Hindawi
Journal of Food Quality
Volume 2022, Article ID 5016795, 12 pages
https://doi.org/10.1155/2022/5016795

Research Article

Antibiotics Resistance Pattern of Food-Borne Bacteria Isolated from Ice Cream in Bangladesh: A Multidisciplinary Study

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Received 9 February 2022; Revised 1 August 2022; Accepted 4 August 2022; Published 31 August 2022

Academic Editor: María B. Pérez-Gago

Ice cream is one of the most popular food items consumed during the summer season in Bangladesh by all ages but mostly school-going students. Due to the ingredients and handling process of ice cream, it acts as a good shelter for pathogenic and non-pathogenic microorganisms. Therefore, we aimed to investigate the microbial count, prevalence, isolate and characterize multidrug-resistant bacteria in ice cream samples collected from nearby shops of schools in Tangail district, Bangladesh. Ice-cream consumer and nonconsumer students were selected by providing questionnaires. Total viable count (TVC) and total coliform count (TCC) were determined by pour plate methods, where conventional methods were performed for bacterial identification. The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility of bacterial isolates. Kado and Liu method, with some modifications, was used to extract plasmid from the isolated bacteria and visualized through gel electrophoresis. The demographic characteristics showed that the degree of symptoms regarding microorganisms mediated disorders and rate of antibiotics intake in ice cream consumers were significantly higher than the nonconsumers. The range of TVC and TCC in the ice cream samples was found 0–9.9×10^9 CFU/ml and 0–900 CFU/ml, respectively. Interestingly, 93.75% of the total ice cream samples also showed fungal positive. A total of 12 different bacterial species were identified, including *Proteus* spp, *E. coli, V.cholera*, *Pseudomonas* spp, *Shigella* spp, *Klebsiella* spp, *Aeromonas* spp, *V. Parahemolyticus. Salmonella paratyphi, Citrobacter*spp, *Plesiomonas*spp, and *Staphylococcus aureus*. The antimicrobial susceptibility assay showed the multiple resistance frequency of these isolates to different antimicrobial drugs. All individual isolates were screened for plasmid DNA, and we found that seven strains harbored a single or more than two plasmids sized approximately between 1.9 and 140 MDa, indicating a possible connection between resistance phenotype pattern and genotype.

1. Introduction

Food-borne disease is an emerging infectious disease caused by microbial pathogens, i.e., fungus and bacteria, leading to threats to global public health in the whole world [1]. Centre for Disease Control reported that around 48 million people get sick from a food-borne illness every year, 128,000 are hospitalized, and 3,000 have died from more than 250 related food-borne diseases globally [2]. Frequencies of food-borne diseases are very common in many developing countries like Bangladesh, and it has become a significant public health-related issue. According to a recent estimate, about 30 million people in Bangladesh are affected by food-borne diseases each year due to adulterated food from vendors, malnutrition, inadequate public health, and sanitation facilities, and other conditions leading to morbidity as well as causes mortality, especially in children [3, 4].

Ice cream is one of the most popular dairy products consumed by all age groups, particularly school-going children worldwide [5]. But most of these ice creams may be

contaminated with microbes during either production or postproduction [6, 7]. Additionally, the components of ice cream like water, fat, sugar, nonfat milk solids, emulsifier and stabilizer, flavoring agents, coloring materials, and thickeners may be a potent source for contamination of ice cream [8]. Additionally, bacteria can cause spoilage of milk and milk products because of extracellular or intracellular thermoresistant enzymes (proteases, lipases, and phospholipases) produced by them [9]. It has also been reported that people are at risk from pathogenic bacteria found in milk, which account for over 90% of all dairy-related disorders [10]. The previous study showed that children who consumed ice cream were suffered from several food-borne diseases like cholera, typhoid, bacillary dysentery, salmonellosis, typhimurium, meningitis, encephalitis, sepsis, hemorrhagic colitis, a hemolytic uremic syndrome in children, gastroenteritis, septicemia, and wound infection, cholera, fever, cough, dysentery, acidity, poisoning, vomiting, dysuria, diarrhea, stomach pain, and weakness [11–15]. Generally, these food-borne diseases have been treated by some common antibiotics, including tetracycline, streptomycin, norfloxacin, amoxicillin, trimethoprim, nitrofurantoin, nalidixic acid, gentamicin, and cefuroxime that can kill and inhibit the growth of the pathogenic bacteria or prevent them from multiplication [16, 17]. However, these commonly used antibiotics are becoming resistant to several bacteria and threat to public health [18]. Johnson et al. reported that several mechanisms are associated with antibiotic resistance, including overuse, inappropriate prescribing, extensive agricultural use, and inadequate regulation [19–22].

Bacteria having plasmid DNA play an essential role in spreading antibiotic-resistant genes to others [23]. Plasmids may contain resistance genes for single or multiple antimicrobial agents, and these mobile genetic elements can be transferred to other nonresistant bacterial cells via horizontal gene transfer mechanisms, including conjugation, transformation, and transduction [24]. In Bangladesh, contamination of ice cream by pathogenic bacteria is becoming an emerging food safety problem.

Bangladesh is still an underdeveloping country, where about 70% of people are educated, so the awareness of food safety and security is vulnerable. As a tropical country, the summer season is very long with hot weather in Bangladesh. Moreover, a huge number of unauthorized ice cream factories are developed here, particularly in the summer season. As a sweetened and cheap food item, ice cream is very popular among school-going students during the summer season. However, contamination of ice cream by pathogenic bacteria and their antibiotic-resistance pattern were not clearly documented in the previous research studies, but little information is reported regarding bacterial load and their biochemical identification [7, 25]. Therefore, in this study, we demonstrated the overall prevalence of bacterial contamination and their characterization with antibiotic susceptibility patterns and the genotypic characteristics of antibiotic-resistant bacterial strains in ice cream samples. We also showed the demographic characteristics of the ice cream consumer and nonconsumer primary and high school students. This is the first report that will assess the microbiological quality and safety of ice cream and will make sense to the regulatory authorities with effective approaches to adopting proper supervision during ice cream preparation for better children and public health management.

2. Materials and Methods

2.1. Questionnaire Preparation, Data Collection, and the Ethical Statement. An empirical study was done to compare isolated microorganisms and observational data. A standardized questionnaire was generated based on some literature search about food-borne diseases caused by several food-borne microorganisms [12]. The variables included in the questionnaire were age, sex, degree of symptoms, antibiotics intake, and body mass index (BMI) of school-going students with their age range 8–14 years, where the total number of student were 232 on. Ethical approval was received from the Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Santosh, Tangail, Bangladesh (MBSTU/BMB/TEST/2014/06(2).

2.2. Collection, Preparation, and Processing of Sample. Sixteen (16) ice cream samples from sixteen (16) different companies, including Bangladesh standards and testing institution (BSTI) approved and non-BSTI approved, were collected during six months period (april, 2019 to september, 2019) by using an icebox from popular ice cream shops as well as steed-vendors ice creams at around nearby schools in the Tangail district, Bangladesh. The respective samples were transported to the microbiology laboratory in aseptic conditions for further investigation [5, 8].

2.3. Mycological (Fungus) Evaluations. The analysis of ice cream samples was done by direct plating to detect micromycetes undiluted 100 μl ice cream samples were inoculated into Potato Dextrose Agar media (HIMEDIA, India) and incubated at optimum growth conditions [26, 27]. Morphology of all fungal isolates was visualized and noted down based on phenotypic characterization, including colony, color, texture, and pigmentation [27].

2.4. Enumeration of Total Viable Count (TVC) and Total Coliform Count (TCC). TVC was determined by traditional methods (serial dilution, spread plate technique, colony count) as described by Shabnum Shaheen et al. [28], and TCC was measured by using MacConkey's agar according to K. M. (2012) [29] and International Organization for Standardization (ISO) rules [30].

2.5. Isolation and Biochemical Identification. Bacterial colonies were isolated by the streak plate method on various selective agar media (EMB, SS, MAC, MSA, PALCAM Listeria identification agar base, and Listeria selective supplement; all from Himedia, India). Growth and morphological characteristics were recorded according to the Biology Libre

Texts technique [31] and the biochemical assay results (Sugar fermentation KIA, Motility, indole, urea, and citrate utilization tests) for the identification of bacterial isolates [32].

2.6. Antimicrobial Susceptibility Testing to Antibiotic. The antibiotics susceptibilities of the isolated bacterial species were determined by using the Kirby-Bauer method on Mueller–Hinton agar plates (merck, germany) according to the clinical and laboratory standard institute [33]. 17 different antimicrobial discs including chloramphenicol (30 μg), kanamycin (30 μg), nalidixic acid (30μ), moxifloxacin (5 μg), cotrimoxazole (5 μg), tetracycline (30 μg), ampicillin (10 μg), levofloxacin (5 μg), ceferoxamine, norfloxacin (10 μg), gentamicin (10 μg), erythromycin (15 μg), streptomycin, azithromycin (15 μg), rifampicin (5 μg), methicillin (5 μg), and ciprofloxacin (5 μg) were used and all were obtained from Oxoid (UK). Antibiotics were selected based on common antibiotics used in Bangladesh [34] and recommended for regular integrated antimicrobial resistance testing by the World Health Organization [35]. Reference strains of *S.typhi* were used for antimicrobial susceptibility studies for quality control [36].

2.7. Extraction, Separation, and Visualization of Bacterial Plasmid DNA. Isolated bacteria were cultured in Luria broth media and incubated overnight at 37°C in a rotatory incubator. Plasmid DNA was isolated with some modifications of the alkaline lysis method of Kado and Liu through repetitive centrifugation [37, 38]. The reagents used for plasmid DNA isolation were Solution I (40 mM Tris-HCl, 2 mM EDTA, pH 7.4 0), Solution II (50 mM Tris-HCl, 3% SDS, pH 12.9, and Solution III (Phenol: chloroform: Isoamyl alcohol (25 : 24 : 1) (manual of icddr,b). Plasmid DNA was electrophoresed in 7% agarose gel; prepared in 1 x TBE (Tris-borate EDTA buffer) and mixed with 0.5 µg/mL of ethidium bromide [23, 39]. Plasmid samples were mixed with 1x dye were electrophoresed for 2.5–3 h at 80 V in 1 x TBE buffer [23]. The molecular mass of the plasmid DNA bands was measured by comparing the mobility of known molecular mass plasmids of *E. coli* V517.

2.8. Statistical Analysis. The parametric data were expressed as the mean ± standard error of the mean (s.e.m.), and the differences between consumer and nonconsumer and comparison of antimicrobial resistances were determined by t-test analysis of variance. Bacteria counts data were transformed to logarithm 10 of colony-forming units per milliliter of the sample (log 10 cfu/ml), and the results were presented as mean ± standard error mean (sem) by t-test. *P-value* of less than 0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using Microsoft Excel 2013 and statistical package for the social sciences (spss) version 20.

3. Result

3.1. Demographic Characteristics of the Ice Cream Consumer and Nonconsumer Students. Table 1 shows the general characteristics of the ice cream consumer ($n = 122$) and nonconsumer ($n = 110$) students in Tangail district, Bangladesh. The participants were selected under the exclusion and inclusion criteria with the consent of their guardians regarding normal hygiene issues during the summer season. Ice cream nonconsumer students were considered a reference group to measure the degree of symptoms related to bacterial infections compared to the ice cream consumer students group. The average BMI level of the nonconsumer and consumer students was 19.76 ± 2.53 and 19.43 ± 2.71, respectively, where the ages were 12.45 ± 1.85 and 12.65 ± 1.87 years. No significant differences were found in average BMI and ages between both groups. The degrees of symptoms of bacterial infections in ice cream consumer students (5.53 ± 1.28) were significantly higher than that in ice cream nonconsumer students (2.27 ± 1.01). On the other hand, the percentage of antibiotics intake in ice cream consumer students was significantly greater than in the nonconsumer group.

3.2. Determination of Total Viable and Total Coliform Count of the Ice Cream Samples. In Bangladesh, BSTI-approved permissible total viable count for ice cream is about less than 4.23 log10 CFU/ml. According to our study, the microbiological load of collected samples was recorded after overnight incubation, and the overall range of TVC was between 0.00 (Polar)—10 (Doikulfi) log10 CFU/ml; about 0–3 fold higher than the safety limits (Figure 1(a)). The TVC of BSTI-approved samples ranged between 0.00 (Polar)—9.53 (Promi Ice Lolly) log10 CFU/ml as against 4.06 (Grameen pipe) to 10 (Doikulfi) log10 CFU/ml in the non-BSTI-approved samples. TVC value of only three ice cream samples was within the BSTI recommended range CFU/ml, where 2 samples (Cocola Mango, Polar) were included in BSTI approved and 1 (Grameen pipe) comprised in non-BSTI approved. The rest of the ice cream samples Lovello Chocobar, Kwality Magnum, Igloo, Kwality Birds, Choco Papa (BSTI) and Ripen Mango, Juice Ripen Litchi Juice, Kironmala, Munni Chocobar, Malai ice cream, Chocobar (Non-BSTI), showed higher microbial load than the normal limit indicated the contamination of ice cream by the pathogenic microorganism.

The coliform counts (TCC) of the total of 7 ice cream samples were crossed the BSTI-approved permissible limits (≤1.17 log10 CFU/ml) of the TCC (Figure 1(b)). Three BSTI-approved samples, including Promi Ice Lolly, Lovello Chocobar, and Kwality Magnum, accounted for the coliform count 1.7, 2.84, and 2.95 log10 CFU/ml, respectively, and the four non-BSTI-approved samples comprise Chocobar, Doi Kulfi, Kironmala, and Choco Papa with coliform count 2.14, 2.85, 2.95, and 2.90 log10 CFU/ml, respectively.

3.3. Prevalence of Bacterial Isolates from Ice Cream Samples. A variety of biochemical assays were conducted to make a comprehensive view, and we identified a total of 26 bacterial strains from which 12 different identical species are listed in

Table 1: Demographic characteristics of ice cream consumer and nonconsumer students.

Character	Ice cream nonconsumer	Ice cream consumer	*P*-value
Age (Years)	12.45 ± 1.85	12.65 ± 1.87	0.736
Students:			
Boys [*n*, (%)]	60 (47.24)	67 (52.76)	0.774
Girls [*n*, (%)]	50 (48.62)	55 (52.38)	
Degree of symptoms[a][*n*, (%)]	2.27 ± 1.01 (29.10)	5.53 ± 1.28 (70.90)	<0.001*
Antibiotics intake [*n*, (%)]			
Yes	28 (25.45)	73 (59.84)	<0.01*
No	82 (74.55)	49 (40.16)	
BMI[a]	19.76 ± 2.53	19.43 ± 2.71	0.678

BMI was calculated as body weight (kg) divided by height squared (m^2). [a]Mean ± SD, and *P-values* were from independent sample *t*-test.

Table 2. The data in Table 2 showed that *Proteus* spp. and *E. coli* were the most frequently (4, 15.38%) identified species. The next magnitude was *Staphylococcus aureus* (3, 11.54%). The frequency and percentage of *Vibrio cholerae*, *Pseudomonas* spp., *Shigella* spp., *Klebsiella* spp,. *Aeromonas* spp., and *V. parahemolyticus* bacterial strains were found 2 and 7.69%, respectively. Some other human pathogenic bacterial strains, including *Salmonella paratyphi* and *Citrobacter* spp. were also identified (1, 3.85%) from several ice cream samples.

3.4. Determination of the Presence of Fungus in the Ice Cream Samples. According to our result, 93.75% of samples flourished with positive results with a high percentage of fungus grown, while the polar alike total viable count of bacteria (Figure 1(a)) did not expose any visible fungal growth. The fungal isolates found in the culture plate differed according to their colony, surface, size, height, shape, pigmentation, and variation of growth rate (Figure 2).

3.5. Antibiotics Susceptibility Pattern of Bacterial Isolates from Ice Cream Samples. The overall bacterial isolate susceptibility profiles have been shown in Table 3. A total of 17 antibiotics were used for our study, where cefuroxime, rifampicin, erythromycin, ampicillin, and methicillin had the highest resistance pattern, which accounted for 100% of resistance, and streptomycin showed the 2nd highest resistance pattern (82.6%). A couple of antimicrobial drugs, including kanamycin, nalidixic acid, and gentamycin, have less resistance behavior with a value of 30.43%, and chloramphenicol, norfloxacin, clotrimazole, ciprofloxacin, tetracycline, and moxifloxacin have least resistance values with high sensitivity. Although the five antibiotics were 100% resistant against 23 different isolates, only one antibiotic, namely levofloxacin, was 100% sensitive against all the isolates. Moxifloxacin, clotrimazole, ciprofloxacin, gentamycin, chloramphenicol, tetracycline, and norfloxacin showed around 75% sensitivity profile against the entire tested strains isolated from the ice cream samples. Our experimental data showed that in the 100% sensitivity and resistance scale of the antimicrobial test, the resistant isolates were significantly higher than the sensitive isolates. However, some antibiotics also showed intermediate values regarding sensitivity and resistance scale against isolated bacterial strains.

The specific antimicrobial resistance rates of the bacterial species are shown in Table 4. The isolates were resistant to one or more antibiotics of different structural classes called multidrug resistance (MDR). *Plesiomonas* spp, a rarely isolated bacterial strain showed the highest resistance rate against 65% of antibiotics used in this study. The other five most common isolates, including *Aeromonas* spp., *V. Parahemolyticus, Citrobacter* spp., *Pseudomonas* spp., and *V. cholera* spp., exhibited a resistance pattern against more than 50% of antibiotics. However, the four isolated bacterial strains, including *Proteus* spp., *S. Paratyphi, Shigella* spp., and *Klebsiella* spp. displayed a resistance pattern between 35 and 47%.

3.6. Determination of Antibiotic-Resistance Patterns in Bacterial Isolates by Plasmid Analysis. Antibiogram profile of bacterial strains isolated from ice cream samples showed multiple antibiotic-resistance patterns (Tables 3 and 4). These phenotypic resistance patterns were validated by the molecular level through the analysis of plasmid DNA extracted from isolated bacterial strains. In this study, a total of 7 (63.63%) from 11 bacterial strains displayed their plasmid DNA in agarose gel and were found MDR-resistant to more than three antibiotics. The plasmid-positive bacterial strains also displayed the seven different plasmid band patterns with varying sizes of molecular weight ranging from 1.9 to 140 MDa (Figure 3). Bacterial strains, including *Paraheamolyticus* spp., *Citrobacter* spp., *Proteus* spp., *E. coli,* and *Proteus* spp. showed only one band size ranging from 1.9 to 140 MDa. The remaining strains had plasmids with a different number of bands. The following isolates *Vibrio, Aeromonas* spp., *Proteus* spp., and *Alcalegenes* spp. showed two bands ranging from above 3.1 MDa to 140 MDa. The plasmid of one isolate, *Klebsiella* spp., showed its bands ranging from 3.4 MDa to 4.8 MDa (Figure 3). The rest of 4 (36.37%) bacterial species did not show any plasmid DNA, but they were resistant to several antibiotics. Our data suggested that plasmid-positive bacterial strains may possess antibiotic-resistant genes and showed their resistance pattern against different antimicrobial drugs. Moreover, our result has also shown that some isolates did not contain any

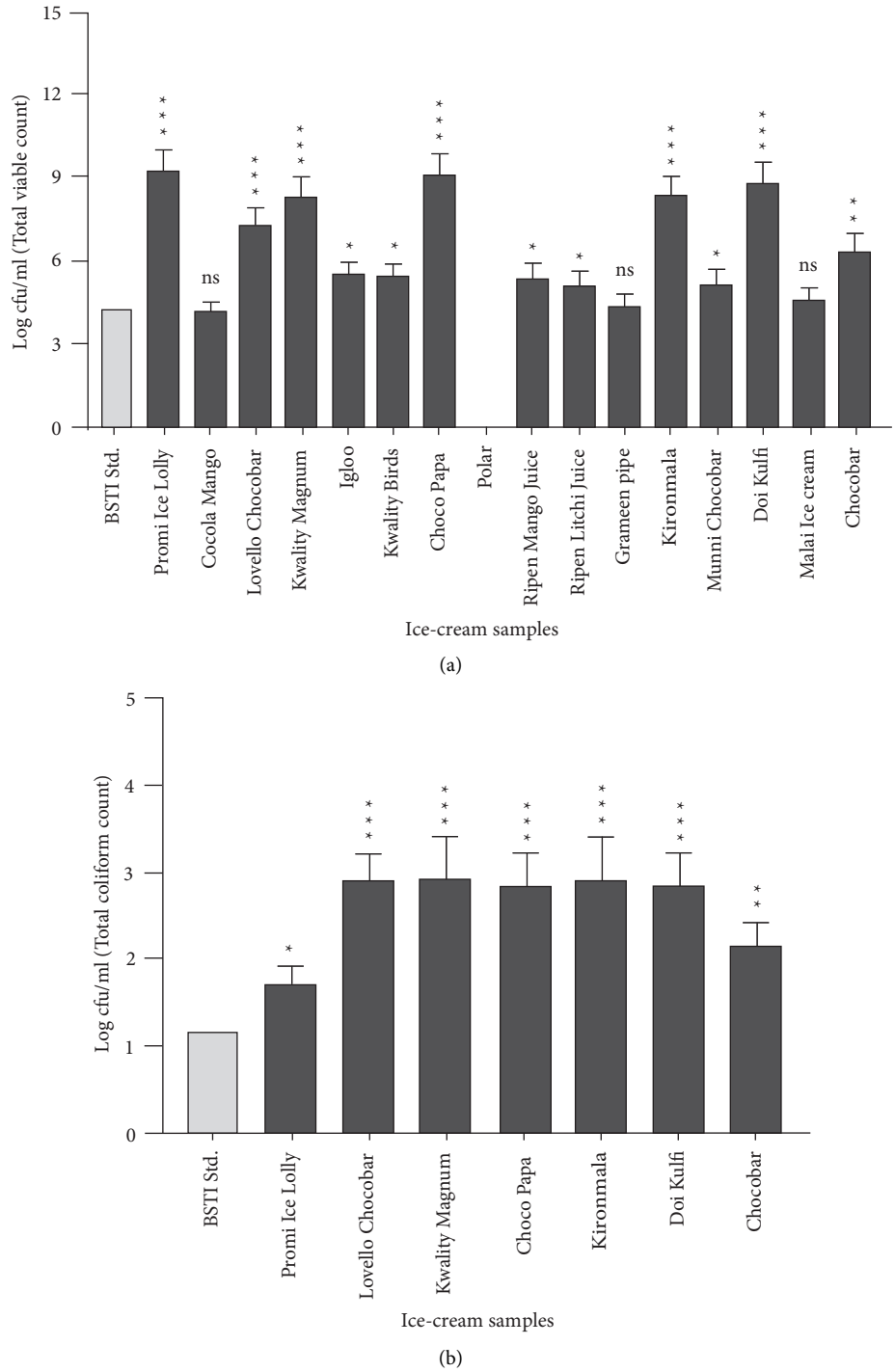

FIGURE 1: Total viable (TVC) and total coliform count (TCC) form ice cream samples. (a) Total viable count (TVC). (b) Total coliform count (TCC). Data are expressed as the mean ± SEM from 3 independent experiments. $^{*}P < 0.05$, $^{*}P < 0.01$ and $^{*}P < 0.001$(t-test).

plasmid but had multidrug resistance phenotypically, which is summarized in Table 5.

4. Discussion

Microbial contaminations are responsible for serious human health concerns worldwide mediated by antibiotic-resistance patterns. Ice-cream is a milk-based product that serves as a potential shelter for microbial growth. Due to the proper nutrient elements as well as nutrient compositions, almost neutral pH (pH 6–7), and extended storage duration, it may easily be contaminated by pathogenic microorganisms. In this study, the data from the demographic characteristics of ice cream consumers and nonconsumers (Table 1) revealed that ice cream consumer students exhibited greater infectious symptoms and used antibiotics in higher amounts than

Table 2: Identification of bacterial isolates from ice cream samples.

Bacterial isolates	Number	Percent
Proteus spp	4	15.38
Vibrio cholera spp	2	7.69
Salmonella paratyphi	1	3.85
Pseudomonas spp	2	7.69
*Shigella*spp	2	7.69
*Klebsiella*spp	2	7.69
E. coli spp	4	15.38
*Aeromonas*spp	2	7.69
V. Parahemolyticus	2	7.69
*Citrobacter*spp	1	3.85
*Plesiomonas*spp	1	3.85
Staphylococcus aureus	3	11.54
Total	26	100

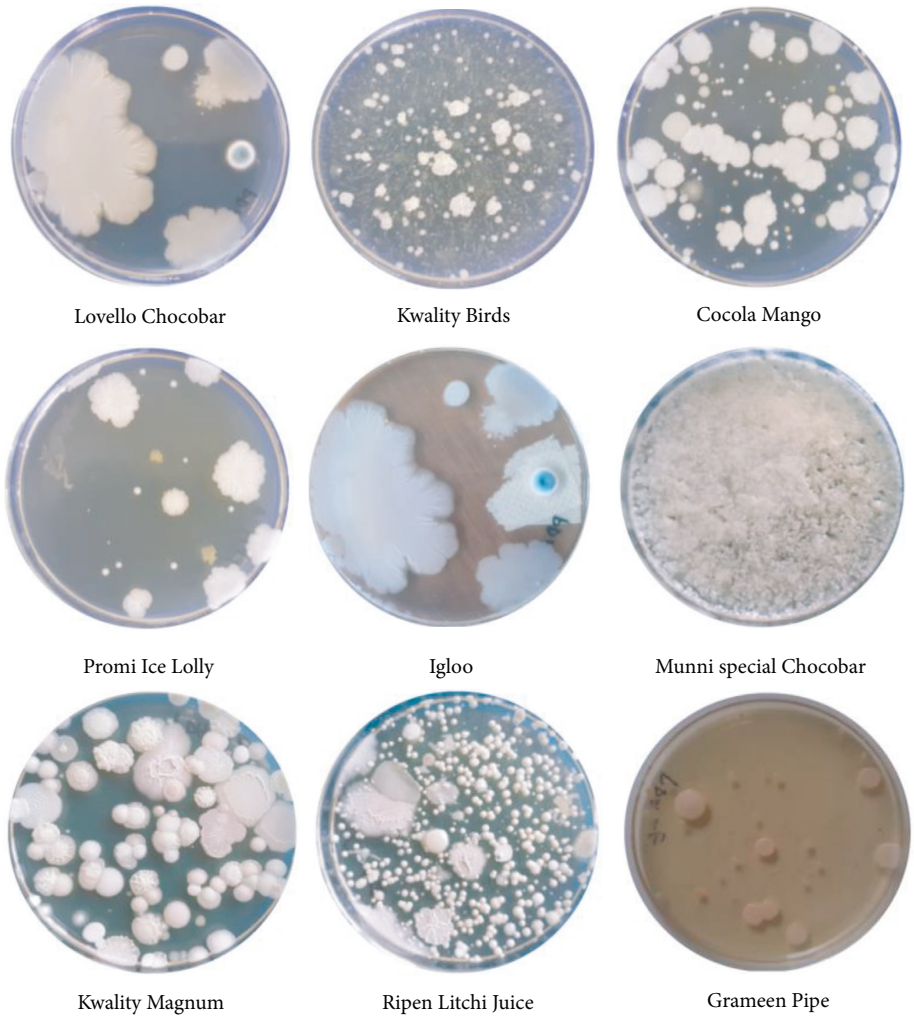

Figure 2: Evaluation of the presence of fungi in the ice cream samples. A numerous types of fungus have been exhibited in 100 μl of different ice cream samples after 5–7 days incubation at 25°C.

Table 3: Overall antibiotics susceptibility pattern of bacterial isolates from ice cream samples.

List of antibiotics	No. of antimicrobials tested	Antibiotics susceptibility patterns		
		Resistant N (%)	Intermediate N (%)	Sensitive N (%)
Chloramphenicol	23	4 (17.4)	3 (13.0)	16 (69.6)
Kanamycin	23	7 (30.4)	11 (47.8)	5 (21.7)
Nalidaxic acid	23	7 (30.4)	6 (26.1)	10 (43.5)
Moxifloxacin	23	1 (4.3)	–	22 (95.7)
Cotrimazole	23	3 (13.0)	2 (8.7)	18 (78.3)
Tetracycline	23	2 (8.7)	6 (26.1)	15 (65.2)
Levofloxacin	23	–	–	23 (100)
Cefuroxime	23	23 (100)	–	–
Norfloxacin	23	4 (17.4)	4 (17.4)	15 (65.2)
Gentamycin	23	7 (30.4)	–	16 (69.6)
Streptomycin	23	19 (82.6)	1 (4.3)	3 (13.0)
Rifampicin	23	23 (100)	–	–
Azithromycin	23	7 (30.4)	5 (21.7)	11 (47.8)
Ciprofloxacin	23	3 (13.0)	4 (17.4)	16 (69.6)
Erythromycin	23	23 (100)	–	–
Ampicillin	23	23 (100)	–	–
Methicillin	23	23 (100)	–	–
P-Value		0.001		

The highest five antibiotics were 100% resistant against 23 different isolates, whereas only one antibiotic was 100% sensitive against all the isolates. In the 100% sensitivity and resistance scale of the antimicrobial test, resistant isolates were significantly higher than sensitive isolates.

the control group, nonconsumers. These data suggested that ice cream consumption has a greater role in food-borne diseases among school-going students in Bangladesh. The rate of antibiotics intake without any prescribed consultant and incomplete the recommended full courses of antibiotics is also a vital issue that influences the generation of antibiotic-resistant bacterial strains. It is generally recommended that patients complete their course of antibiotics even if they feel better, preventing reinfection with the same species and reducing the risk of becoming resistant to antibiotics [40].

Our experimental data regarding bacterial load showed high levels of TVC and TCC both in BSTI-approved and non-BSTI-approved ice cream samples, deviating from a permissible limit of BSTI standard (Figures 1(a) and 1(b)). A total of 93.75% ice cream samples were also possess fungal positive. However, such a high microbial load in the ice cream samples recorded in our study indicates the contamination of the samples by pathogenic microorganisms [28, 41–43]. The occurrences of indicator organisms in the ice cream constitute a severe threat to the ice cream consumers and are responsible for various food-borne diseases, including salmonellosis, hemorrhagic colitis, listeriosis, shigellosis *perfringens* poisoning, and campylobacteriosis.

In the overall prevalence study (Table 2), the most frequently isolated strains *Proteus* spp. and *E. coli* imply that they may have come from the contaminated water used in the ice cream industry. These types of pathogens cause a series of diseases like diarrhea and gastrointestinal illness [44]. Milk is the major source of *Staphylococcus aureus* in the dairy industry that cause numerous food-borne diseases by producing toxins, especially when the dairy product is kept at room temperature. In our study, we also found that the tested ice cream samples were contaminated by *Vibrio cholera*, possibly due to improper handling, undercooking, and washing with unhygienic water [45]. Contamination of ice cream by this bacterium may cause diarrheal diseases, nausea, stomach cramping, and fever among ice cream consumers. Milk, different raw materials, fruit juice, and fruits pulps are frequently used in the ice cream industry, which are significant sources of *Klebsiella* spp. and *Aeromonas* spp. and contribute to developing pneumonia, septicemia, wound infections, and gastroenteritis like diarrhea, abdominal pain, headache, vomiting, or fever [46, 47]. However, some other bacteria such as *Salmonella paratyphi, Citrobacter* spp., and *Shigella* spp. may contaminate ice cream from utensils, use of raw or undercooked components, and improper pasteurization process during ice cream production and may cause diarrhea, gastroenteritis, and vomiting among the consumers; school-going children [44]. Moreover, some of these identified bacterial species are also associated with neonatal sepsis and meningitis; few are the causes of sporadic pneumonia and sometimes cause milk-borne infections [48, 49].

The disc diffusion method showed a relatively higher antibiotic-resistance frequency among the identified bacterial isolates (Tables 3 and 4). Interestingly, we found that the most frequent isolated bacteria were highly resistant to cefuroxime, rifampicin, erythromycin, ampicillin, and methicillin, indicating that these commercial drugs did not possess antimicrobial activity and failed to halt the bacterial growth in human bodies. *Proteus* spp., *Aeromonas* spp., *and Plesiomonas* spp. were found to be resistant to cefuroxime, rifampicin, erythromycin, ampicillin, and methicillin but sensitive to levofloxacin, ciprofloxacin, norfloxacin, moxifloxacin, clotrimazole, and gentamycin. In contrast, the different antibiotic-resistant patterns reflect that the ice cream sample from the different retail shops might be contaminated with antimicrobial-resistant bacteria. The use of inadequate doses,

Table 4: In vitro antimicrobial efficacy against isolated bacterial strains from ice cream samples.

Antibiotics (17)	Proteusspp N (%R)	V.cholera N (%R)	S.Paratyphi N (%R)	Pseudomonaspp N (%R)	Shigellaspp N (%R)	Klebsiellaspp N (%R)	E. coli N (%R)	Aeromonasspp N (%R)	V.Parahemolyticus N (%R)	Citrobacterspp N (%R)	Plesiomonasspp N (%R)
Chloramphenicol	1 (25)	—	—	1 (50)	—	—	—	—	—	1 (100)	1 (100)
Kanamycin	—	2 (100)	1 (100)	—	1 (50)	1 (50)	—	—	1 (50)	—	1 (100)
Nalidaxic acid	—	2 (100)	—	1 (50)	1 (50)	—	—	2 (100)	—	—	1 (100)
Moxifloxacin	—	—	1 (100)	—	—	—	—	—	—	—	—
Cotrimazole	—	—	—	—	—	—	—	2 (100)	—	—	1 (100)
Tetracycline	—	—	—	—	—	—	—	1 (50)	—	—	1 (100)
Levofloxacin	—	—	—	—	—	—	—	—	—	—	—
Cefuroxime	4 (100)	2 (100)	1 (100)	2 (100)	2 (100)	2 (100)	4 (100)	2 (100)	2 (100)	1 (100)	1 (100)
Norfloxacin	—	—	—	—	—	—	4 (100)	—	—	—	—
Gentamycin	—	—	—	—	—	—	4 (100)	—	2 (100)	1 (100)	—
Streptomycin	3 (75)	2 (100)	—	2 (100)	—	2 (100)	4 (100)	2 (100)	2 (100)	1 (100)	1 (100)
Rifampicin	4 (100)	2 (100)	1 (100)	2 (100)	2 (100)	2 (100)	4 (100)	2 (100)	2 (100)	1 (100)	1 (100)
Azithromycin	1 (25)	—	1 (100)	1 (50)	—	—	1(25)	2 (100)	1 (50)	—	—
Ciprofloxacin	—	2 (100)	—	—	—	—	—	—	—	1 (100)	—
Erythromycin	4 (100)	2 (100)	1 (100)	2 (100)	2 (100)	2 (100)	4 (100)	2 (100)	2 (100)	1 (100)	1 (100)
Ampicillin	4 (100)	2 (100)	1 (100)	2 (100)	2 (100)	2 (100)	4 (100)	2 (100)	2 (100)	1 (100)	1 (100)
Methicillin	4 (100)	2 (100)	1 (100)	2 (100)	2 (100)	2 (100)	4 (100)	2 (100)	2 (100)	1 (100)	1 (100)
Number of S/R (%)	9/8 (53/47)	8/9 (47/53)	9/8 (53/47)	8/9 (47/53)	10/7 (65/35)	10/7 (59/41)	8/9 (47/53)	7/10 (41/59)	8/9 (47/53)	8/9 (47/53)	6/11 (35/65)

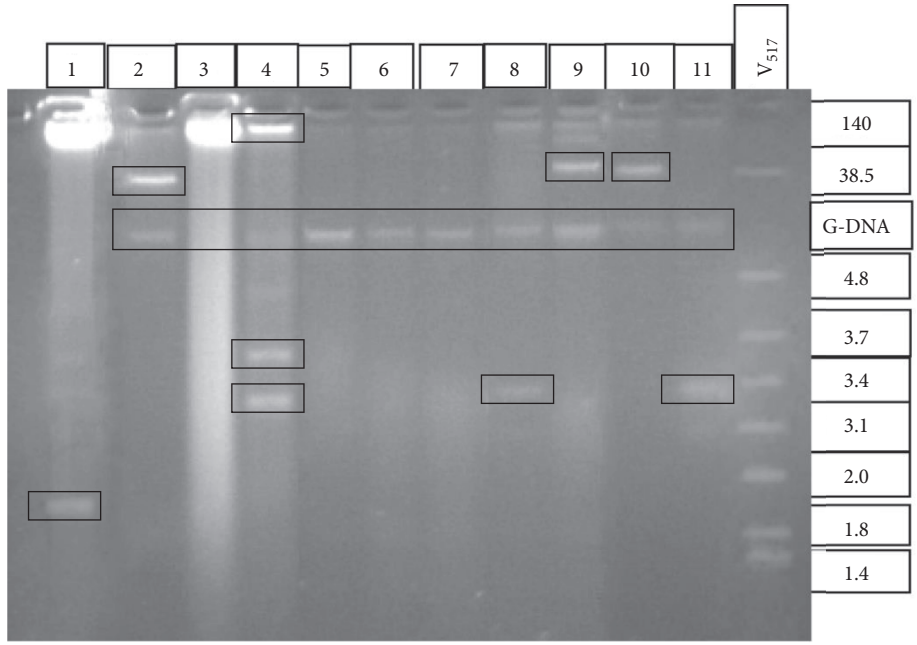

Figure 3: Gel electrophoresis of plasmid DNA from isolated bacteria. Numerous plasmid bands were found to be different sizes compared to plasmids in the corresponding *E.coli* strain V517. G-DNA expresses the genomic DNA of the bacterial isolates.

Table 5: Correlation between phenotype and genotype of antibiotics resistance.

Slot No.	Bacterial strain	Resistance phenotype pattern (Code)	No. of band agarose gel	Plasmid size (MDa)
1	*V. Paraheamolyticus*	CXM, MET, RIF, S, AMP, AZM, E	01	1.9
2	*Citrobacterspp*	C, CXM, MET, RIF, GEN, S, AMP, CIP, E	01	38.8
3	*Plesiomonaspp*	C, K, CXM, MET, COT, TE, RIF, S, AMP, E, NA	00	—
4	*Klebsiellaspp*	CXM, S, MET, RIF, AMP, E	03	140, 3.6 & 3.4
5	*V.cholerae*	K, NA, CXM, MET, RIF, S, CIP, AMP, E	00	--
6	*Pseudomonas spp*	CXM, S, RIP, E, AMP, MET	00	--
7	*S. Paratyphi*	K, MO, CXM, RIF, AZM, E, AMP, MET	00	--
8	*E.coli*	CXM, NOR, GEN, S, RIF, E, AMP, MET	01	3.3
9	*Aeromonasspp*	NA, COT, CXM, S, RIF, AZM, E, AMP, MET	02	38.50
10	*Proteus spp*	CXM, RIF, E, AMP, MET	02	38.8
11	*Shigellaspp*	CXM, RIF, E, AMP, MET	01	3.4

CXM (Cefuroxime), MET (Methicillin), RIF (Rifampicin), S (Streptomycin), AMP (Ampicillin), AZM (Azithromycin), E(Erythromycin), C (Chloramphenicol), GEN (Gentamycin), CIP (Ciprofloxacin), K (Kanamycin), COT (Cotrimazole), TE (Tetracycline), NA (Nalidaxic acid), MO (Moxifloxacin).

inappropriate prescribing, extensive agricultural use, and inadequate regulation of antibiotics intake may have a vital role in achieving and generating antibiotic-resistant genes to the commonly infected bacteria.

A significant correlation was also found between the phenotypes and genotypes of antibiotic resistance in bacteria that may indicate bacteria contain a pool of mobile genetic elements (Figure 3), and the transfer of antibiotic-resistance genes can easily occur between bacteria through conjugation or transformation. In this study, the isolated bacterial strains contain different sizes of plasmid DNA that may indicate the presence of antibiotic-resistance genes [23].

The phenotypic properties indicated that maximum bacteria were resistant to a couple of antibiotics (Table 5). Van TT (2007) conducted an experimental study and reported that high-molecular-weight plasmids are conjugative and contain many antibiotic-resistance genes [36]. Bacteria can also contain a number and size of plasmids for a long time and possesses antibiotic-resistant genes that can be transferred to the daughter cells in equal numbers [50]. Our electrophoresis data showed that the identified bacterial strains have large plasmids, so it may contain antibiotic-resistance genes and could have contributed to the spread of resistance genes. Previous studies have reported that plasmids with lower molecular weight are nonmotile or may lose during conjugation but have antibiotic-resistant genes [23, 51]. However, in this study, we found that some of the isolated strains did not possess any plasmid band in gel electrophoresis but showed their antibiotic-resistant pattern phenotypically. Therefore, we may conclude that some of these isolated bacterial strains indicated that multidrug-resistant determinants were due to chromosomal DNA or other reasons instead of plasmid DNA [23], and it could be a potential risk for public health issues.

The data of this study will provide essential information regarding safety and quality issues of ice cream and ice cream like food items. These may also help the regulatory authorities to take effective steps to adopt proper supervision during ice cream preparation and marketing for better children and public health management.

5. Conclusion

Most of the isolated bacterial strains from the ice cream samples are pathogenic. Therefore, it can be concluded that microorganisms in ice cream have the potential role in causing severe illness, increased risk of complications, and hospital admission by consuming these types of food items. The correlations between the phenotypic and the genotypic susceptibility were also explored that the majority of the antibiotic resistances were due to acquiring plasmid-carrying antibiotic-resistance genes. This situation is alarming for Bangladesh, where health care facilities, surveillance for antibiotics medication, and facilities to detect MDR are underdeveloped. Our results highlighted the need to improve the hygiene level during pre- and postproduction, distribution, and retail storage practices to ensure the microbiological safety of ice cream and other food items.

Data Availability

The data used to support all the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

AAM and MS contributed to the conceptualization and study designing; MS, MA, and MFH conducted all laboratory experiments, manuscript writing, and draft preparation; MS, MJI, and AI equally participated in data analysis, table and figure generation; SM and KI provided their logistics support; AAM participated in the writing, reviewing, editing, visualization, and supervision.

Acknowledgments

The authors would like to thank the Department of Biochemistry and Molecular Biology and the Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Santosh, Tangail, Bangladesh, for giving them the permission to use their lab facilities. They also thank Sourav Sarker and Dip Kumar for their support and help in the laboratory work.

Supplementary Materials

Materials and method. Isolation and biochemical identification: Bacterial colonies were isolated by the streak plate method on Mannitol salt agar and PALCAM Listeria identification Agar base mixed with Listeria selective supplement (HIMEDIA, India) for the isolation of Gram-positive bacteria *Staphylococcus aureus* and *Listeria* spp. Growth and morphological characteristics were recorded according to the Biology Libre Texts technique [30]. The isolated colonies were identified based on some biochemical assays including sugar fermentation, KIA, Motility, indole, urea, and citrate utilization tests [31]. Isolation and biochemical identification of Gram-positive bacteria *Staphylococcus aureus* and Listeria spp. from ice cream samples. Initially a total of five from sixteen ice cream samples (Kironmala, Doi Kulfi, Cocola Mango, Grameen Pipe, and Chocobar) were positive for bacteria culture and the rest of the samples did not show any colony (Supplemental Figure 1). After performing a biochemical assay, we identified that three (Kironmala, Doi Kulfi, and Chocobar) of the five colony-positive samples possess Gram-positive bacteria *Staphylococcus aureus* and the remaining two were unidentified (Supplemental Figure 2). On the other hand, we did not find any positive colony on the culture plate of PALCAM Listeria identification Agar base (mixed with Listeria selective supplement) media for the isolation and identification of Gram-positive bacteria Listeria (Supplemental Figure 3). (*Supplementary Materials*)

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