WATER USED IN STREET FOOD STALLS: A POSSIBLE SOURCE OF TRANSMISSION OFANTIBIOTIC RESISTANCE BACTERIA

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Abstract: *Street foods, namely fuska, chotpoti, shamucha, fruit juices, and tea, are quite popular everywhere in Bangladesh. Though in some cases the overall environment of these street food shops looks unhygienic, people consume them anyway, overlooking this fact and the health risk Therefore, this study was intended to explore the microbiological quality of the dishwashing water used by the street food vendors to wash their food serving dishes, plates, cups, and other utensils. In addition, their association with the development of antibiotic resistance was explored. Results showed that the presence of E. coli and Pseudomonas is at a high percentage, and a non-deniable percentage of Salmonella spp. and Klebsiella spp. were found as well. Antibiotic resistance results revealed that the identified bacteria are capable of developing resistance to many commonly used antibiotics like ampicillin, ciprofloxacin, tetracycline, cefoxitin, cefuroxime. From the sensitivity test report, it is clear that people are at risk of transmission of these antibiotic-resistant bacteria through the regular consumption of street foods from these kinds of food stalls.*

Key Words: *Street Foods, Microbiological Quality, Serving Dish Washing Water, Transmission of Bacteria, Antibiotic Resistance*

Introduction

Street food has become a common phenomenon in most countries, including Bangladesh. In Dhaka, the capital city of Bangladesh, the term "street food" is considered to be foods or beverages that are sold by the informal sector's small entrepreneurs.[1] Street food vendors contribute to the economy by generating self-employment and jobs for others.[2-4] A study on street foods in the capital found that around 70 percent of people eat them.[5] Similarly, a survey in 2017 found that 60 lakh people of the city eat street foods daily from several thousand vendors.[6] In Dhaka city, street foods are much in demand because they are tasty, easily available, low cost, ready to eat. Poor people or day laborers in the city find no alternative but to fill up their stomachs with street foods. Students and young people mostly love to eat street foods.[7] Vendors sell foods in the open sky nearby any road or market to sell several cheap and tasty local foods like tea, jhalmuri, panipuri, bhelpuri, fuska, chotpoti, noodles, fruit juice, piyajo, peties, chicken fry, french fry, roll, burger, sugarcane juice, and so on. Food and water are the major transporters for the transmission of bacteriological hazards. Water and foodborne illness are major public health problems associated with the consumption of street foods.[8]

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It is also recognized that street-food vendors are often poor, uneducated, and lack knowledge in safe food handling practices, the environment, sanitation, and hygiene, modes of food display, food service, hand washing, sources of raw materials, and the use of portable water. Consequently, street foods are perceived to be a major public health risk.[9]

Previously published reports show that consumers of street food suffer from many diseases that are directly or indirectly related to water quality, for example, cholera, diarrhea, dysentery, enteric fever, hepatitis A, B, typhoid, and so on.[10] In most cases, running water is not continuously supplied for hand- and dishwashing, cooking, or drinking, leading the street vendors to store water in plastic buckets or containers under vulnerable conditions subject to microbial contamination. [11-13]

Food contamination with antibiotic-resistant bacteria can also be a major threat to public health since the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially complicating the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades. [14-17] Resistance to antibiotics in foodborne pathogens may create problems for disease or illness treatment, while antibiotic susceptibility leads to healing of the illness that the organism caused.[18] Travelers diarrhea is a major inconvenience to visitors arriving in developing countries from more industrialized areas. [19,20] Antimicrobial resistance (AMR) is a growing global threat to the health of people, animals, and the environment. Multidrug-resistant (MDR) bacteria, also referred to as "superbugs," have developed, spread, and persisted. [21-23]

Several studies have shown that the prevalence of harmful microorganisms, especially bacteria in varieties of street foods and juices, may increase the risk of antibiotic resistance alarmingly among the people living in Dhaka city. [24-26] The water used for washing serving dishes, plates, glasses, and cups at street food stalls can also be a potential source of transmission of various infectious bacteria among the consumers.

This study is based on the fact that water use for the washing of serving dishes, glasses, and plates by the vendors of various street food shops in Dhaka, Bangladesh, can be a common source for spreading water-borne diseases among the customs. Especially young children and students who are used to having these kinds of street foods might be at high risk of various microbial diseases as well as the development of antibiotic resistance.

Materials and Methods

Study Design and Collection of Samples:

It was a laboratory-based study. A total of 24 (4x2x3 =24) samples of dishwashing water were collected from 4 different categories of street food stalls, *i.e.*, tea stalls, fuska, fruits, and fried food stalls, in 3 different zones of Dhaka, Bangladesh. At least six samples from each category of food stall were collected for microbial quality analysis in the laboratory. In the laboratory, all samples were examined sequentially to explore the existence of any microorganism, especially bacteria. Then the isolation and detailed characterization of all bacteria have been done following a series of biochemical tests such as gram staining, oxidase test, motility test, colony count etc. In addition, the antibiotic resistance capability of the identified bacteria was tested. Food stalls were selected where the foods were served on plates, cups, or any other dishes. The stalls don't have a system for washing food serving plates and glasses in bucket water, which was not selected.

Chemicals and Reagents:

Blood agar (40.0 g/L), MacConkey agar (51.5 g/L), Salmonella-Shigella (56.68 g/L), Muller-Hinton agar media (3 g/L), and antibiotic discs for sensitivity testing were purchased from Oxoid Ltd., UK. In addition, Muller-Hinson agar broth media and oxidase dies were obtained from Himedia Laboratories, India. Other reagents like crystal violet, grams of iodine, and carbon fuchsin were obtained from DLC, Bangladesh. 95% Ethanol was purchased from SUPELCO, UK. Hand gloves, distilled water, test tubes, Petri dishes, and plastic containers (for sample collection) were collected from local suppliers in Bangladesh. For antibiotic sensitivity tests, commercial antibiotic discs containing specific concentrations of ampicillin, amoxicillin-clavulanate, ciprofloxacin, cefuroxime, cefixime, tetracycline and cefoxitin available in the local.

Preparation of Media and Culture of Water Samples for Microbiological Analysis:

A spread-plate culture technique was used to grow the bacteria in water samples. For the culture and isolation of microorganisms, more specifically bacteria, three agar media were used. They are: blood agar (40.0 g/L), MacConkey agar (51.5 g/L), and Salmonella-Shigella (56.68 g/L), respectively. All the media were prepared by following appropriate laboratory procedures. At first, agar powder was measured with an electrical balance and taken into a conical flask. Then mix with 1 liter of distilled water. The rest was heated in the autoclave at 121°C for 15 minutes, except the Salmonella-Shigella media. The Salmonella-Shigella agar medium was placed in a water bath that was heated at 45–40°C. Mix the medium with gentle swirling and completely dissolve the medium. Cool to 45–40 °C before pouring into the sterile petri dishes.

10 ml of the collected water from each sample were centrifuged, and the sediment was used to spread over the agar media. Total of 24 samples were tested for microbial analysis using 3 selective media. Each sample was spread on selected media using the spread plate method and a sterile swab stick. Then the plates were incubated for 24 hours at 37°C.

Biochemical Tests for the Identification of Bacteria in Water Samples

To identify the bacteria, present in the water samples, biochemical tests were performed after culture by performing the following tests:

Gram staining:

For this test, crystal violet and Gram's iodine were used as the primary and moderate stains, respectively. 95% ethanol was used for decolorization, and safranin was used for counterstain. The result is interpreted according to the purple and red colors for gram positive and gram negative, respectively, by observing under a light microscope with oil immersion.

Oxidase Test:

A wet filter paper was used to differentiate E. coli from Pseudomonas spp. A freshly made 1% solution of dimethyl-p-phenylene-diamine dihydrochloride in water was soaked on filter paper. A small amount of bacterial growth was rubbed on it with an aluminum loop. An immediate purple color indicates the bacterial colony is Pseudomonas spp. No color on the filter strip indicates the bacteria in E. coli.

Motility Test:

The motility test is used to determine whether an organism is motile or not. Motile organisms contain flagella, which help them to travel beyond the point of inoculation. Motile bacteria are generally bacilli, although a few motile cocci do exist A small drop of a cultured bacteria colony was taken from the petri dish and placed into the cavity slide with 1 drop of saline. A thin, small smear was created and was ready to be observed through the electronic microscope with a 40X objective.

Colony Count:

The single colony-forming unit (CFU) of bacteria was counted following the plate count method. In this case, a 10 μL sample was taken, and the serial dilution technique was applied up to the dilution factor of 10^6 for forming a single colony. After allowing the bacteria to grow on the plates for 24 hours at 37 °C, individual colonies are counted on a plate.

Antibiotic Sensitivity Test:

The Kirby-Bauer method was used to examine the in vitro susceptibility of isolated bacteria to various antimicrobial drugs, utilizing antibiotic disc diffusion on Muller-Hinton agar (3 g/L).[27] It enabled the determination of the antibiotic's effect, which demonstrates the pathogen's inhibition to a degree proportionate to the diameter of the zone of inhibition produced by the antimicrobial's diffusion encircling the disc onto the agar medium. Commercial antibiotic discs containing **AMP** = ampicillin (10 g); **AMC** = amoxicillin-clavulanate (10 g); **CIP** = ciprofloxacin (5 g); **CXM** = cefuroxime (30 g); **TE** = tetracycline (10 g); **FOX** = cefoxitin (15 g) were used in this study. In brief, a pure culture of a specific strain was added to 5 mL of Mueller-Hinton broth and incubated at 37° C for an overnight period. The 0.5 McFarland standard was used to account for the turbidity of broth cultures that were actively growing.[28]

Results

Sample Analysis, Identification, and Characterization of Bacteria:

In this study, microbial quality samples of dishwashing water were collected from 4 different categories of street food stalls, including a tea stall, a fusca stall, a fried food stall, and a fruit stall, in three different zones of Dhaka, Bangladesh (**Figure 1**). At least six samples from each category of food stall were collected for microbial quality analysis in the laboratory. After collection, the samples were sealed immediately to avoid environmental contamination and labeled. The samples were taken to the lab with proper precautions for microbial quality analysis and reporting about the profile of antibiotic resistance of the bacteria identified in those water samples.

Figure 1. Representative photos of the street foods shop from where water samples were collected (fellow circle) for microbial analysis. (A) tea stall; (B) Fuska stall and (C) A fruit-selling stall.

Figure 2 shows representative images of patterns of growth of bacteria using three selective media i.e., blood agar, MacConkey agar, and Salmonella-Shigella agar, respectively. Images confirm the successful growth of bacteria present in the collected water samples.

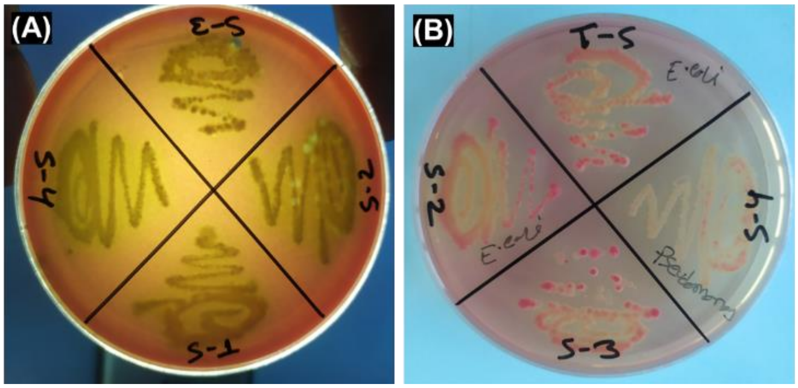

Figure 2. Growth patterns of bacteria exist in various water samples. The spread-plate culture technique was followed using various culture media: (A) Blood Agar Media and (B) MacConkey Agar Media.

The spread-plate approach was employed to assess the microbial load in samples, as listed in **Table 1**. The results of biochemical tests showed a wide variety of microbial organisms were present in all kinds of foods. It was also found that water samples from tea stalls were contaminated by *E. coli*, *Pseudomonas spp.*, and Salmonella typhi. *E. coli* was common for all samples. *Klebsiella spp*. was identified in fruits and fried food shops (samucha, chop, dal puri, et cetera). Moreover, *Pseudomonas spp*. and *Enterobacter spp*. were found in fried food stalls. All of the bacteria are motile except *Klebsiella spp.* and show negative results in the catalase test except *Pseudomonas spp*. In addition, all bacteria are gram-negative.

For more characterization, after the isolation and identification of bacteria that grew on multiple culture plates, colonies were counted manually and an average number of colonies was calculated per unit. The colony count for each identified bacteria is also given in **Table 1**. Dishwashing water samples from fruits, fusilli, and fried foods stalls were found to be maximally loaded with bacteria (6.8×10^5 to 7.6×10^5 cfu/g). Water in a tea stall contains significantly less bacteria (1.9×10^5 to 3.2×10^5 cfu/g).

Table 1. Biochemical profiles of identified bacteria from various water samples used for washing dish, plates and cups of various streets foods stalls in various zone of Dhaka city in Bangladesh

Source of water sample	Catalase test	Motility	Gram Staining Test	Identified Bacteria	Colonies per unit (cfu/g)
Tea stall	-	+	-	*E. coli*	3.2×10^5
	+	+	-	*Pseudomonas spp.*	3.0×10^5
	-	+	-	*Salmonella typhi*	1.9×10^5
Fruits stall	+	+	-	*Pseudo-monas spp.*	6.8×10^5
	-	+	-	*E. coli*	4.2×10^5
Fuska stall	-	+	-	*E. coli*	6.9×10^5
	-	-	-	*Klebsiella spp.*	3.7×10^5
Fried foods (Samucha, dal puri, chop etc)	-	+	-	*E. coli*	3.4×10^5
	+	+	-	*Pseudo-monas spp.*	7.6×10^5
	-	+	-	*Entero-bactor spp.*	5.1×10^5
	-	-	-	*Klebsiella spp*	6.6×10^5

The summarized biochemical profiles of identified bacteria in **Table 1**, a clear view of the microbial contamination of the dishwashing waters used by the street food vendors was confirmed.

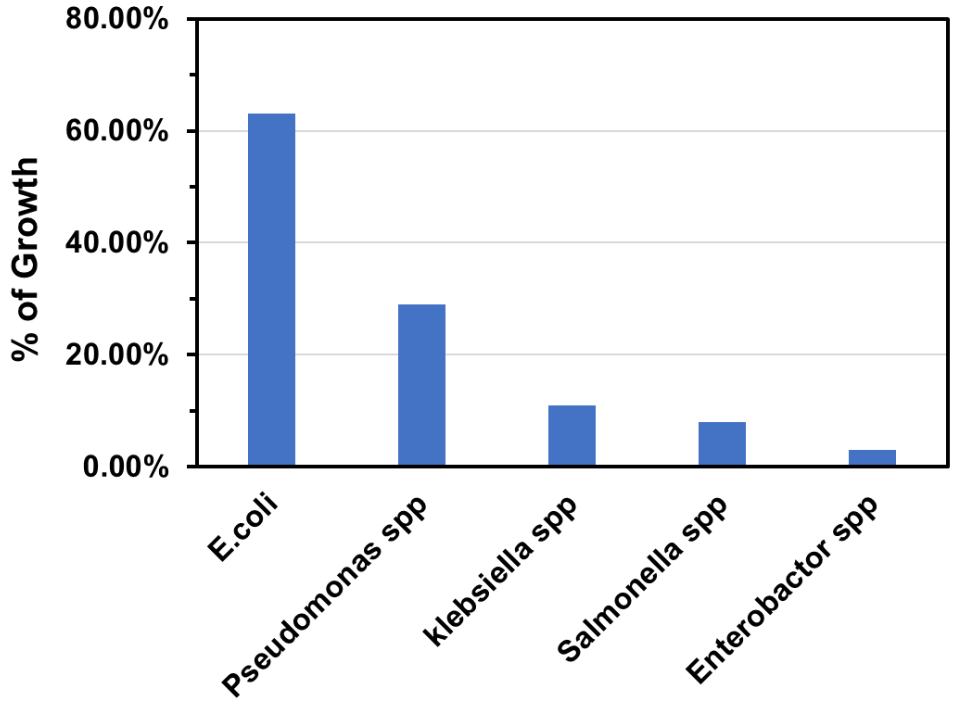

Figure 3. Comparative growth rate of bacteria obtained from various water samples following spread-plate culture technique.

In **Figure 3**, it is shown that the growth rate of *E. coli* was the highest (63%), followed by the growth of *pseudomonas spp.* (29%). The growth patterns of *Klebsiella spp.* (11%) and *Salmonella spp.* (8%) show a significant health risk through their contamination. *Enterobacter spp.'s* (3%) growth percentage is comparatively low. This study found no identified bacteria in 13% of sample. It means 87% of the water sample was contaminated with at least one or more of the bacteria mentioned above.

Antibiotic Resistance Profile:

To investigate the antibiotic resistance of the isolated bacteria from the dishwashing water, zone of inhibition screening is a quick, qualitative way to assess an antimicrobial agent's capacity to prevent the growth of microorganisms. As shown in **Figure 4**, with pathogen inhibition proportional to the size of the zone of inhibition brought on by antimicrobial diffusion into the agar medium around the disc, it was possible to determine how effective the antibiotic was, while no zone of inhibition indicates the antibiotic is not able to stop bacterial growth.

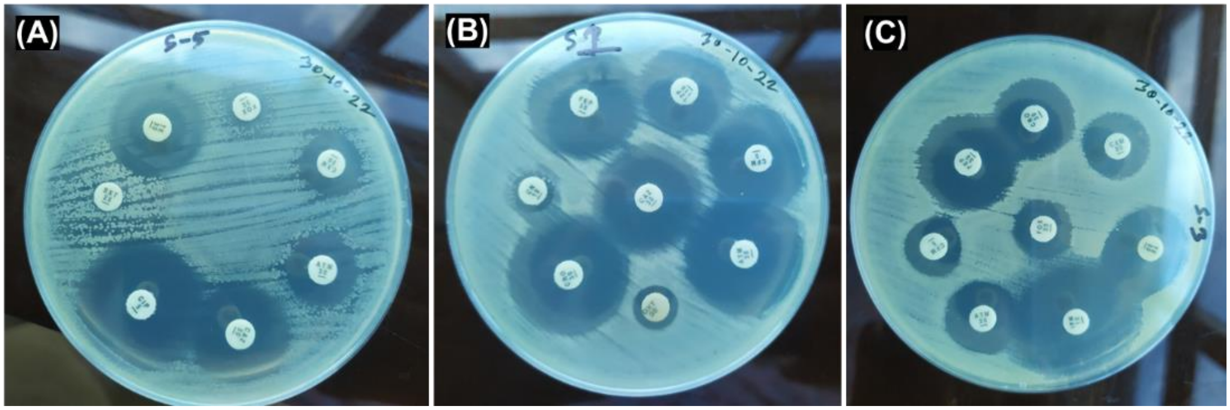

Figure 4. Images of antibiotic sensitivity test showing a clear zone of inhibition against the antibiotics (A) Amoxicillin; (B) Ciprofloxacin; and (C) Cefuroxime.

In addition, **Table 2** shows that E. coli was found in a tea stall and showed the highest resistance to ampicillin and cefuroxime. *Pseudomonas spp* was not assessed as having significant antibiotic resistance except for ciprofloxacin. *Salmonella* shows resistance to ampicillin, ciprofloxacin, and cefuroxime. In the case of *Klebsiella spp* from Fuska and fried foods shops, the highest amount of resistance was observed except for tetracycline.

Table 2: Summary of antibiotic resistance profiles against various bacteria responsible for the contamination of dishwashing water used by the street foods vendors. The sign (+) means that bacterial growth was suppressed (antibiotic sensitive) due to the presence of antibiotics, and the (-) sign indicates antibiotic resistance.

Nome of Food stall	**Identified bacteria**	**Name of Antibiotic**					
		AMP	**AMC**	**CIP**	**CXM**	**TE**	**FOX**
Tea Stall	*E. coli.*	–	+	+	–	–	–
	Pseudo -monas spp.	+	+	–	+	+	+
	Salmonella spp.	–	+	–	–	+	+
Fuska Stall	*E. coli.*	–	+	+	–	–	–
	Klebsiella spp.	–	–	–	–	+	–
Fried foods Stall	*E. coli.*	–	–	+	–	+	–
	Pseudo -monas spp.	+	+	–	+	+	+
	Enterobactor spp	–	+	–	+	+	–
	Klebsiella spp	–	–	–	–	–	–
Fruits stall	*E. coli.*	–	+	+	+	+	–
	Pseudo -monas spp.	+	+	–	+	+	+

As shown in **Table 2,** the bacteria present in the water used in fried food stalls were *E. coli, Pseudomonas spp, Enterobacter spp*, and *Klebsiella spp* one strain of *E. coli* was found, and it showed resistance to amoxicillin-clavulanate, ciprofloxacin, ampicillin, and cefoxitin. One *pseudomonas spp* was found that showed resistance only to ciprofloxacin. One *Enterobacter spp* was also found in the water of fried food, and it showed resistance to ampicillin, amoxicillin, and cefoxitin. Water samples were found to contain *E. coli* from the fruit-selling stall and were resistant to ampicillin and cefoxitin.

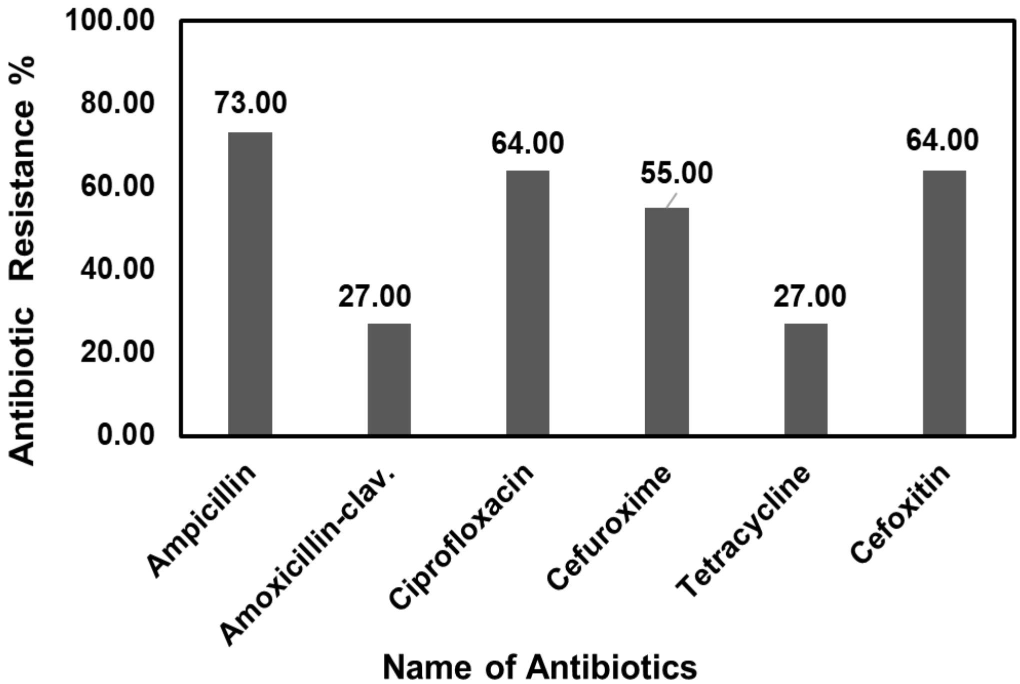

Figure 5. Comparative % of resistance of various commercial antibiotics against bacteria exist in various water samples.

From **Figure 5**, we can see that the resistance of ampicillin is quite significant (73%). Ciprofloxacin and cefoxitin have shown modest percentages (64%) of resistance. Cefuroxime has the ability to resist half of the bacterial growth (55%). Amoxicillin-clavulanate and tetracycline have shown the lowest percentage of resistance (27% each).

Discussion

Bangladesh has a high insistence on street foods, mostly in the capital city of Dhaka. The demand is increasing day by day. The reasons behind the popularity of street foods, are easily available, lots of variations and different types of foods also low in price which is under the rang of students.

A recent study shows that the majority of microbiological diseases are caused by a variety of food-borne infections. [29, 30] Because of overcrowding, high demand, and some other factors, street food vendors are unable to maintain their hygiene and food safety, especially with the water they use to make foods, serve drinks, and wash dishes. Using unhygienic and contaminated water to make foods or to wash dishes can cause some common water-related illnesses like diarrhea, giardiasis, dysentery, typhoid fever, *E. coli* infection, and salmonellosis. Those diseases can have adverse health effects, including pain, gastrointestinal infection, reproductive problems, neurological problems, and other symptoms.[31]

Eighty seven percent (87%) of collected samples of this study were identified contaminated with various pathogenic bacteria, like *Salmonella typhi*, *E. coli*, *Pseudomonas spp*, *Klebsiella spp*, and *Enterobacter spp*.

Therefore, results show that eating these foods can easily lead to serious health issues. *Escherichia coli (E. coli)* can cause a variety of intestinal and extra-intestinal infections, such as diarrhea, urinary tract infection, meningitis, peritonitis, septicemia, and gram-negative bacterial pneumonia.[31] A serious infection can cause symptoms like high fever, a lung infection, chills, confusion, and shock. *Klebsiella spp. is a* gram-negative bacterium that can cause different types of healthcare-associated infections. It can lead to a range of illnesses, including pneumonia, a bloodstream infection, meningitis, and so on. *Salmonella spp.* infections can be more life-threatening and lethal.[32] It causes diarrhea that can be bloody, stomach cramps, a high fever, and occasionally nausea and vomiting.[33] *Enterobacter* species are responsible for causing many nosocomial infections and less commonly community-acquired infections, including urinary tract infection (UTI), respiratory infection, soft tissue infection, osteomyelitis, endocarditis, and many others.[34]

The results for antibiotic resistance show that commonly used antibiotics like ampicillin and ciprofloxacin are highly resistant (73% and 64%, respectively). This means that, while eating these street foods, people can easily become infected by the above-mentioned antibiotic-resistant bacteria. The result of the study also showed that the consumers of those foods and waters have a significant risk of contracting illnesses as well as developing multi-drug resistance.[18] This set of circumstances will be more lethal for small children and the general public. An extension in apprehension between food manufacturers, suppliers, customers, and inspection authorities may improve food safety.

Conclusion

This study revealed that the water used by the street food vendors for washing food serving dishes, plates, cups, etc. is contaminated by various disease-causing bacteria. Therefore, customers who are eating varieties of street foods are at high risk of developing diseases. Particularly, young people and students are under threat of being infected by microorganisms. Moreover, antibiotic susceptibility tests demonstrated that multi-drug-resistant organisms may also develop due to the resistance to growth inhibition for most of the antibiotics against these bacterial pathogens in foods. This means, water used in street foods shop can be one of the very common sources of developing antimicrobial residence of regularly eating customers. The study's findings could be attributed to the increased social awareness of improper processing, poor handling techniques, and contaminated foods, which can endanger consumers' health.

Recommendation

It is highly mandatory to used fresh and clean water by the street food manufacturers. Proper hygiene and food safety should be maintained. Inspection authorities should be more active. People awareness should be increased also those kinds of street foods and drinks must try to avoid. If we need to prevent antibiotic resistance problems, steps can be taken at all levels of society to reduce the impact and limit the spread of resistance. Moreover, food safety rules and hygiene must be maintained by the street food vendors during the preparation and serving of foods to customers. Above all, the use of contamination-free water for the preparation of street foods, serving, and washing of utensils is very important.

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