JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

ORIGINAL ARTICLE

J Adv Biotechnol Exp Ther. 2025 Jan; 8(1): 18-31
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2025.d02
Published by www.bsmiab.org

Occurrence of multi-antibiotic resistant bacteria isolated from food handlers' hands and utensils at different restaurants in Dhaka, Bangladesh

Sumi Akter[1], Ratna Akter[2], Md. Abdus Salam[3,*]

[1]Department of Botany, Jagannath University, Dhaka, Bangladesh
[2]Department of Marketing, University of Development Alternative, Dhaka, Bangladesh
[3]Directorate of Secondary and Higher Education, Ministry of Education, Dhaka, Bangladesh

***Corresponding author**
Md. Abdus Salam
Directorate of Secondary and Higher Education, Ministry of Education, Dhaka, Bangladesh.
Email: sami.11081978@gmail.com

Academic editor
Md Nurul Haider Rasel, PhD
Bangladesh Agricultural University, Bangladesh

Article info
Received: 09 July 2024
Accepted: 22 October 2024
Published: 12 November 2024

Keywords
Bangladesh, Food handlers, Multidrug-resistant bacteria, MDR, Restaurants, Utensils

ABSTRACT

Restaurant customers consume food contaminated by germs, increasing the risk of foodborne disease. In this investigation, food handlers' hands and utensils from many restaurants near Sadarghat launch station in Dhaka, Bangladesh, were tested to determine bacterial contamination incidence. Standard procedures were followed in isolation and identification of bacterial isolates. Of the 60 analyzed swabs, 46.7% showed a positive culture for one or more potential bacterial contaminants. The occurrence of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Vibrio cholerae* in the hands of food handlers was 50%, 33.4%, 33.4%, and 23.4%, respectively, and those in utensils was 33.4%, 16.7%, 16.7%, and 10%, respectively. The majority of tested isolates of *P. aeruginosa* (100%) showed the highest resistance phenomenon to ampicillin and tetracycline, *V. cholerae* (100%) to ampicillin, *E. coli* (80%) to ampicillin and streptomycin, and *S. aureus* (100%) to ciprofloxacin. All of the examined isolates of *P. aeruginosa* were found to be most sensitive to piperacillin-tazobactam, *E. coli* to imipenem and nitrofurantoin, *S. aureus* to gentamicin, amikacin, tetracycline and chloramphenicol, and *V. cholerae* to piperacillin-tazobactam, imipenem and amikacin. Remarkably, 100% of *V. cholerae* isolates were multidrug-resistant (MDR), with the highest multiple antibiotic resistance index (MARI) score of 0.428. Among the examined isolates, 75% of bacterial isolates showed MDR phenotype, while 90% of isolates recorded a MARI value greater than 0.2. Due to the increased risk of food contamination or cross-contamination from food handlers' hands and utensils, the findings of this study indicate that restaurant hygiene standards are inadequate and might have detrimental effects on the general public's health. Consequently, this highlighted the significance of further training to enhance food workers' understanding of proper hand-washing techniques.

INTRODUCTION

Restaurant customers consume food contaminated by germs, increasing the risk of foodborne disease [1]. Globally, foodborne illness is thought to be a serious public health concern [2, 3]. According to World Health Organization (WHO) data as of April 30, 2020, 420,000 people annually die out because of foodborne illnesses, and about one in ten individuals become sick as a result of eating food that was contaminated [4]. In Bangladesh, foodborne diseases result in around 30 million illnesses annually [5]. Food handlers are a major source of foodborne disease transmission and a health risk to the general public [4]. According to WHO definitions, food handlers handle food or food-related items throughout their regular workdays or may come into touch with handling food and utensils that are not intended for personal use [3, 4, 6]. Food handlers may introduce bacteria into the food through their skin, nasal secretions, and bowel movements, as well as through contaminated food that they prepare or serve [3]. Food contamination can come from a variety of sources, but food workers' hands, the

most common source of microorganisms to contaminate food, have been demonstrated to act as vectors in the spread of foodborne illness due to poor personal hygiene, a lack of knowledge and practice regarding food hygiene, or cross-contamination [3, 7, 8, 9].

People frequently come into contact with their surroundings through their hands. As a result, different bacteria can enter the mucous membranes of the mouth, nose, eyes, and genitalia through them, which can lead to an epidemic of foodborne sickness [10]. Unclean utensils, inadequate storage cleaning, food preparation spaces, and the hands of food workers can all contaminate raw and cooked meals [11, 12]. Neglecting hygiene and handling food improperly can lead to an increase in infections, which can ultimately lead to consumer sickness. About 10% to 20% of foodborne illness outbreaks are caused by food handler contamination [8, 13]. Further, 97% of food poisoning cases were caused by workers in food service sectors handling food improperly [14, 15]. According to statistics, the catering business is responsible for 70% more food poisoning cases than any other industry [14]. It has been reported that about 89% of outbreaks were caused by contaminated foods, and pathogens were transferred to food by workers' hands [16]. According to previous reports, foodborne disease encompasses a broad spectrum of illnesses caused by bacterial, viral, parasite, or chemical contamination of food [4, 17]. The most frequent cause of foodborne diseases is pathogenic bacteria [18]. One typical way that bacterial infections are spread and cause foodborne illnesses is through fecal-oral transmission. Further, hands, nails, food, and water can all serve as vectors for the spread of these illnesses [4].

Previous research has demonstrated that the hands of food workers are considerably contaminated with a variety of dangerous bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter*, *Klebsiella*, *Pseudomonas areugenosa*, and *Vibrio* spp. [3, 4, 8, 14]. On the hand surface, *S. aureus* is a common colonizer. Unclean hands sometimes cause episodes of diarrheal sickness because bacteria like *E. coli* and *S. aureus*, which are frequently linked to foodborne illnesses, may persist on hand surfaces for days [4]. These bacteria have been linked to foodborne infections, which have increased patient mortality and morbidity, prolonged hospital stays, and increased patient cost burden [4]. An estimated 600 million illnesses and 420,000 fatalities are attributed to diarrheal diseases each year, resulting in the loss of 33 million healthy daily life years [4].

Numerous food sellers run their businesses in railway stations, bus terminals, markets, industrial zones, and streets [19]. One of Bangladesh's biggest and busiest river ports is Sadarghat Launch Terminal, which is located in the southern portion of Dhaka the city. Hundreds of boats and launches arrive at and depart from Sadarghat every day, mostly enabling connectivity with the areas to the south. Numerous businesses, including wholesale stores, street food vendors, restaurants, and fruit and vegetable markets, are founded around this bustling port [20]. Thousands of travelers travel every day for their doctrine of necessity. A vast number of travelers have food at various restaurants around those places. Unwashed hands of food handlers and unclean utensils can spread germs directly or indirectly to people, and consequently, a large proportion of travelers may suffer by verities of foodborne diseases. To the best of our knowledge, no previous study exclusively emphasizes this area and research point. The goal of this study was to identify and isolate bacteria from the hands of food handlers and utensils, as well as to determine the prevalence of bacterial contamination among them in various restaurants near the Sadarghat launch station in Dhaka, Bangladesh.

MATERIALS AND METHODS

Study area

The research locations were a number of street restaurants near Sadarghat launch terminal in Dhaka, Bangladesh.

Sample collection

From 15 different street restaurants near the Sadarghat launch terminal in Dhaka, Bangladesh, a total of 60 swab samples of food workers' hands and utensils were collected. Four samples were obtained from each restaurant by collecting samples of the two person's hands and utensils. Samples were taken by a sterile cotton wool swab moistured in 5 ml of phosphate buffer solution (PBS) (HIMEDIA, M1452, Pune) from different parts of the palm, between fingers, fingernails of each food handlers' and surface of dishes for bacteriological analysis. Following that, the swab was put back into its sterile PBS (HIMEDIA, M1452, Pune) tube, which was then labeled and sealed with adhesive tape.

This study was approved by the Institutional Ethical Review Committee (ERC) of Jagannath University, Dhaka, Bangladesh (No. JnU/ERC/2023/010).

Transportation of collected samples

The collected samples were moved to the lab in an ice-packed cool box. The temperature during storage was kept within 0-4°C, and the transportation time to the laboratory was within 15 minutes to 1 hour. When the samples arrived at the lab, the analyses were started right away.

Identification of *P. aeruginosa* and *E. coli*

The swab stick containing the sample was inoculated onto Cetrimide agar (CA; Himedia, Mumbai, India) for isolating the *P. aeruginosa* and MacConkey agar (MAC; Himedia, Mumbai, India) of *E. coli*. Following that, the inoculation plates were incubated for around two days at 37 °C. The morphological traits, color, and texture of the colonies growing on this medium were examined after 24 and 48 h of incubation. *E. coli* bacteria were considered as pink or brick red colonies on MAC (Himedia, Mumbai, India) agar and green colonies on CA (Himedia, Mumbai, India) agar as *P. areugenosa*. Pink or brick red color colonies picked from MAC agar (Himedia, Mumbai, India) were then subsequently screened and subcultured once more on plates of MAC agar (Himedia, Mumbai, India) and Eosin Methylene Blue (EMB; Oxoid, Basingstoke, England) agar. For twenty-four hours, all plates were incubated at 37 °C. The distinct bacterial isolates seen on the selective media plates following the incubation period were pink on MAC agar (Himedia, Mumbai, India) and dark centered with a green metallic sheen on EMB (Oxoid, Basingstoke, England) agar. For precise identification, isolated strains of *P. aeruginosa* and *E. coli* were subjected to microscopic, physiological, and biochemical examinations [21, 22]. The tryptic soy agar (TSA; Himedia, Mumbai, India) plates were used to maintain the identified bacterial isolates, and Luria Bertani (LB) broth (Himedia, Mumbai, India) supplemented with 30% glycerol (Nepa, Tangerang, Indonesia) for preserving it.

Identification of *S. aureus* and *V. cholerae*

The sample containing the swab was inoculated onto Mannitol Salt Agar (MSA; Himedia, Mumbai, India) to isolate the *S. aureus*. After incubating at 37 °C for about 48 h, yellow-color colonies considered as *S. aureus* were observed on MSA (Himedia, Mumbai, India) plates. After that, the swab containing the sample was placed into incubation for overnight at 37 °C after being dipped into 5 ml of alkaline peptone water with a pH of 8.5. Finally, thiosulfate citrate bile sucrose agar plates (TCBS; Merck, Darmstadt, Germany) were infected with a loopful of pre-enrichment and incubated for 24 h at 37 °C. Vibrios have been described as yellow, green, and/or blue-green colonies that appeared on TCBS agar (Merck, Darmstadt, Germany). Single yellow-colored colonies were picked up for patch inoculation on TCBS (Merck, Darmstadt, Germany) and CHROMagar™ (CHROMagar, Paris, France) media, and they were incubated at 37°C for a full day period. Colonies that appeared yellow on TCBS (Merck, Darmstadt, Germany) and green-blue to turquoise blue on CHROMagar™ (CHROMagar, Paris, France) medium were considered as *V. cholerae*. Afterward, the chosen bacterial strains were subsequently injected into Gelatin Agar (GA; Himedia, Mumbai, India) plates in order to assess the gelatinase enzyme's activity for further confirmation. Luria Bertani (LB) broth (Himedia, Mumbai, India) with 30% glycerol (Nepa, Tangerang, Indonesia) was employed for preserving the pure culture of all bacterial isolates, whereas TSA (Himedia, Mumbai, India) plates were utilized for maintenance. Following the previously established methodology [21, 22], isolated bacterial strains of *S. aureus* and *V. cholerae* were identified by a series of physiological and biochemical tests and subsequently by PCR method.

For further molecular identification, isolated colonies of *S. aureus* on MSA (Himedia, Mumbai, India) and *V. cholerae* on TCBS (Merck, Darmstadt, Germany) and CHROMagar™ (CHROMagar, Paris, France) plates were chosen. One ml of a pure culture of *S. aureus* and *V. cholerae* grown separately in LB broth (Himedia, Mumbai, India) was centrifuged at 13,000 x g for 5 min and harvested the centrifuged bacterial cells. Using the RED Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA), complete genomic DNA was extracted from centrifuged bacterial cells as per the manufacturer's instructions. In order to confirm the presence of *S. aureus* molecularly, the *nuc* gene, which is the gold standard, was detected in the genomic DNA extracted from centrifuged bacterial cells. The species-specific *nuc* gene of *S. aureus* was identified by the simplex PCR approach with the use of specific primers (Nuc-F: GCG ATT GAT GGT GAT ACG GTT, NucR: AGC CAA GCC TTG ACG AAC TAA AGC) as previously mentioned [23]. The primers were utilized in a 25 µl reaction containing 12.5 µl of Dream Taq™ Green PCR Master mix (2x) (Thermo Scientific, Life Technologies Inc., Carlsbad, California, United States) 1 µl of each primer, 1 µl of DNA template and 9.5 µl of nuclease-free water. Amplification was performed by using a BIO-RAD T100 Thermal Cycler (Bio-Rad, California, United States) with the following thermal profile: 4 min at 94°C for initial denaturation of DNA, and 37 cycles, each consisting of 1 min at 94°C, 0.5 min at 55°C, and 1.5 min at 72°C, with a final extension for 3.5 min at 72°C. The species-specific *toxR* gene and virulence *ctxA* gene for *V. cholerae* were detected using the multiplex PCR technique with particular primers (toxR-F: CCTTCGATCCCCTAAGCAATAC, toxR-R: AGGGTTAGCAACGATGCGTAAG; ctxA-F: CGGGCAGATTCTAGACCTCCTG, ctxA-R: CGATGATCTTGGAGCATTCCCAC) on the isolated genomic DNA from the bacterial cells [24, 25]. The strain of *V. cholerae* (ATCC BAA-238) served as the positive control. PCR amplification of the target DNA was carried out in a BIO-RAD T100 Thermal Cycler (Bio-Rad, California, United States) with a 25-µl reaction mixture containing 12.5 µl of GoTaq®Green master mix (Promega, Madison, WI, USA), 1 µl of each of the two primer pairs, 2 µl of DNA template, and 6.5

µl of nuclease-free water. Amplification was conducted using the following conditions: 5 min at 94°C for initial denaturation of DNA and 30 cycles, each consisting of 1 min at 94°C, 1 min at 57°C, and 2 min at 72°C, with a final round of extension for 10 min at 72°C. The bands from the electrophoresis of all amplified PCR products were visible under a UV lamp using a GelDoc Go imaging system (Bio-Rad, California, United States). The bands were separated by 1.5% agarose gel.

Antibiotic susceptibility study

By employing the disk diffusion technique, the antibiotic susceptibility patterns of the bacterial isolates were assessed [26]. For each isolation, bacterial suspension was prepared from 24 h fresh culture and incubated for 2-3 h. Then, a bit of suspension was used to make a loan with the help of a cotton swab over the Mueller Hinton Agar (MHA; Merck, Darmstadt, Germany) medium, and this process was done for each isolate individually in respective plates. Then antibiotic discs of different concentrations were placed over the medium containing culture suspension. The antibiotic disc (Oxoid, Thermo Scientific, New York, USA) containing the following antibiotics was used: ampicillin (10 µg), piperacillin-tazobactam (110 µg), cefotaxime (30 µg), cefuroxime (30 µg), imipenem (10 µg), gentamicin (30 µg), amikacin (30 µg), streptomycin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), trimethoprim-sulfamethoxazole (25 µg), and cholramphenicol (30 µg) and nitrofurantoin (F-300 µg) . Then, for 24 h, the MHA (Merck, Darmstadt, Germany) plates were incubated at 37°C. The Clinical Laboratory Standards Institute (CLSI) [27] guidelines were followed for measuring and interpreting the inhibition zones following a 24-hour incubation period.

MDR phenotypes and multiple antibiotic resistance indices (MARI) in bacterial isolates

The multidrug resistance capacity of the isolates was assessed according to Gurung *et al*. [28]. Any isolate that showed resistance to ≥3 different classes of antibiotics was considered a multidrug-resistant (MDR) strain [28]. A microorganism's MARI was calculated by dividing its resistance to all antibiotics by the total number of antibiotics it has been exposed [29].

Statistical analysis

Data collected from samples were entered into a spreadsheet (Microsoft Excel 2010) and transferred into a statistical software program (STATA, version 18, Stata Corporation 2023, College Station, TX: StataCorp LLC.) for further analysis. Descriptive statistics were used to compute the occurrence of isolated bacteria, the percentage of antibiotic resistance, and the frequency of MDR and MARI.

RESULTS

Occurrence of *P. aeruginosa*, *E. coli*, *S. aureus*, and *V. cholerae* in hands and utensils

In this study, 30 food worker hands and 30 utensils were examined. Of the 60 samples examined, 46.7% (28/60) of hands and utensils samples were carriers of some pathogenic bacteria. Among the pathogenic bacteria analyzed, *E. coli* (41.7%) was the most commonly present in both types of samples, while *V. cholerae* (16.7%) was the least frequently present. The incidence levels of *P. aeruginosa* and *S. aureus* in all samples were 25% (Figure 1 and Table 1).

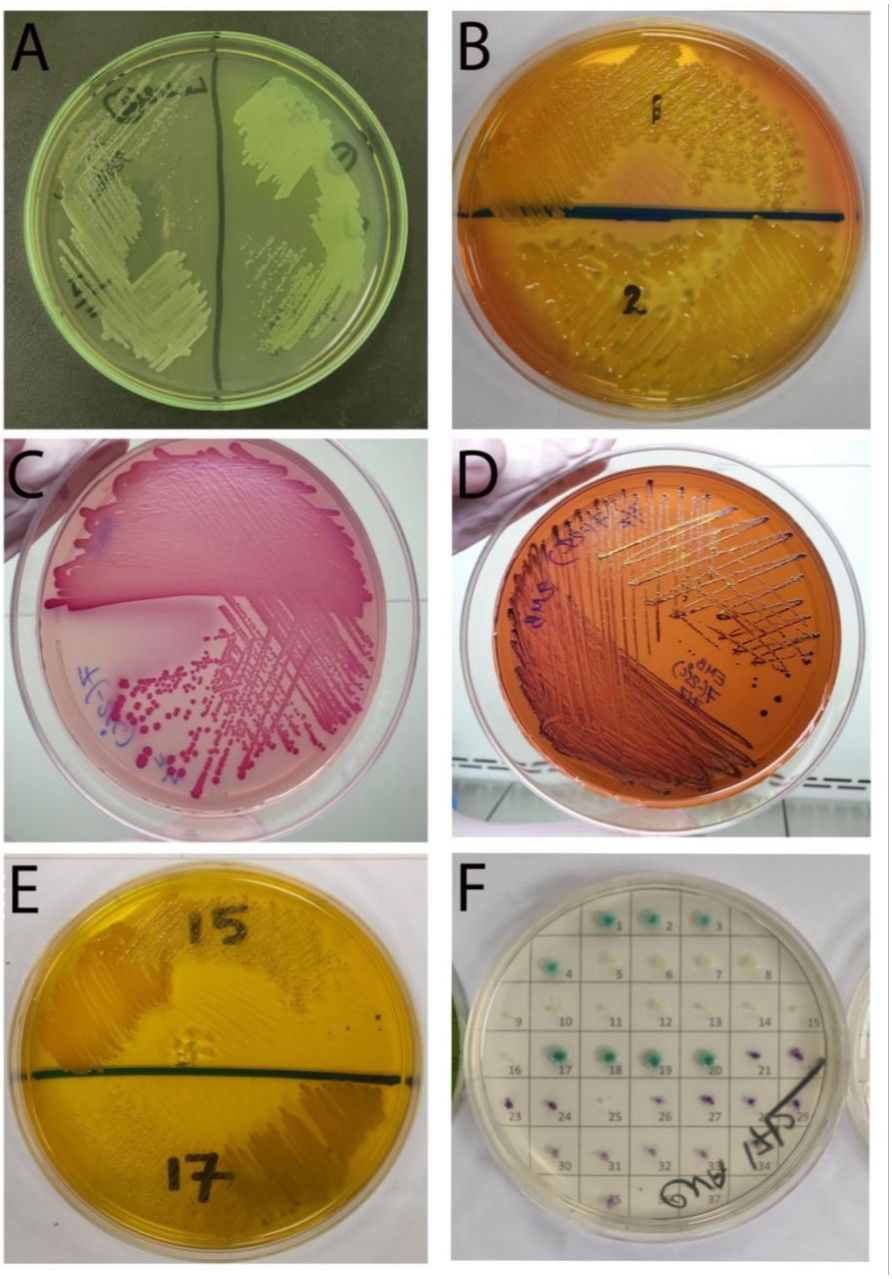

Figure 1. Bacteria colonies on selective media plates. A) Colonies of *P. aeruginosa* (Green) on Cetrimide agar plate B) *S. aureus* (Yellow) on Mannitol Salt agar plate, C) *E. coli* (Pink) on MacConkey agar plate, D) *E. coli* (Dark centered with a green metallic sheen) on Eosin methylene blue agar plate E) *V. cholerae* (Yellow) on thiosulfate citrate bile sucrose agar plate F) *V. cholerae* (turquoise blue) on CHROMagar™ plate.

Table 1. Showed the occurrence of bacteria isolated from the hands of food handlers and utensils.

	Food handler's hands			Utensils			Total	
Bacteria isolated	**No of sample**	**Positive**	**Contamination rate in (%)**	**No of sample**	**Positive**	**Contamination rate in (%)**	**Positive**	**Contamination rate in (%)**
P. aeruginosa	30	10	33.4	30	5	16.7	15	25
E. coli	30	15	50	30	10	33.4	25	41.7
S. aureus	30	10	33.4	30	5	16.7	15	25
V.cholerae	30	7	23.4	30	3	10	10	16.7

Molecular confirmation of pathogenic bacteria in hands and utensils

Using specific primers, simplex PCR was used to analyze the extracted DNA from the *S. aureus* isolates for the presence of a species-specific *nuc* gene, confirming the molecular identification of *S. aureus* (Figure 2). Using a multiplex PCR-based assay, it was possible to identify the species-specific *toxR* gene and the virulence *ctxA* gene for *V. cholerae*. Of the 13 isolates tested, 10 tested positive for the *toxR* gene and among those isolates 2 tested positive for the *ctxA* gene (Figure 3). Among *ctxA*-positive isolates, one (10%) in food worker's hands and one (10%) in utensils samples were exhibited from same street restaurant shop (Figure 3).

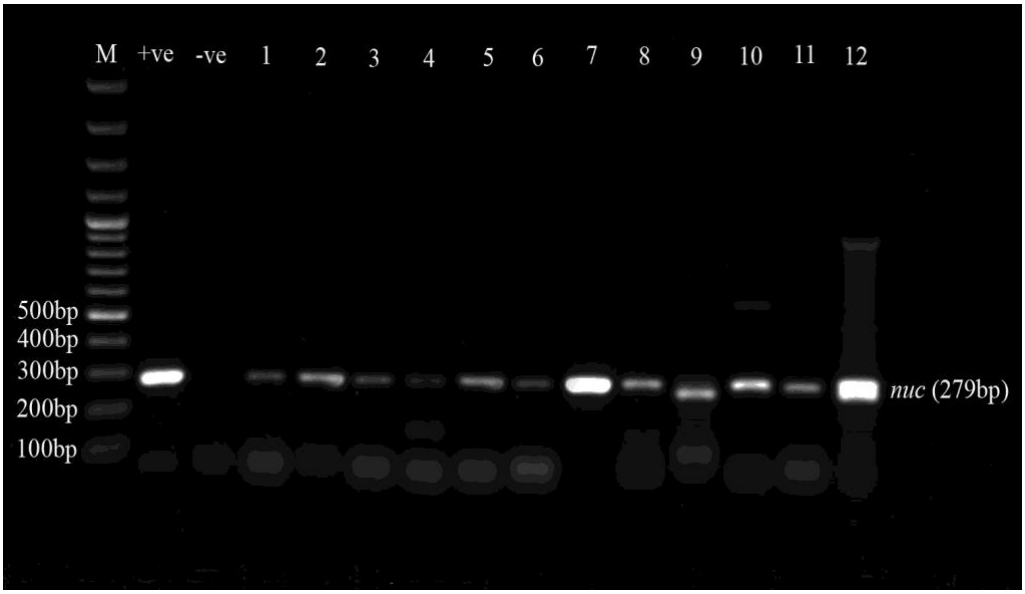

Figure 2. Simplex PCR of *S. aureus* isolated from the swabs of food handlers' hands and utensils. Marker (M): 100 bp DNA ladder; + ve control: *S. aureus* ATCC 29213; Lane 1-12: *nuc* (279 bp) gene amplification visualize band; Lane 1-10: *nuc* positive isolates recovered from food handlers' hands and Lane 11-12: *nuc* positive isolates recovered from utensils.

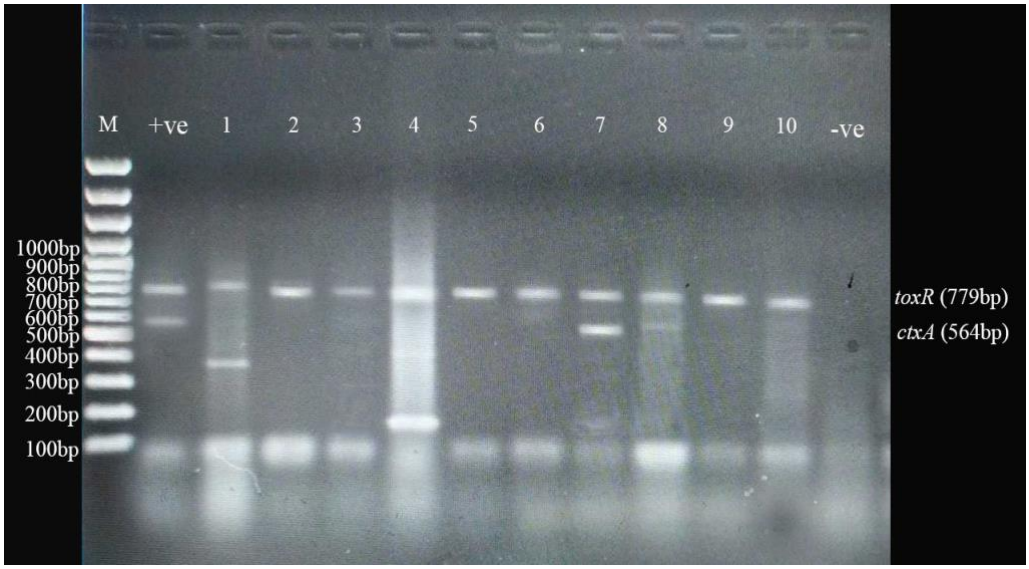

Figure 3. Multiplex PCR of *V. cholerae* isolated from swabs of food handlers' hands and utensils. Marker (M): 100 bp DNA ladder; + ve control: *V. cholerae* ATCC BAA-238; Lane 1-10: *toxR* (779 bp) gene amplification visualize band; Lane 7, 8: *ctxA* (564 bp) gene amplification visualize band; Lane 1-7: *toxR* positive isolates recovered from food handlers' hands and Lane 8-10: *toxR* positive isolates recovered from utensils.

Antibiotic susceptibility profile of bacterial isolates in hands and utensils

A test for antibiotic sensitivity was performed on isolated strains of *P. aeruginosa*, *E. coli*, *S. aureus*, and *V. cholerae*. Table 2 displays the antibiotic-resistant antibiogram profile of the investigated isolates. With the exception of *S. aureus* to ciprofloxacin (100%), all examined isolates of *P. aeruginosa* (100%), *V. cholerae* (100%), and *E. coli* (80%) showed the highest resistance phenomena to ampicillin. Furthermore, it was discovered that ten *P. aeruginosa* isolates under examination were resistant to tetracycline, eight *E. coli* isolates to streptomycin, and nine *V. cholerae* isolates to nitrofurantoin and nalidixic acid. The results of the antibiotic sensitivity test revealed that ten isolates of *P. aeruginosa* (100%) were most sensitive to piperacillin-tazobactam, *E. coli* (100%) to imipenem and nitrofurantoin, *S. aureus* (100%) to gentamicin, amikacin, tetracycline and chloramphenicol, and *V. cholerae* (100%) to piperacillin-tazobactam, imipenem and amikacin (Table 2).

MDR isolates and MARI in hands and utensils

The MARI of all tested and isolated bacterial species varied from 0.083 to 0.428. One isolate of *V. cholerae* (UV3) exhibited the highest MAR index (0.428), showing resistance to six different antibiotics among five classes. According to the current analysis, the MARI for *V. cholerae* isolates varies from 0.214 to 0.428. 100% of the *V. cholerae* isolates had MARI greater than 0.2, and 100% of them displayed MDR characteristics (Table 3).

Table 2. Antibiotic resistance profiles of bacteria isolates.

		P. aeruginosa, n=10 (%)			*E. coli*, n=10 (%)			*S. aureus*, n=10 (%)			*V. cholerae*, n=10 (%)		
Antibiotics	**Disc Code**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
Ampicillin (10 µg)	AMP	10 (100)	0 (0.0)	0 (0.0)	8 (80)	0 (0.0)	2 (20)	8 (80)	0 (0.0)	2 (20)	10 (100)	0 (0.0)	0 (0.0)
Piperacillin-tazobactam (110 µg)	TZP	0 (0.0)	0 (0.0)	10 (100)	2 (20)	1 (10)	7 (70)	ND	ND	ND	0 (0.0)	0 (0.0)	10 (100)
Cefotaxime (30 µg)	CTX	2 (20)	0 (0.0)	8 (80)	1 (10)	0 (0.0)	9 (90)	3 (30)	0 (0.0)	7 (70)	2 (20)	0 (0.0)	8 (80)
Cefuroxime (30 µg)	CXM	3 (30)	0 (0.0)	7 (70)	7 (70)	0 (0.0)	3 (30)	6 (60)	2 (20)	2 (20)	4 (40)	0 (0.0)	6 (60)
Imipenem (10 µg)	IPM	2 (20)	0 (0.0)	8 (80)	0 (0.0)	0 (0.0)	10 (100)	ND	ND	ND	0 (0.0)	0 (0.0)	10 (100)
Gentamicin (10 µg)	CN	5 (50)	0 (0.0)	5 (50)	3 (30)	3 (30)	4 (40)	0 (0.0)	0 (0.0)	10 (100)	1 (10)	0 (0.0)	9 (90)
Amikacin (30 µg)	AN	4 (40)	0 (0.0)	6 (60)	3 (30)	0 (0.0)	7 (70)	0 (0.0)	0 (0.0)	10 (100)	0 (0.0)	0 (0.0)	10 (100)
Streptomycin (10 µg)	S	ND	ND	ND	8 (80)	2 (20)	0 (0.0)	ND	ND	ND	2 (20)	0 (0.0)	8 (80)
Tetracycline (30 µg)	TE	10 (100)	0 (0.0)	0 (0.0)	1 (10)	2 (20)	7 (70)	0 (0.0)	0 (0.0)	10 (100)	2 (20)	0 (0.0)	8 (80)
Ciprofloxacin (5 µg)	CIP	1 (10)	4 (40)	5 (50)	2 (20)	3 (30)	5 (50)	10 (100)	0 (0.0)	0 (0.0)	1 (10)	0 (0.0)	9 (90)
Nalidixic Acid (30 µg)	NA	1 (10)	1 (10)	8 (80)	1 (10)	1 (10)	8 (80)	ND	ND	ND	9 (90)	0 (0.0)	1 (10)
Trimethoprim-sulfamethoxazole (25 µg)	SXT	1 (10)	0 (0.0)	9 (90)	1 (10)	2 (20)	7 (70)	2 (20)	0 (0.0)	8 (80)	4 (40)	0 (0.0)	6 (60)
Cholramphenicol (30 µg)	C	ND	ND	ND	0 (0.0)	4 (40)	6 (60)	0 (0.0)	0 (0.0)	10 (100)	0 (0.0)	2 (20)	8 (80)
Nitrofurantoin (300 µg)	F	0 (0.0)	2 (20)	8 (80)	0 (0.0)	0 (0.0)	10 (100)	2 (20)	0 (0.0)	8 (80)	9 (90)	0 (0.0)	1 (10)

R, Resistant; I, Intermediate; S, Sensitive

Table 3. Multidrug resistance and MARI of the isolated bacteria.

Tested bacteria	Number of antibiotics tested	Frequency of MDR isolates (%)	MARI range	MARI of < 0.2 (%)	Tested bacterial isolates showed highest MARI	Total antibiotic resistance	Antibiotic-resistant pattern
P. aeruginosa	12	4 (40)	0.083-0.416	6 (60)	HP4	5	AMP-CTX-CXM-CIP-SXT
E. coli	14	8 (80)	0.214-0.357	10 (100)	HE1	5	AMP-CTX-CXM-CIP-NA
S. aureus	10	8 (80)	0.2-0.4	10 (100)	HS3, US3, US12	4	AMP-CTX-CXM-CIP
V.cholerae	14	10 (100)	0.214-0.428	10 (100)	UV3	6	AMP-CTX-CXM-NA-SXT-F

AMP, Ampicillin; CTX, Cefotaxime; CXM, Cefuroxime; CIP, Ciprofloxacin; SXT, Trimethoprim-sulfamethoxazole; NA, Nalidixic Acid; F, Nitrofurantoin; and MARI, multiple antibiotic resistance index.

DISCUSSION

Food handlers are a major source of bacteria, and poor handling techniques can lead to food contamination and foodborne illnesses, which can be dangerous for the general public's health [4]. In order to test for the presence of bacterial contamination, swab samples from food workers' hands and utensils were taken from different restaurants in Sadarghat. Twenty-eight swab samples showed bacterial growths on different culture plates exhibiting a high level of bacterial contamination (46.7%), which is indicative of inadequate personal hygiene habits, unsanitary environments, a scarcity of clean water, and a lack of knowledge about health promotion strategies [6]. According to the findings of the current investigation, food handlers' hands and utensils were contaminated with harmful germs, including *P. aeruginosa*, *E. coli*, *S. aureus*, and *V. cholerae*. In earlier research, similar kinds of bacterial contaminants were found in different countries [3, 4, 6, 8, 10, 14, 16]. In contrast to studies by Assefa et al. [16] and Nasrolahei et al. [6], the study revealed the highest incidence of *E. coli* 41.7% in hands, followed by *S. aureus* and *P. aeruginosa* 25%.

Hand washing is a simple way to eliminate enteric pathogens among the transitory hand flora. Given that it may spread by hand-to-mouth contact or passive transfer through water and food items, the presence of these bacteria may be a sign of poor sanitation procedures among food handlers. Bacteria such as *E. coli* can cause diarrhea, gastroenteritis, cholera, dysentery, and other digestive system disorders when consumed in food or water [30]. The isolation of *E. coli* exhibited a faeces-to-hand transmission and indicated that the food handlers were not practicing good hygiene [16]. The presence of *E. coli* is indicative of inadequate hand hygiene standards and fecal contamination among food workers [16]. In the present study, *E. coli* was identified from hands of 50% food handlers, which is higher than 41.7%, 41.4%, 29.2%, 29.2%, 20.3%, 10.9%, 7.8%, and 6.8% reported in Egypt [3], Indonesia [31], India [4], Iran [6], Nigeria [8], Ethiopia [16], Turkey [16] and Brazil [16] respectively. The source population, kind of food establishment, sampling strategies, and contaminants detection methodologies may have contributed to the discrepancy between our findings and those of the other investigations [3, 16]. Foodborne infections can result from *E. coli* that spreads from the hands of food handlers and utensils to the food consumed by consumers [8, 30, 31].

The current investigation revealed the second-highest incidence of *S. aureus* (33.4%), which is understandable given that this organism is prevalent in the skin's resident microflora and 40–50% of healthy individuals carry *S. aureus* in their anterior nostrils [4]. The prevalence rate of *S .aureus* detected on the hands of food handlers in this study is nearly comparable to 31% isolation rate reported in Egypt [16], 29.2% in Egypt [3], 25% in India [4], 23.5% in Ethiopia [16], and higher than 17.5% in Saudi Arabia [32], 16.5% in Gondar [16], 14.7% in Nigeria [8], and lower than 42% in Mexico [16], 46% in Iran [6], and 70% in Turkey [16]. Food handlers can easily introduce *S. aureus* into food if they have open wounds or skin infections, sneeze or cough while preparing food, or fail to properly wash their hands after touching their nose, sticking fingers in it, or using the restroom [3, 6]. Contamination of food with *S. aureus* may also lead to staphylococcus food intoxication [4, 6, 8, 9, 33, 34]. Foods such as raw milk, chicken meat, ready-to-eat meals, fish, and eggs may be potential sources of pathogenic *S. aureus* with important medical consequences, research done by Ballah et al. found *S. aureus* in foods and food handlers [34]. Food workers who neglect to wash their hands often can contaminate food, which increases the risk of foodborne disease in others who consume that food [4, 9, 33, 34].

In the current study, *P. aeruginosa* was found in the hands of 33.4% of food handlers. This is higher than the 6.6% and 0.6% isolation rates reported in Iran [6] and Turkey [7], respectively. *Pseudomonas* bacteria were isolated from the hand by Larson *et al.* in 1986, whereas Sheena and Stiles in 1983 were isolated as transient bacteria from the hand [35, 36]. In 2023, a study was conducted by Badawy *et al*. in Egypt on detecting *P. aeruginosa* from the hands of dairy farm workers [37]. It has been reported previously that the individuals' fingernails may have carried *P. aeruginosa* due to contact with contaminated raw foodstuffs, such as raw meat, poultry, fish, and unwashed fruits and vegetables [6]. Due to their widespread presence in nature, *Pseudomonas* species are frequently found as food contaminants. It has been reported that *P. aeruginosa* causes food poisoning [35]. Food handlers can easily contaminate their hands with *P. aeruginosa* during the food preparation period with contaminated raw products. Consequently, while they serve food to consumers, it also leads to contamination of the consumer's food and reasons for food poisoning.

Water contaminated with faeces is the main way that vulnerable individuals get cholera, which is caused by the *V. cholerae* bacteria. A significant means of cholera transmission,

in addition to water, has been identified as food. Foods are likely to be contaminated with faecal materials particularly by infected food handlers in an unhygienic environment during preparing and serving to consumers [38]. A present study revealed that food handler's hands were contaminated with pathogenic *V. cholerae* strains. Using PCR to identify the *toxR* gene, a total of 10 *V. cholerae* strains were confirmed. During the search for potentially pathogenic strains, two strains (19%) containing the *ctxA* gene were also found. Previous studies also exhibited that food handler's hands were contaminated with *V. cholerae* [8, 38]. In the current investigation, *V. cholerae* was found in the hands of 23.4% of food handlers, which is more than the 2.6% reported in Nigeria [8]. These results may indicate that hands were contaminated by fecal matter and probably by touching contaminated raw food materials like vegetables or contaminated utensils. These bacteria attached to the hands moved into food and consuming of this food by consumers may lead to gastroenteritis [8].

The results of this investigation also showed that 33.4% of *E. coli*, 16.7% of *S. aureus*, 16.7% of *P. aeruginosa*, and 10% of *V. cholerae* were detected in 36.7% (11/30) of the utensil samples. A study in Indonesia done by Djaja et al. reported that utensils were contaminated with 35.7% *E. coli* which is consistent with current study results [31]. Previous studies exhibited unclean utensils that showed contamination by several pathogenic microorganisms, which are considered a major source of contamination of foods as well as the hands of food handlers [9, 12, 31, 33, 39]. Cross-contamination can also occur significantly from unclean utensils [9, 39]. Bacterial transmission to hands may take place through cross-contamination of food and utensils when food workers process raw foods and serve foods to consumers [9, 12, 31, 33, 40]. A study carried out in Japan also showed the possibility of *Staphylococcus* bacteria spreading from human hands to utensils in food processing areas [41]. Utensils do not clean well, pathogens will contaminate, and it could be a serious threat to food safety [9, 31, 33].

Pathogens with antimicrobial and MDR are becoming a major worldwide health and economic problem [42, 43]. Worldwide, antibiotic-resistant bacteria have been identified in common bacterial infections such as diarrhea [44]**.** Regretfully, the majority of bacterial infections linked to diarrhea have been discovered in developing nations [44]. Increased prevalence of MDR microorganisms in both human and environmental samples has been revealed by recent investigations conducted in Bangladesh [44]. A rising prevalence of microorganisms resistant to antibiotics suggests that AMR and MDR are becoming a major and widespread issue in Bangladesh [44]. In examined isolates, MDR of *V. cholerae* has the maximum proportion of 100%, while *P. aeruginosa* has the lowest rate of 40%, according to the present research. Two researches conducted in Nepal in 2015 and 2019, as well as a five-country study conducted in sub-Saharan Africa in 2015, had found 100% MDR *V. cholerae* rates [43]. In 2017, a study conducted in Bangladesh in which 93% of the *V. cholerae* strains tested proved to be MDR [45]. In this study, the majority portion of tested *E coli, S. aureus*, *P. aeruginosa*, and *V. cholerae* isolates showed MDR phenotypes, which is supported by previous studies in Bangladesh, India, Nigeria, Pakistan, Iran, and Egypt [34, 37, 44]. Bacterial characteristics, such as selective stress from antimicrobial agents, spontaneous mutation, horizontal gene transfer within different bacterial pathogens, and overuse of antibiotics during treatment, are some of the factors driving the increased spread of genes linked to antibiotic resistance from their natural environments to pristine and clinical settings [34, 37, 44, 46]. AMR is concerningly rising among pathogens, mostly as a result of uncontrolled antibiotic abuse in people and animals [42]. Antimicrobial residues in the environment are increasing as a result of the extensive use of antibiotics in modern medicine, aquaculture, agriculture, dairy production, and poultry farming [47]. Antibiotic resistances amongst environmental bacterial species pose threats to both

humans and the environment as there is a possibility of transferring or sharing MDR genes amongst potential pathogens via horizontal gene transfer [48]. The duration of diarrhea and the excretion of pathogenic bacteria may be reduced by the effective use of antibiotics in controlling diarrheal disease through overdose or effective dosage, but there is a chance this will also raise the risk of developing MDR strains [37, 46]. The MARI greater than 0.2 was found in 90% of the studied strains, indicating that the bacteria likely originated from a setting where several antibiotics were abused or used excessively, perhaps resulting in faecal contamination. Since the development of antibiotics, it has been shown that bacteria have developed resistance to them, which presents a significant issue when selecting antibiotics for medicinal purposes [48]. The CDC advises routinely monitoring bacterial isolates from any setting for antibiotic resistance since performing so is crucial to interpreting and preventing the emergence of resistance [49].

Molecular confirmation of virulent genes of MRSA such as *sea*, *seb*, *sed*, *hla*, *hld*, *tst*, *eta*, *etb*, and *LukS/F-PV* genes are needed to determine the virulent capacity of all positive MRSA isolates. Moreover, a biofilm-forming assay is required in future studies to provide more precise and valuable information on the resistance and pathogenesis of *S. aureus* isolates. In *S. aureus*, the ability to form biofilms is directly related to the presence of virulence factors such as adhesins, and this structure contributes to the dissemination of resistance genes in the cells therein [50].

CONCLUSIONS

In this investigation, bacterial contaminants were isolated from the food handler's hands and utensils. This study results revealed that food handlers' hands and utensils in different restaurants in the Sadarghat area had a high prevalence of potentially pathogenic bacterial infestation (46.7%), which is indicative of inadequate personal hygiene habits of food handlers, infected utensils, unsanitary environments, a scarcity of clean water, and a lack of knowledge about health promotion strategies. The responsibility to stop illnesses and eradicate pathogens is for everyone involved in the food-producing and serving process. To improve the quality of food and environment at restaurants and prevent outbreaks of foodborne illnesses, some steps also should be taken, such as proper attention of the relevant local and higher authorities on safe food in restaurants around the launch terminal of Sadarghat and arranging regular training and health education of these food handlers in all aspects of food hygiene and safety.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

SA and MAS designed outlines and drafted the manuscript. SA and RA collected study samples and SA operated the experiments. SA, RA, and MAS analyzed the results data. All authors of the present study reviewed and approved the final article.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] Jones TF, Angulo FJ. Eating in restaurants: a risk factor for foodborne disease?. Clin Infect Dis. 2006;43(10):1324-1328.

[2] Bondi M, Messi P, et al. Emerging microbial concerns in food safety and new control measures. Biomed Res Int. 2014;2014:251512.

[3] Allam HK, Al-Batanony MA, et al. Hand contamination among food handlers. Brit Microbiol Res J. 2016;13(5):1–8.

[4] Sharma A, Gangopadhyay S, et al. Hand contamination among food handlers: A study on the assessment of food handlers in canteen of various hospitals in Solapur City, Maharashtra. J Pure Appl Microbiol. 2021;15(3):1536-1546.

[5] FAO. National food policy capacity strengthening programme. Final report PR, 2012.

[6] Nasrolahei M, Mirshafiee S, et al. Bacterial assessment of food handlers in Sari city, Mazandaran Province, north of Iran. J Infect Public Health. 2017;10:171-176.

[7] Aycicek H, Aydogan H, et al. Assessment of the bacterial contamination on hands of hospital food handlers. Food Control. 2004;15(4):253–259.

[8] Dahiru JY, Abubakar FA, et al. Bacterial contamination of food handlers at various restaurants in Kano State Metropolis, Kano Nigeria. Int J Curr Microbiol App Sci. 2016;5(5):165–170.

[9] Bencardino D, Amagliani G, et al. Carriage of *Staphylococcus aureus* among food handlers: An ongoing challenge in public health. Food Control. 2021;130.

[10] Tan SL, Lee HT, et al. Microbiological quality of food handlers hands at primary school in hulu Langat district Malaysia. IFRJ. 2013;20(5):2973-2977.

[11] Davis CE, Samuel C. Detecting Bacterial Contamination. Bio Tech Int Inc. 1997;31:364–396.

[12] Ahmed MA. Isolation, identification, and characterization of pathogenic bacteria from food and surfaces of selected restaurants in Iraq. IJBPAS. 2019;8(1):132-146.

[13] Fariba R, Gholamreza JK, et al. Knowledge, attitude, and practice among food handlers of semi-industrial catering: a cross sectional study at one of the governmental organization in Tehran. J Environ Health Sci Eng. 2018;16(2):249–256.

[14] Aa L, Is H, et al. Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries in South Africa. Pak J Med Sci. 2014;30(4):755-758.

[15] Erdoğan M, Pamuk S. Microbial contamination in food, food-handlers' hands and surfaces and evaluation of contamination sources by the similarity between isolates. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2020;67:73-79.

[16] Assefa T, Tasew H, et al. Contamination of bacteria and associated factors among food handlers working in student cafeteria of Jimma university main campus Jimma south west Ethopia. J Community Med Health Educ. 2015;5(2):1-8.

[17] Honua MHM. The bacterial contamination of food handlers hands in Wad madani city restaurants, Sudan. Int J Community Med Public Health. 2018;5(4):1270–1273.

[18] Jay JM. Modern Food Microbiology. 4 th Ed. Van Nostrand Reinhold, New York, 2000.

[19] Suneetha C, Manjula K, Depur B. Quality assessment of street foods in Tirumala. Asian J Biological Sci. 2011;2(2):207-211.

[20] "Port of Dhaka" *Wikipedia, the free encyclopedia*, 19 July 2024, https://en.wikipedia.org/wiki/Port_of_Dhaka

[21] Barrow GH, Feltham RKA. Cowan and Steel's manual for identification of medical bacteria. 3rd Ed., Cambridge university press, Cambridge, 331, 1993

[22] Cheesbrough M. Medical laboratory manual for tropical countries in microbiology.1st ed. English Language Book Society, London. 1985.

[23] Brakstad OG, Aasbakk K, et al. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J Clinical Microbiol. 1992;30(7):1654-1660.

[24] Collin B, Rehnstam-Holm AS. Occurrence and potential pathogenesis of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* on the South Coast of Sweden. FEMS Microbiol Ecol. 2011;78(2):306–313.

[25] Waturangi DE, Pradita N, et al. Prevalence and molecular characterization of *Vibrio cholerae* from ice and beverages sold in Jakarta, Indonesia, using most probable number and multiplex PCR. J food protect. 2012;75(4):651–659.

[26] Bauer AW, Kirby WMM, et al. Antibiotic susceptibility testing by a standardized single disk method. Ame J Clin Pathol. 1966;45(4):493-496.

[27] Clinical and Laboratory Standards Institute [CLSI]. Performance Standards for Antimicrobial Susceptibility Testing. in CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2017

[28] Gurung RR, Maharjan P, et al. Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from pediatric patients. *Future science OA*. 2020;6(4):FSO464.

[29] Ayandele AA, Oladipo EK, et al. Prevalence of multi-antibiotic resistant *Escherichia coli* and *Klebsiella* species obtained from a tertiary medical institution in Oyo State, Nigeria. Qatar med J. 2020;2020(1):9.

[30] Herawati C, Endayani H, et al. Sanitary hygiene and behavior of food handlers in the presence of *Escherichia coli* bacteria. J Pure Appl Microbiol. 2023;17(4):2098-2103.
[31] Djaja IM, Puteri AR, et al. *Escherichia coli*'s contaminated food from Faculty Canteen in University X, Jakarta. J Pure Appl Microbiol. 2018;*12*(2):*223*-227.
[32] Zaglool DA, Khodari YA, et al. Prevalence of intestinal parasites and bacteria among food handlers in a tertiary care hospital. Nigerian med J. 2011;52(4):266-70.
[33] Beyene G, Mamo G, et al. Nasal and Hand Carriage rate of *Staphylococcus aureus* among food handlers working in Jimma Town, Southwest Ethiopia. Ethiopian J Health Sci. 2019;29(5):605-612.
[34] Ballah FM, Islam MS, et al. Virulence determinants and methicillin resistance in biofilm-forming *Staphylococcus aureus* from various food sources in Bangladesh. Antibiotics (Basel, Switzerland), 2022;11(11):1666.
[35] Sheena AZ, Stiles ME. Efficacy of germicidal hand wash agents against transient bacteria inoculated onto hands. J Food Protect. 1983;46(8):722-727.
[36] Larson E, McGinley KJ, et al. Physiologic, microbiologic, and seasonal effects of handwashing on the skin of health care personnel. Am J Infect Control. 1986;14(2):51-59.
[37] Badawy B, Moustafa S, et al. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* isolated from dairy cattle, milk, environment, and workers' hands. Microorganisms. 2023; 11(11):2775.
[38] Rabbani GH, Greenough WB 3rd. Food as a vehicle of transmission of cholera. J Diarrhoeal Dis Res. 1999;17(1):1-9.
[39] Chen Y, Jackson KM, et al. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. J Food Protect. 2001;64(1):72-80.
[40] Coates D, Hutchinson DN, et al. Survival of thermophilic campylobacter's on fingertips and their elimination by washing and disinfection. Epidemiol Infect. 1987;99(2):265-274.
[41] Wakabayashi Y, Kumeda Y, et al. Prevalence of *Staphylococcus argenteus* among food handlers, kitchen utensils, and food samples in Japan. LettAppl Microbiol. 2024;77(4):ovae031.
[42] Ventola CL. The antibiotic resistance crisis: causes and threats. P T. 2015;40(4): 277–283.
[43] Garbern SC, Chu TC, et al. Multidrug-resistant enteric pathogens in older children and adults with diarrhea in Bangladesh: epidemiology and risk factors. Trop Med Health. 2021;49(1):34.
[44] Sharif N, Ahmed SN, et al. Multidrug resistance pattern and molecular epidemiology of pathogens among children with diarrhea in Bangladesh, 2019-2021. Scientific Reports. 2023;13(1):13975.
[45] Rashed SM, Hasan NA, et al. *Vibrio cholerae* O1 with reduced susceptibility to ciprofloxacin and azithromycin isolated from a rural coastal area of Bangladesh. Front Microbiol. 2017;8:252.
[46] Uddin M, Akter T, et al. Isolation, identification and antimicrobial susceptibility profile analysis of *Vibrio cholerae* O1 from stool samples of Bangladesh. Advan Microbiol. 2018;8(3):188-196.
[47] Akter S, Zuhura FT, et al. Detection of multi-antibiotic resistant *Vibrio parahaemolyticus* isolated from fresh produce in Dhaka, Bangladesh. J Adv Biotechnol Exp Ther. 2024; 7(2): 303-313.
[48] Igere BE, Okoh AI, et al. Antibiotic susceptibility testing (AST) reports: A basis for environmental/epidemiological surveillance and infection control amongst environmental *Vibrio cholerae*. Int J Environ Res Public Health. 2020;17(16):5685.
[49] Abioye OE, Nontongana N, et al. Antibiotic resistance and virulence genes profiling of *Vibrio cholerae* and *Vibrio mimicus* isolates from some seafood collected at the aquatic environment and wet markets in Eastern Cape Province, South Africa. PloS one. 2023;18(8):e0290356.
[50] Savage VJ, Chopra I, et al. *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. Antimicrob Agents Chemother. 2013;57(4): 1968-1970.