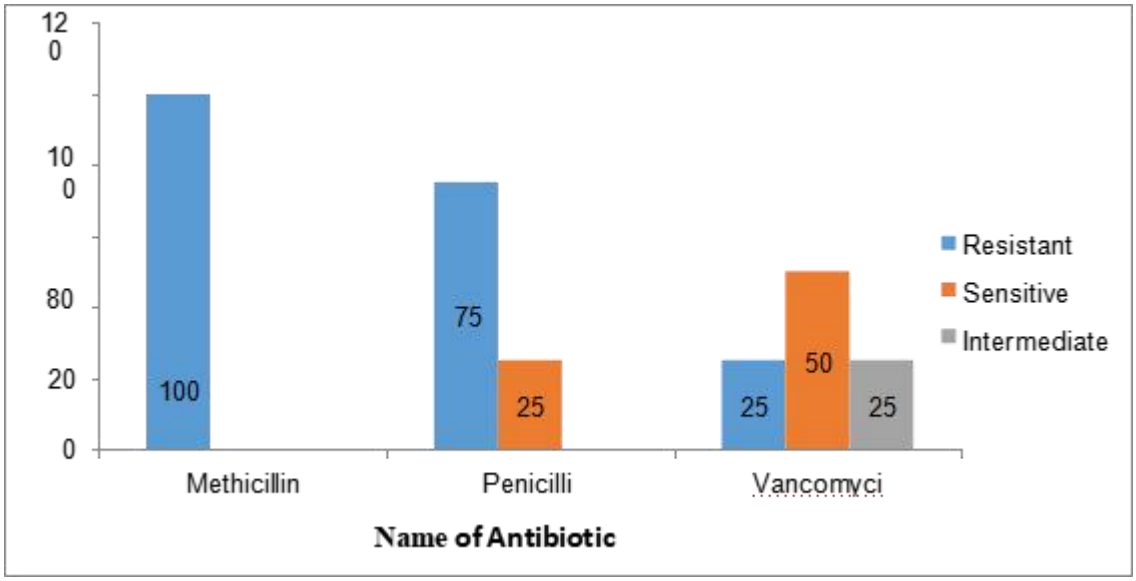

Figure 4. Graphical Representation of Antibiotic sensitivity pattern of Staphylococcus aureus.

In this experiment, Methicillin (5µg), Penicillin (10 µg), Vancomycin (30 µg) were used to measure the susceptibility & resistance level of Staphylococcus aureus**.**

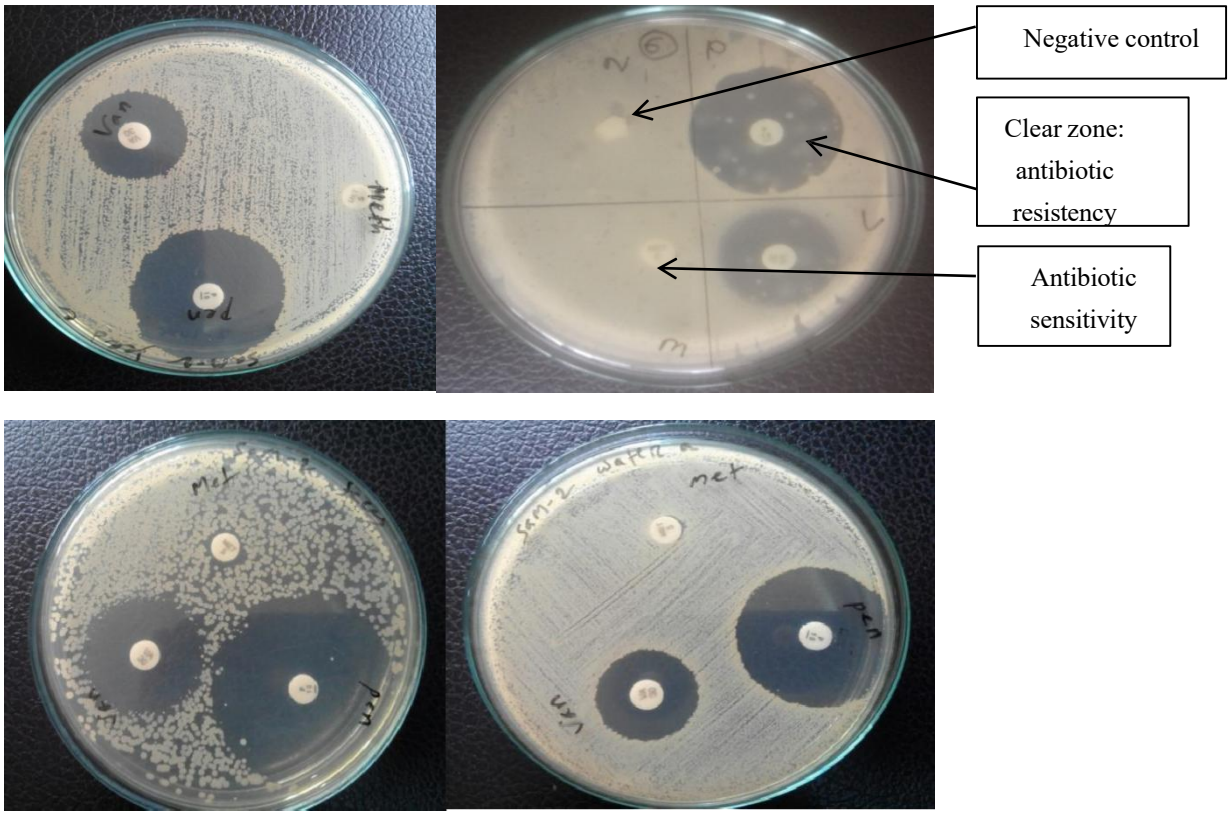

Figure 5. Antibiogram of Staphylococcus aureus.

4. Discussion

The study revealed a wide variation in the total viable bacterial count across different samples, with the highest count of 32×10^6 cfu/g observed in cake samples, and the lowest count of 2×10^6 cfu/g[10]. This is significantly higher than the maximum threshold set by food quality agencies, which recommend a maximum of 10,000 cfu/g for aerobic bacteria. The elevated bacterial load, particularly in cake samples, can be attributed to inadequate hygiene practices in doughnut factories and bakeries[11]. Workers often neglect proper protective clothing such as gloves, which facilitates contamination during preparation, baking, and packaging. Additionally, the machines used in the manufacturing process may contribute to bacterial contamination if not adequately sanitized[12].

The bread samples showed a lower bacterial count of 1.519×10^7, likely due to better packaging methods employed by most bread bakers, which help to reduce exposure to contaminants. The high bacterial count in baked goods, however, poses a risk of foodborne illnesses and food poisoning, with *Staphylococcus aureus* being the most common pathogen associated with such outbreaks. This bacterium is ubiquitous in nature and can proliferate under improper handling conditions[13].

The isolation and identification of *S. aureus* from bakery samples were key objectives of this study. Isolates showed typical colonies on mannitol salt agar, and biochemical tests such as catalase, oxidase, motility, and methyl red tests confirmed the presence of *S. aureus*. The gram-positive cocci-shaped microorganism was further confirmed through Gram staining, which revealed its characteristic purple staining. The identification of *S. aureus* is of critical importance in ensuring the safety of food products, as this pathogen is often implicated in foodborne infections[14].

Antibiotic susceptibility testing of *S. aureus* isolates from bakery samples revealed that antibiotic-resistant strains were present in all food samples. This highlights the growing concern of antibiotic resistance in foodborne pathogens, making it more challenging to control and treat infections caused by *S. aureus*. Poultry and bakery products are potential reservoirs for these antibiotic-resistant strains, which can pose significant health risks to consumers[15].

To mitigate the risk of *S. aureus* contamination, strict hygiene practices should be enforced, including thorough handwashing and the use of protective clothing by workers involved in food preparation. Regular cleaning and sanitization of equipment and surfaces in bakeries are essential to reduce bacterial contamination. Additionally, public awareness campaigns on food safety and hygiene, especially in food processing environments, should be promoted to minimize the risk of foodborne illnesses caused by *S. aureus* and other pathogens.

5. Conclusion

The study found bacterial and fungal contamination in filled bakery products, possibly due to poor handling, preparation, and marketing. The microbiological status was slightly good, but other samples were contaminated with disease-causing bacteria. This raises concerns about public health safety and the potential for food-borne illnesses and poisoning. Regular monitoring of food quality in Bangladesh is necessary by BSTI and the Ministry of Health.

Conflict of interest

The authors declare no conflict of interest.

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