Prevalence of Antibiotic Resistant Bacteria in Ready to eat Food Sold by Street Vendors in Bangladesh

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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 *Staphylococcaceae*), deoxycholate-hydrogen sulfide-lactose (DHL, for *Enterobacteriaceae*), and NaCl glycine Kim Goeppert 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.

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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, bhel puri, samucha, jhalmuri, daal puri, 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

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 (TS) agar (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 petiesh, coconut khaja, velpuri (2), singara, samosa (2), nimki, piaju, anthon, egg chop	14	5.44±0.61	0.61±0.27
Cooked food	Payesh, suji/halwa (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), isabgol juice.	12	4.54±0.85	0.53±0.49
Cereal based food	Jilapi, pauruti, pantavaat, corn flakes, muri, jhal muri (3), puffed rice ball, naan, jorda vaat	11	5.64±0.87	0.89±0.34
Pickle/achar	Boroi/kul chatni (9), mango achar (6), jam chatni (2), tamarind achar, pineapple chatni, mix fruits achar, dates achar (3), kasundi, guava pickle	25	3.86±0.99	0.90±0.35
RTE betel leaves	(paan) betel leaf with areca nut (8)	8	4.53±0.29	0.67±0.31
RTE tobacco	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		141		

*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		Viable counts (Log CFU/g)				Detected No. after enrichment		
		TSA	DHL	MSA	NGKG	DHL	MSA	NGKG
Cream based food (n=8)	Detected samples	8	2			5	6	7
	Log CFU g ⁻¹	4.92	2.91					
	SD	0.67	0.12					
Egg-based food (n=6)	Detected samples	6	2	1	5	5	6	6
	Log CFU g ⁻¹	5.42	4.3	8.11	4.5			
	SD	1.93	2.14		1.03			
Milk-based food (n=13)	Detected samples	13	7	9	5	8	10	9
	Log CFU g ⁻¹	6.33	4.89	5.02	5.79			
	SD	1.71	0.97	1.78	0.13			
Non fried dry food (n=15)	Detected samples	15	8	8	5	10	8	8
	Log CFU g ⁻¹	5.50	5.37	5.79	6.13			
	SD	1.44	0.66	0.44	0.22			
Fried dry food (n=14)	Detected samples	14	11	11	7	10	12	8
	Log CFU g ⁻¹	6.23	4.75	6.21	4.99			
	SD	1.16	1.77	1.72	1.37			
Cooked food (n=20)	Detected samples	19	13	19	8	12	19	9
	Log CFU g ⁻¹	6.54	4.54	5.66	4.98			
	SD	0.96	1.20	1.22	1.07			
Fruit juice and liquid food (n=12)	Detected samples	12	9	11	8	5	10	9
	Log CFU g ⁻¹	5.71	4.81	4.45	5.04			
	SD	1.69	1.38	1.28	1.48			
Cereal based food (n=11)	Detected samples	11	3	6	1	5	9	6
	Log CFU g ⁻¹	5.36	7.13	5.60	3.12			
	SD	1.96	0.28	1.51				
Pickle/Achar (n=25)	Detected samples	19	6	14	7	8	15	7
	Log CFU g ⁻¹	5.52	5.52	4.41	4.41			
	SD	1.44	1.17	1.44	1.47			
RTE betel Leaves (n=8)	Detected samples	8	8	6	4	7	8	4
	Log CFU g ⁻¹	7.11	5.57	5.32	4.8			
	SD	1.25	2.27	1.84	1.32			
RTE Tobacco (n=9)	Detected samples	7	1	5	1	2	7	4
	Log CFU g ⁻¹	4.19	4.14	3.59	4.69			
	SD	1.15		1.11				
Total detected samples		132	70	90	51	77	110	77

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://apiweb.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://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-alor 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, CPFX, 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, 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 to*	Resistant to*	Classified names with API 32E
Milk-based food	Borhani	Dhaka	E-27	ABPC, TC, VCM	FOM	<i>Enterobacter cloacae</i>
	Borhani	Dhaka	E-28	ABPC, TC, VCM	FOM	<i>Enterobacter aerogenes</i>
	Mattha		E-21	ABPC, FOM, VCM		<i>Cronobacter malonaticus</i>
	Doi-fuska	Chittagong	E-29	ABPC, FOM, VCM, ST	FOM, TC	<i>Pseudomonas aeruginosa</i>
Non fried dry food	Vapa pitha-2	Cox'sbazar	E-40	ABPC, TC, VCM	FOM	<i>Escherichia coli</i>
	Vapa pitha-4	Cox'sbazar	E-41	ABPC, TC, VCM	FOM	<i>Klebsiella pneumoniae</i>
Fried dry food	Samosa-1	Chittagong	E-55	ABPC, FOM, VCM	CPFX	<i>Enterobacter aerogenes</i>
	Samosa-2	Dhaka	E-47	ABPC, FOM, VCM		<i>Enterobacter aerogenes</i>
	Nimki	Dhaka	E-53	ABPC, FOM, VCM	TC	<i>Salmonella Typhimurium</i>
	Velpuri-1	Dhaka	E-49	ABPC, FOM, VCM		<i>Burkholderia cepacian</i>
Cooked food	Fuska-1	Chittagong	E-51	ABPC, FOM, VCM, ST, CP		<i>Pseudomonas aeruginosa</i>
	Chotpoti-3	Dhaka	E-65	ABPC, TC, VCM, ST	CPFX, FOM	<i>Klebsiella pneumoniae</i>
Fruit juice and liquid food	Basil seed juice-1	Chittagong	E-35	ABPC, VCM, ST		<i>Hafnia alvei</i>
	Fuska tok-1	Chittagong	E-79	ABPC, TC, VCM, ST		<i>Citrobacter farmer</i>
	Cereal based food	Jhal muri-1	Chittagong	E-72	ABPC, FOM, VCM	
Jhal muri-3		Dhaka	E-104	ABPC, FOM, VCM		<i>Raoultella planticola</i>
Pickle/Achar	Guava pickle	Cox'sbazar	E-84	ABPC, TC, VCM, ST	CPFX, OFLX, LVFX	<i>Citrobacter braakii</i>
	Jam pickle-1	Chittagong	E-42	ABPC, TC, VCM	CPFX, FOM	<i>Klebsiella pneumoniae</i>
RTE Leaves	Paan-1	Chittagong	E-30	ABPC, VCM, ST		<i>Escherichia coli</i>
RTE Tobacco	Paan masala-2	Chittagong	E-82	ABPC, FOM, VCM		<i>Enterobacter cloacae</i>

* CFFX: 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 *Staphylococcaceae*-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-alor chop and one samosa were higher than 8 log CFU/g. With the enrichment culture, 78% (110/141) of the tested samples were positive for *Staphylococcaceae*-like bacteria. As shown in Fig. 1C and Table IV, more than 70% of *Staphylococcaceae*-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 aureus*, 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 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
		Anthon	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-54	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
	Fried dry food	Chittoi pitha	Cox'sbazar	B-12	ABPC, FOM, ST	TC, VCM
		Patishapta pitha-2	Dhaka	B-13	ABPC, FOM, ST	VCM
		Samosa-2	Dhaka	B-17	ABPC, FOM, ST	VCM
		Anthon	Dhaka	B-18	ABPC, FOM, VCM	TC
		Fuska-3	Dhaka	B-19	ABPC, FOM, ST, TC	
	Cooked food	Chicken roll-2	Chittagong	B-21	ABPC, FOM, ST	VCM
		Chotpoti-3	Dhaka	B-22	ABPC, FOM, ST	TC, VCM
		Basil seed juice-1	Chittagong	B-23	ABPC, FOM, ST	VCM
	Fruit juice and liquid food	Aloe vera juice	Chittagong	B-28	ABPC, FOM, ST	VCM
		Sugarcane juice-2	Dhaka	B-29	ABPC, FOM, ST	VCM
		Jhal muri-1	Chittagong	B-30	ABPC, FOM, ST	VCM
	Cereal based food	Pan masala-2	Chittagong	B-31	ABPC, FOM, ST	TC, VCM

* CFFX: 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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