ABSTRACT

Bangladesh has a vibrant street food culture, with easily available ready-to-eat (RTE) foods. In this study, a total of 221 isolates were obtained from 141 Bangladeshi RTE food samples, such as fried, non-fried, and cooked foods; egg-, milk-, cereal-, and cream-based foods; pickles/achar; fruit; and RTE leaves, through culture on trypticase soy (for aerobic plate count), mannitol salt (MS for *Staphylococcus*), deoxycholate-hydrogen sulfide-lactose (DHL, for *Enterobacteriaceae*), and NaCl glycine Kim Goepfert agar (NGKG, for *Bacillus cereus*-like bacteria) agar plates. The aerobic plate counts ranged from undetectable to 8.5 log CFU/g. After enrichment with peptone water, contaminated bacteria detected on DHL, MS, and NGKG agar plates, from 77, 110, and 77 samples, respectively. Twenty out of 111 isolates on DHL agar, and 17 of 32 isolates on NGKG agar clearly showed resistance against three or more drugs. Through 16S ribosomal DNA sequencing analysis, six selected isolates from DHL agar were identified as *Pseudomonas nitroreducens*, *Citrobacter braakii*, *Klebsiella pneumoniae* subsp. *pneumoniae*, and *Serratia marcescens*. One selected isolate from NGKG agar was identified as *Bacillus cereus*-like bacteria. The results suggest that additional safety measures and regulations are necessary to ensure the quality and safety of the RTE foods in Bangladesh.

Keywords: antibiotic resistant bacteria, food safety, ready to eat food, street vendor.

I. INTRODUCTION

Bangladesh is one of the most densely populated countries in the world, and it has a vibrant street food culture, with ready-to-eat (RTE) foods, beverages, and snacks easily found at almost any time of the day [1]. The general Bangladeshi population’s traditional eating habits are changing due to rapid urbanization [2]. Changing lifestyles, including an increasing number of women working outside the home and changes in family structures, encourage more people to consume RTE foods than home-cooked food, which was the standard practice in the last century [3]. There are 128 varieties of street foods found in Dhaka, Bangladesh, among which chotpoti, bhelpuri, samucha, jhalmuri, daalpuri, lassi, pakura, and halim are the most popular [4]. Traditionally RTE foods and beverages are produced at the commercial or household level; unfortunately, such food items are sold by grocery stores, department stores, vendors, and supermarkets without supervision by authorities [5]. Bangladesh has long been facing problems associated with microbiological contamination of various foods due to the lack of awareness on hygiene followed by the defective legislative action [6].

In developing countries, bacterial diarrhoea causes high mortality [7]. Controlling microorganisms is essential to addressing the microbial safety issues associated with RTE foods. The World Health Organization estimates that foodborne and waterborne diseases together globally kill approximately 2.2 million people annually, including 1.9 million children [8], [9]. However, in Bangladesh, a dependable assessment of the public health impact of food contamination is not available due to the absence of a regular monitoring system. Limited data from the International Centre for Diarrhoeal Disease Research, Bangladesh, indicates that 501 hospital visits for diarrhoea treatment per day were attributable to food- and waterborne illnesses [10]. Multi-drug resistant (MDR) bacterial infections are a public health problem worldwide [11]. It can be considered that multi-drug resistance genes of bacteria found in foods can be horizontally transferred to human gut bacteria, including opportunistic pathogens [12], [13]. Many researchers have reported the role of street foods as vectors of pathogenic bacterial transmission to humans [14]. Most studies focus on microbiological and hygiene quality; however, only few efforts have been made to identify antibiotic-resistant organisms, although the status of multi-drug resistance plays an important role in fighting foodborne pathogens [15], [16]. Several studies have assessed foodborne pathogens in RTE foods in other countries. For example, among 154 foodborne *Staphylococcus aureus* isolates analysed in Turkey, 39 (25.3%) were found to be MDR [17]. In a study on vegetables collected in Switzerland, 78.3% (47/60) of samples contained MDR extended-spectrum β-lactamase-producing *Enterobacteriaceae* isolates [18]. In Bangladesh, some research teams have focused on the microbiological quality of RTE foods; however, few efforts have been made to determine antibiotic resistance levels and associated

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resistance mechanisms in these foods [19]. Therefore, in this study, to confirm the existence of multi-drug resistance and the potential contribution of food to the dissemination of antibiotic-resistant pathogens, various RTE foods (141 samples) were collected mainly from street vendors in the densely populated cities of Dhaka, Chittagong, and Cox’s Bazar in Bangladesh. The level of multi-drug resistance of a total of 221 isolates was determined using classical disc diffusion assay, and the strains having high resistance against multiple drugs were selected and identified.

II. MATERIALS AND METHODS

A. Collection of RTE Food Samples

A total of 141 food samples (Table I) were collected from randomly selected vendors operating in Dhaka, Chittagong, and Cox's Bazar between June and December 2019. All samples were handled aseptically during transportation and kept in sterile containers until they were prepared for bacteriological analysis. The information of each sample is shown in Table I. The products were preserved in the refrigerator (4 °C), except for the RTE tobacco. Food product pH was measured using a pH meter (B-711 LAQUAtwin, Horiba, Kyoto, Japan), and salinity was measured with a salinity analyser (B-721 LAQUAtwin, Horiba).

B. Aerobic Plate Counts

A 3 g sample of each food was homogenised in 30 mL of phosphate-buffered saline (Nissui Pharmaceutical, Tokyo, Japan) containing 0.1% (w/v) agar. The serial diluted samples (0.03 mL) were plated on trypticase soy agar (TSA) (Nippon Becton Dickinson, Tokyo, Japan) plates and incubated at 30 °C for isolation of aerobic bacteria. Plates of deoxycholate hydrogen sulfide lactose (DHL) agar (for Enterobacteriaceae, Eiken Chemical, Tokyo, Japan), mannitol salt (MS) agar (for Staphylococcaceae, Eiken Chemical, Tokyo, Japan), and NaCl-glycine Kim and Goepfert (NGKG) agar (for Bacillus cereus-like bacteria, Nissui Pharmaceutical) were incubated at 30 °C (NGKG) or 37 °C (DHL, MS) for 24 h under aerobic conditions. Viable cell counts were calculated from the number of developed colonies.

C. Isolation and Antibacterial Resistance Test

Each sample (3 g) was enriched at 37 °C for 18 h using 30 mL of buffered peptone water (BPW, Nippon Becton Dickinson) and streaked on DHL, MS, and NGKG agar. After 24 h incubation, as described above, typical colonies from the plates were isolated. The susceptibility of the isolates to different antimicrobial agents was determined with the disc diffusion assay using 10 or 11 antibiotic discs: ciprofloxacin (CPFX, 5 μg), ampicillin (ABPC, 10 μg), fosfomycin (FOM, 50 μg), ofloxacin (OFLX, 5 μg), tetracycline (TC, 30 μg), gentamicin (GM, 10 μg), vancomycin (VCM, 30 μg), levofloxacin (LVFX, 5 μg), chloramphenicol (CP, 30 μg), trimethoprim/sulfamethoxazole (ST, 23.75 μg/1.25 μg), and cefoxitin (CFX for methicillin-resistant staphylococci, 30 μg) (BD Sensi-Disc™, Nippon Becton Dickinson) on Mueller-Hinton agar (Oxoid, Basingstoke, UK). The assay allows the determination of bacterial resistance to the antibiotic on the disc, based on the relative diameter of the zone of inhibition surrounding the disc. Briefly, isolated colonies were inoculated into the nutrient broth, incubated at 37 °C for 24 h, and then spread (0.1 mL) on the test plates. The diameters of the inhibition zones (Fig. 2) were measured using precision callipers 24 h after incubation. The resistance was as resistance (R), intermediate resistance (IR), and susceptible (S) according to manual of the disk products and the British Society for Antimicrobial Chemotherapy (BSAC) standard disc susceptibility [20]. Additionally, among the R group, isolates that showed no clear zone were defined as no clear zone (NCZ).

**Table I: Values of pH and Salinity in Ready to Eat (RTE) Foods Distributed in Bangladesh**

| Groups                  | Sample name (number of samples) | Samples/ group | pH*            | Salinity (%) |
|-------------------------|---------------------------------|----------------|----------------|--------------|
| Cream-based food        | Cream bun (3), cream cake, layer cake, sandwich sponge cake, butter cake (2) | 8              | 5.58±0.50      | 0.90±0.27    |
| Egg-based food          | Egg cake (3), egg alor chop, vanilla egg bun, custard cake | 6              | 5.24±0.32      | 1.00±0.24    |
| Milk based food         | Lassi, matha, borhani, shandesh (2), dahi (3), laddu (2), shonpapri, modhu-sandesh, doi-fuska | 13             | 5.14±1.10      | 0.91±0.72    |
| Non fried dry food      | Toast, biscuit, dry cake (2), coconut fudge ball, pinuts cookies, vapa pitha (4), patishapta pitha (2), chitoi pitha, taler pitha | 15             | 5.81±0.79      | 1.05±0.25    |
| Fried dry food          | Fried pitha (3), jhal petesh, coconut khaja, velpuri (2), singara, samosa (2), nimki, piaju, anthon, egg chop | 14             | 5.44±0.61      | 0.61±0.27    |
| Cooked food             | Payesh, sujhi/vala (2), chicken roll (3), chicken samosa, chicken sandwich, chotpoti (4), fuska (5), noodles, pizza, crab-jhal | 20             | 4.67±0.46      | 1.18±0.42    |
| Fruit juice and liquid food | Mango juice, basil seed juice (2), aloe vera juice, sugarcane juice (3), shorbot, tetul juice (3), isabol juice. | 11             | 5.64±0.87      | 0.89±0.34    |
| Cereal based food       | Jilapi, pauruti, pantavaat, corn flakes, muri, jhal muri (3), puffed rice ball, naan, jorda vaat | 25             | 3.86±0.99      | 0.90±0.35    |
| Pickle/achar            | Boros/kal chani (9), mango achar (6), jam chatni (2), tamarind achar, pinapple chatni, mix fruits achar, dates achar (3), kasundi, guava pickle (paan) betel leaf with areca nut (8) | 8              | 4.53±0.29      | 0.67±0.31    |
| RTE betel leaves        | Swagat, kuber, zit, pan parag, pan masala (2), baba 120 plus, dilber, special gul, supari | 9              | 7.98±0.98      | 1.86±0.36    |
| Total                   |                                  |                | 48             |              |

*Values are expressed as the mean ± standard deviation
TABLE II: ViABLE BACTERIA COUNT AND DETECTION WITH THE SELECTIVE AGAR PLATES WITH ENRICHMENT IN THE RTE FOOD SAMPLES

| Groups                        | Detection samples | TSA counts (Log CFU/g) | DHL | MSA | NGKG |
|-------------------------------|-------------------|------------------------|-----|-----|------|
|                               |                   | Log CFU g⁻¹            |     |     |      |
|                               |                   | SD                     |     |     |      |
| Cream based food (n=8)         | Detected samples  | 8                      | 2   | 5   | 6    |
|                               |                   | Log CFU g⁻¹            | 4.92| 2.91|      |
|                               |                   | SD                     | 0.67| 0.12|      |
| Egg-based food (n=6)           | Detected samples  | 6                      | 2   | 1   | 5    |
|                               |                   | Log CFU g⁻¹            | 5.42| 4.3 | 8.11 |
|                               |                   | SD                     | 1.93| 2.14| 1.03 |
| Milk-based food (n=13)         | Detected samples  | 13                     | 7   | 9   | 5    |
|                               |                   | Log CFU g⁻¹            | 6.33| 4.89| 5.02 |
|                               |                   | SD                     | 1.71| 0.97| 1.78 |
| Non fried dry food (n=15)      | Detected samples  | 15                     | 8   | 8   | 8    |
|                               |                   | Log CFU g⁻¹            | 5.50| 5.37| 5.79 |
|                               |                   | SD                     | 1.44| 0.66| 0.44 |
| Fried dry food (n=14)          | Detected samples  | 14                     | 11  | 11  | 7    |
|                               |                   | Log CFU g⁻¹            | 6.23| 4.75| 6.21 |
|                               |                   | SD                     | 1.16| 1.77| 1.72 |
| Cooked food (n=20)             | Detected samples  | 19                     | 13  | 19  | 8    |
|                               |                   | Log CFU g⁻¹            | 6.54| 4.54| 6.66 |
|                               |                   | SD                     | 0.96| 1.20| 1.22 |
| Fruit juice and liquid food (n=12) | Detected samples | 12                    | 9   | 11  | 8    |
|                               |                   | Log CFU g⁻¹            | 5.71| 4.81| 4.45 |
|                               |                   | SD                     | 1.69| 1.38| 1.28 |
| Cereal based food (n=11)       | Detected samples  | 11                     | 3   | 6   | 1    |
|                               |                   | Log CFU g⁻¹            | 5.36| 7.13| 5.60 |
|                               |                   | SD                     | 1.96| 0.28| 1.51 |
| Pickle/Achar (n=25)            | Detected samples  | 19                     | 6   | 14  | 7    |
|                               |                   | Log CFU g⁻¹            | 5.52| 5.52| 4.41 |
|                               |                   | SD                     | 1.44| 1.17| 1.44 |
| RTE betel Leaves (n=8)         | Detected samples  | 8                      | 8   | 6   | 4    |
|                               |                   | Log CFU g⁻¹            | 7.11| 5.57| 5.32 |
|                               |                   | SD                     | 1.25| 2.27| 1.84 |
| RTE Tobacco (n=9)              | Detected samples  | 7                      | 1   | 5   | 1    |
|                               |                   | Log CFU g⁻¹            | 4.19| 4.14| 3.59 |
|                               |                   | SD                     | 1.11|     |      |

D. Identification of Selected Strains

In the case of isolates from DHL and NGKG agar plates, strains that showed NCZ to at least three classes of antimicrobial agents were selected. In the case of isolates from MS agar plates, bacteria that showed R to at least three classes of antimicrobial agents, including CFX, were selected. The selected isolates from DHL agar were preliminarily classified using commercial API ID 32E kits (BioMerieux, Marcy-l’Etoile, France) for bacterial identification according to the manufacturer’s instructions, and the data were analysed with apiweb™ (https://apilab.biomerieux.com/). Among that, 11 isolates that showed resistance to the largest having number of the antibiotics were identified based on their 16S rRNA gene sequences. Following the amplification of the 16S rRNA genes using the PCR primers 27F and 1492R, the PCR products were sequenced by Macrogen Japan Corp. (Tokyo, Japan). The homology search was performed using BLASTn, which was from the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/blast/blastn).

III. RESULTS AND DISCUSSION

A. Values of pH, Salinity, and Total Aerobic Plate Counts of RTE Food Samples

About the total 141 RTE food samples sold by street vendors in Bangladesh, the average pH of the 11 RTE food groups ranged from 3.86 to 7.98 (Table I). Zit and kuber (RTE tobacco) had the highest pH (9.80 and 9.70, respectively, Table II), whereas the lowest pH (3.86) was observed in the RTE pickle group. The groups’ salinity ranged from 0.53% to 1.86%, and the highest salinity (2.3%) was observed in borhani (fermented milk).

In this study, the average total aerobic plate counts (APCs) with TS agar plate of the RTE food groups ranged from 4.19 to 7.11 log CFU/g (Table II). Among the sample APCs, APCs for 6.4% (9/141) were <2 log CFU/g, 16% (22/141) ranged from 2 to 4 log CFU/g, 38% (53/141) ranged from 4 to 6 log CFU/g, and 40% (57/141) were >6 log CFU/g. The bacterial load of RTE betel leaf (7.11 log CFU/g) was the highest among the 11 types of RTE foods. However, several samples of other sample groups, such as egg-aloo chop, lassi, borhani, sandesh (milk-based sweet), samosa, chicken roll, crab jhal (curry taste), and basil seed juice, showed a high APC (>8 log CFU/g).

B. Antibiotic Resistance of Isolates

As summarised in Fig. 1A (raw data is shown in Table III), all 111 isolates formed NCZ in the presence of VCM, whereas 49%, 14%, and 9.0% of isolates formed NCZ in the presence of ABPC, FOM, TC, and ST, respectively. Resistance was most commonly observed in FOM (45%) and ABPC (23%). Furthermore, 34%, 18%, 17%, 14%, and 11% of isolates showed IR to GM, CP, CFX, ABPC, and FOM, respectively. Among the isolates, 15, 4, and 1 were resistant...
to three, four, and five antibiotics, respectively, as indicated by NCZ formation (Fig. 1B). Origin samples and the 20 isolates showing NCZ against three and more antibiotics are summarised in Table III. There was no correlation between the resistance and RTE food materials and sampling areas.

Preliminary classified names using API ID 32E kit are also shown in Table III. Seven, three, two, two, and two isolates were estimated as Enterobacter spp., Klebsiella spp., Escherichia coli, Citrobacter spp., Enterobacteriaceae spp., and Pseudomonas spp., respectively.

Fig. 1. Numbers of isolates from DHL agar (A, B), mannitol salt (MS) agar (C, D), and NGKG agar (E, F) plates showing no clear zone (NCZ: closed column), resistance (R: semi-closed column), and intermediate resistance (IR: open column) against antibiotics. CPFX: ciprofloxacin, ABPC: ampicillin, FOM: fosfomycin, OFLX: ofloxacin, TC: tetracycline, GM: gentamicin, VCM: vancomycin, LVFX: levofloxacin, ST: trimethoprim-sulfamethoxazole, CP: chloramphenicol, CFX: cefoxitin.

**TABLE III: ISOLATES FROM DHL AGAR PLATES SHOWING NO CLEAR ZONE (NCZ) AGAINST THREE OR MORE DRUGS**

| Groups                    | Sample Name | Area      | Strain ID | No clear zone | Resistant to | Classified names with API 32E |
|---------------------------|-------------|-----------|-----------|---------------|--------------|--------------------------------|
| Milk-based food           | Borhani     | Dhaka     | E-27      | ABPC, TC, VCM | FOM          | Enterobacter cloacae          |
|                           | Borhani     | Dhaka     | E-28      | ABPC, TC, VCM | FOM          | Enterobacter aerogenes        |
|                           | Martha      |           | E-21      | ABPC, FOM, VCM|              | Cronobacter malonaticus       |
| Non fried dry food        | Vapa pitha-2| Cox’sbazar| E-40      | ABPC, TC, VCM | FOM          | Escherichia coli              |
|                           | Vapa pitha-4| Cox’sbazar| E-41      | ABPC, TC, VCM | FOM          | Klebsiella pneumoniae         |
| Fried dry food            | Samosa-1    | Chittagong| E-55      | ABPC, FOM, VCM|              | Enterobacter aerogenes        |
|                           | Samosa-2    | Dhaka     | E-47      | ABPC, FOM, VCM|              | Enterobacter aerogenes        |
|                           | Nimki       | Dhaka     | E-53      | ABPC, FOM, VCM| TC           | Salmonella Typhimurium        |
|                           | Velpuri-1   | Dhaka     | E-49      | ABPC, FOM, VCM|              | Burkholderia cepacia          |
| Cooked food               | Fuska       | Chittagong| E-51      | ABPC, FOM, VCM| ST, CP       | Pseudomonas aeruginosa        |
|                           | Chotpoti-3  | Dhaka     | E-65      | ABPC, FOM, VCM| ST, CP       | Klebsiella pneumoniae         |
| Fruit juice and liquid    | Basil seed  | Chittagong| E-35      | ABPC, VCM, ST |              | Hafnia alvei                  |
| food                      | juice-1     |           |           |               |              |                                |
|                           | Fuska tok-1 | Chittagong| E-79      | ABPC, TC, VCM | ST           | Citrobacter freundii          |
| Cereal based food         | Jhal muri-1 | Chittagong| E-72      | ABPC, FOM, VCM|              | Enterobacter aerogenes        |
|                           | Jhal muri-3 | Dhaka     | E-104     | ABPC, FOM, VCM|              | Raoultella planticola         |
| Pickle/Achar              | Guava pickle| Cox’sbazar| E-84      | ABPC, TC, VCM | ST, CP       | Citrobacter braakii           |
|                           | Jam pickle-1| Chittagong| E-42      | ABPC, TC, VCM |              | Klebsiella pneumoniae         |
| RTE Leaves                | Paan-1      | Chittagong| E-30      | ABPC, VCM, ST |              | Escherichia coli              |
|                           | RTE Tobacco | Paan masala-2| E-82  | ABPC, FOM, VCM|              | Enterobacter cloacae          |

* CPFX: Ciprofloxacin, ABPC: Ampicillin, FOM: Fosfomycin, OFLX: Ofloxacin, TC: Tetracycline, GM: Gentamicin, VCM: Vancomycin, LVFX: Levofloxacin, ST: Trimethoprim-sulfamethoxazole, CP: Chloramphenicol.
Based on direct detection analysis, 64% (90/141) of samples were contaminated with *Staphylococcus aureae*-like bacteria, including 18% (26/141) with between 2 and 4 log CFU/g, and 19% (27/141) with >6 log CFU/g (Table II). Notably, the counts in egg-alnor chopp and one samosa were higher than 8 log CFU/g. With the enrichment culture, 78% (110/141) of the tested samples were positive for *Staphylococcus aureae*-like bacteria. As shown in Fig. 1C and Table IV, more than 70% of *Staphylococcus aureae*-like isolates were susceptible to seven antimicrobials, including SXT, GM, TC, LVFX, CFX, CP, and FOM. Resistance was most commonly observed to VM (59 isolates, 75%), ABPC (46 isolates, 58%), CFX (12 isolates, 15%), TC (11 isolates, 14%), and FOM (6 isolates, 8%). NCZ formation in the presence of FOM was observed for 7 isolates, 16 of 79 isolates were resistant to at least 3 different classes of antimicrobials, and 7 isolates (8.8%) were resistant to more than 4 antimicrobials (Fig. 1D). Origin samples and the selected 15 isolates showed apparent R against three and more antibiotics, including CFX, summarised in Table IV. There was no correlation between the resistance and RTE food materials and sampling areas. Strain S-54 showed R against the most (seven) of the antibiotics. As shown in Table II, based on direct detection analysis, 36% (52/141) of samples were contaminated with bacteria detected on NGKG agar, including 9.9% (14/141) with counts between 2 and 4 log CFU/g, and 6.4% (9/141) with counts >6 log CFU/g. After enriching the 141 food samples, 55% (77/141) of samples showed presence of bacteria that grew on NGKG agar. From the NGKG agar plates, a total of 32 typical *B. cereus*-like morphological (White turbid colony and red discoloration of the medium) colonies were selected and isolated. Of these, 81%, 72%, and 69% formed NCZs in the presence of ABPC, ST, and FOM, respectively (Fig. 1E and Table IV). Fifteen isolates (48.4%) resistant to three antibiotics and one isolate (3.2%) resistant to four antibiotics formed NCZs and were considered MDR (Fig. 1F). Origin samples and the selected 17 isolates showing NCZ against three and more antibiotics are summarised in Table IV. There was no correlation between the resistance and RTE food materials and sampling areas. Besides one strain (B-6), most strains were observed as gram-positive rods with spores. Strain B-19 showed NCZ with four antimicrobial drugs.

One of the most critical aspects of food safety is the emergence of antimicrobial-resistant bacterial strains [21]. Resistant strains are capable of horizontal transfer of resistance genes from environmental and animal food sources to normal human indigenous microbiome and pathogens through the food chain [22], [23]. VCM is regarded as a drug for gram-positive bacteria as it cannot penetrate their outer membrane of gram-negative bacteria [24]. In this study, all isolates from DHL agar (mainly *Enterobacteriaceae*) showed NCZ with VCM (Fig. 1A). Most of the isolates from MS and NGKG showed clear “R” against VCM (Fig. 1C and E). Furthermore, VCM has been regarded as a silver bullet against MDR gram-positive bacteria; however, issues with VCM-resistant gram-positive bacteria, not only enterococci but also *Staphylococcus aureae*, have been reported [25].

In this study, most isolates from NGKG agar and a proportion of isolates from DHL agar showed NCZ with ABPC, FOM, and ST (Fig. 1A and E). Resistances of *Bacillus spp.* against these antibiotics has previously been reported [26]. Additionally, an increase in ampicillin-resistant *Enterobacteriaceae* is regarded as an alarming issue. Of note, adverse effects of ampicillin dosage on ampicillin resistance in *Enterobacteriaceae* in swine faeces were reported [27]. Moreover, 16/78 isolates from MS agar showed CFX resistance (Fig. 1C). CFX resistance is regarded as a surrogate marker for detecting methicillin-resistant *Staphylococcus aureus* [28]. The present study’s results suggest that antibiotic-resistant bacteria are ubiquitous in RTE foods distributed in Bangladesh.

C. Identification of Selected Strains with 16S rRNA Gene Sequencing

Finally, six, three, and one typical and selected MDR isolates from DHL (E-6, 29, 51, 65, 79, and 84 in Table III), MS (S-3, 54 and 57 in Table IV), and NGKG (B-19 in Table IV) agar plates, respectively, were subjected to 16S rRNA gene sequencing to confirm the results. The selected isolates from DHL were identified as four *Enterobacteriaceae* species — *Klebsiella pneumoniae* subsp. *pneumoniae* (E-65, Accession number: LC572262), *Enterobacter cloacae* (E-79, LC572263), *Citrobacter braakii* (E-84, LC572264) and *Serratia marcescens* (E-29, LC572258) — and two species of *Pseudomonas*: *P. plecoglossicida* (E-6, LC572260) and *P. nitroreducens* (E-51, LC572261). Three isolates from MS agar having resistance to CFX and three other antibiotics were identified as two *Staphylococcus* species — *S. gallinarum* (S-3, LC572265) and *S. sciuri* (S-54, LC572267) — and *Aerococcus viridans* (S-57, LC572266). The rRNA sequence of a strain isolated from NGKG (B-19, LC572259) was similar (99.7%) to those of several species of *B. cereus*-like bacteria. With 16S rDNA and BLASTn search, four MDR *Enterobacteriaceae* isolates in this study were identified as *K. pneumoniae*, *E. cloacae*, *C. braakii*, and *S. marcescens*. These were reported as indigenous bacteria and opportunistic virulent bacteria in *pneumoniae*, *bacteraemia*, and other infections [29]. The correlation of the virulence and antibiotic-resistant properties is essential for the therapy, though the interplay between resistance and virulence is not understood well [30]. There are reports on antibiotic resistance of *P. plecoglossicida* and *P. nitroreducens* in aquaculture, wastewater, sludge, and soils [31]. The excretion of antibiotics into these environments and the consequent development of resistance are regarded as a major issue in the food supply. The MDR *S. gallinarum* and *S. sciuri* were detected in both farms and hospitals, and their susceptibility against drugs, including CFX, varies [32]. Furthermore, *B. cereus* and the related species have been isolated from RTE foods [33].

The current study indicates the poor microbiological quality of several RTE food types and food products prepared by vendors and sold in open markets or supermarkets in Bangladesh. Some of the bacterial species isolated as MDR in the present study and related species have been reported as pathogenic bacteria; however, most isolated bacteria have no virulence. However, as mentioned earlier, horizontal gene transfer of antibiotic resistance to pathogenic bacteria is possible. The present study’s results suggest that additional safety measures and regulations are necessary for the preparation and manufacture of RTE foods to ensure food quality of several RTE food types and food products prepared by vendors and sold in open markets or supermarkets in Bangladesh.
quality and safety. Genetic analyses of multi-drug resistance of the selected strains by whole genome sequencing are in progress.

| TABLE IV: ISOLATES FROM MS AND NGKG AGAR PLATES SHOWING NO CLEAR ZONE (NCZ) AND RESISTANCE (R) AGAINST THREE OR MORE DRUGS |
|---|---|---|---|---|---|
| Isolated from | Groups | Sample Name | Area | Strain ID | No clear zone to* | Resistant to* |
| MS agar | Cream based food | Cream bun-2 | Dhaka | S-3 | FOM | CPFX, ABPC, VCM, CFX |
| | Egg-based food | Egg-Alor chop | Dhaka | S-9 | ABPC, FOM, OFLX, VCM, CFX |
| | Milk-based food | Sweet dahi | Chittagong | S-15 | ABPC, FOM, VCM, CFX |
| | Non fried dry food | Vapa pitha-3 | Chittagong | S-27 | ABPC, FOM, VCM, CFX |
| | Patishapta pitha-2 | Dhaka | S-30 | FOM, TC, VCM, CFX |
| | Fried dry food | Egg chop | Dhaka | S-43 | FOM, VCM, CFX |
| | | Anthong | Dhaka | S-44 | FOM, VCM, CFX |
| | Cooked food | Suji/Halwa-2 | Dhaka | S-46 | FOM | ABPC, VCM, CFX |
| | | Fuska-1 | Chittagong | S-47 | FOM | ABPC, VCM, CFX |
| | | Fuska-3 | Dhaka | S-49 | FOM | VCM, CFX |
| | | Chicken roll-1 | Dhaka | S-52 | FOM, VCM, CFX |
| | | Chotpoti-1 | Chittagong | S-57 | FOM | ABPC, VCM, CFX |
| | Fruit juice and liquid food | Shorbath | Cox’sbazar | S-50 | FOM | ABPC, VCM, CFX |
| | Cereal based food | Jhal muri-3 | Dhaka | S-62 | ABPC, FOM, VCM, CFX |
| | RTE Leaves | Paan-2 | Cox’sbazar | S-64 | ST | CPFX, OFLX, GM, VCM, LVFX |
| NGKG agar | Egg-based food | Egg Cake-2 | Dhaka | B-3 | ABPC, FOM, ST | TC, VCM |
| | | Egg-Alor chop | Dhaka | B-6 | ABPC, FOM, TC, ST | VCM |
| | Milk-based food | Lassi | Chittagong | B-7 | ABPC, FOM, ST | VCM |
| | Non fried dry food | Vapa pitha-2 | Cox’sbazar | B-10 | ABPC, FOM, ST | TC, VCM |
| | | Vapa pitha-4 | Cox’sbazar | B-11 | ABPC, FOM, ST | TC, VCM |
| | | Chitpai pitha | Cox’sbazar | B-12 | ABPC, FOM, ST | TC, VCM |
| | | Patishapta pitha-2 | Dhaka | B-13 | ABPC, FOM, ST | VCM |
| | Fried dry food | Samosa-2 | Dhaka | B-17 | ABPC, FOM, ST | VCM |
| | | Anthong | Dhaka | B-18 | ABPC, FOM, ST | VCM |
| | Cooked food | Fuska-3 | Dhaka | B-19 | ABPC, FOM, ST, TC | VCM |
| | | Chicken roll-2 | Chittagong | B-21 | ABPC, FOM, ST | VCM |
| | | Chotpoti-3 | Dhaka | B-22 | ABPC, FOM, ST | TC, VCM |
| | Fruit juice and liquid food | Basil seed juice-1 | Chittagong | B-23 | ABPC, FOM, ST | VCM |
| | | Aloe vera juice | Chittagong | B-28 | ABPC, FOM, ST | VCM |
| | | Sugarcane juice-2 | Dhaka | B-29 | ABPC, FOM, ST | VCM |
| | Cereal based food | Jhal muri-1 | Chittagong | B-30 | ABPC, FOM, ST | VCM |
| | RTE Tobacco | Pan masala-2 | Chittagong | B-31 | ABPC, FOM, ST | TC, VCM |

* CFLEX: Ciprofloxacin, ABPC: Ampicillin, FOM: Fosfomycin, OFLX: Ofloxacin, TC: Tetracycline, GM: Gentamicin, VCM: Vancomycin, LVFX: Levofloxacin, ST: Trimethoprim-sulfamethoxazole, CP: Chloramphenicol, CFX: Cefoxitin

CONFLICT OF INTEREST

No conflict of interest declared.

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