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Microbial Load Assessment of Street-Vended Foods and Utensils in Selected Markets of Islamabad

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ABSTRACT

Background: Street-vended foods have become a considerable part of the urban food culture. In Islamabad, street-food vending not only represents the showcase of multiple culinary cultures, but it also proves to be a major source of nutrition that is affordable for everyone. However, their popularity does not eradicate the potential risks they pose to public health which is due to the possibly contaminated environments in which they are prepared and served. A significant contributor of contamination in these foods and serving utensils is the poor sanitary conditions.

Objectives: The main purpose of this study conducted is to assess the microbial contamination load (due to various contributing factors) on both food and utensils in different sectors of Islamabad including H-8, I-8, G-6, G-9, F-6 and F-7, where street-food is popularly sold.

Methods: Before collecting the samples and completing the observational survey, Informed consent was taken from all the vendors . Standard microbiological techniques were employed, including homogenization, preparation of serial dilution, determination of total viable count via the pour-plate method, bacterial isolation using quadrant streaking on MacConkey agar and blood agar, Gram staining, and biochemical identification through Catalase, Oxidase, Indole and Coagulase tests. Results were statistically analyzed to conclude findings in terms of percentages.

Results: Significant microbial contamination was reported in both street-foods as well as utensils which was primarily due to inadequate hygiene practices and lack of compliance to guidelines. Regression analysis demonstrated a non-significant association between hygiene compliance score and microbial load (P=0.192), suggesting that hygiene practices were not independent predictors of contamination level in this study.

Conclusions: Limited food safety knowledge and lack of regulatory oversight highlight the urgent need for providing vendors with appropriate facilities and improved surveillance to protect public health.

Keywords: Street food, microbial contamination, Islamabad, food safety, compliance, utensil swabs, food sample

INTRODUCTION

Street food culture is owned by many populations around the world; particularly in the continents like Africa, Asia and Latin America.[1] Street food vending has considerably evolved globally during the recent times due to their affordability among different social classes.[2] It is an informal small scale setup, a common practice all around the world.[3] During the recent years, the rapid urbanization of cities and fast-paced lifestyle has caused street food to become a valid choice of nutrition for a larger population, with both its pros and cons.[4] Although street food consumption has become quite common worldwide, it can be highly contaminated due to multiple factors such as the environment within which it is being prepared, stored and served. These informal urban settings may lack access to potable water and appropriate waste disposal facilities that can surely facilitate microbial growth.[5] Despite the economic importance of such foods, they are extremely prone to health-associated risks.[6] Contaminated food consumption can lead to various forms of food-borne illnesses ranging from minor gastrointestinal discomfort to severe and fatal infections.[8] According to WHO statistics, almost 600

million people annually suffer from food-borne diseases world-wide, in which inefficient hygienic practices and lack of regulatory enforcement act as major contributors of contamination.[7]

In Islamabad , street-food caters to the nutritional needs of migrants from different areas of the country, students, tourists as well as locals. The density of food vendors is also very rich in Islamabad but it can be clearly observed that there is a lack of knowledge regarding hygiene and foodborne risks among most of these vendors. This observation states an urgent need of public health surveillance related to street food consumption. Despite the importance, there is limited study conducted on this subject in Pakistan, specifically there is scarcity of localized research and data in Islamabad.[8]

Our study aims to assess the quality of street-vended foods along-with the cleanliness of utensils being used in different popular street-food vending zones of Islamabad. By evaluating contamination patterns alongside associated factors, we aim to provide evidence-based policy-making recommendations in order to prevent public health problems linked to contaminated food consumption.

MATERIAL AND METHODS

This pilot study took place over three months, from March to May 2024, in six bustling commercial areas of Islamabad: G-6, G-9, F-6, F-7, I-8, and H-8. These locations were chosen for their high population density and the abundance of street food vendors.

We selected 10 vendors through random sampling, focusing on those who were easily accessible and willing to take part. The sample size of 10 vendors was selected as this was a pilot study aimed at assessing feasibility and generating preliminary data rather than producing statistically powered estimates. Pilot studies commonly use small, pragmatically chosen samples without formal sample size calculations, as supported by methodological guidance.[9,10] For each vendor, we collected one food sample and one utensil sample, total of 20 samples (10 food and 10 utensils) . Those operating in indoor, enclosed, or formally registered establishments were not included in the study. Similar to previous studies that have used structured surveys to assess food safety issues among the public,[11] we conducted a 10–15 minutes observational survey at each vendor site to assess hygiene, food handling, and environmental conditions including utensil hygiene , food safety practices , vendor health and waste management.[12] The survey focused on utensil hygiene, food safety, vendor health, and waste management. Observations supported lab findings by linking visible poor practices to microbial contamination. The food items we gathered featured popular local delicacies like shawarma, channa chaat, mango milkshake, and gol gappay.[13] All samples were collected using sterile gloves, swabs, and sealed containers. For utensils, a 10 cm^2 area was swabbed using sterile swabs moistened with 0.85% saline. Food samples (25 g) were taken directly from vendor stalls into sterile containers. All samples were put in proper labeled sterile polyethylene bags, carefully transported in an ice box, and delivered to the microbiology lab at National Skills University within 2 hours.[14] In order to test for microorganisms like *E. coli*, Salmonella, *Staphylococcus aureus* and to measure total viable counts we performed microbiological analysis.[15]

We conducted all microbiological procedures under strict aseptic environment. Culture media, glassware, and instruments were autoclaved at 121°C (15 psi, 15–20 min). During the process, Inoculating loops were sterilized by flame before and after each use. We disinfected all inoculation and plating which were done in a Class II biosafety cabinet with 70% ethanol before and after use. Every working individual wore sterile gloves and lab coats, and 70% ethanol was used to clean the work surfaces regularly. Using these measures ensured that the sample analysis were reliable and contamination-free. Homogenization for every sample was done in sterile saline and serially diluted (2-fold and 3-fold) in triplicates. One milliliter from every dilution was transferred on properly labeled Nutrient Agar plates using the pour plate method to calculate total viable count (TVC). The plates were then incubated at 37°C for approximately 18 hours. Colonies with 30–300 range were manually counted, and CFU/mL was calculated by multiplying the average count by the dilution factor.

After observing the initial growth, we took representative colonies and sub-cultured them by using Quadrant streaking method on MacConkey Agar , this helped us to isolate lactose fermenting and non lactose fermenting gram negative bacteria, with a thorough focus on *E. coli*. This is used to evaluate hemolysis patterns and to support the growth of fastidious organisms on the blood agar. After this process, we again sent the plates for incubation at 37°C for 24 hours, and followed by noting the colony morphology, including traits like color, opacity, hemolysis, edge, and elevation. To differentiate bacteria by cell wall structure, we performed Gram staining. By mixing colonies, we prepared smears with 0.85% saline on clean slides, air dried, and heat fixed. Crystal violet was used to stain the slides (1 min), followed by the treatment with Iodine (1 min), decolorized with ethanol, and counterstained with safranin (30 to 60 sec). After air-drying, we examined the slides under a 100x oil immersion lens, and then we recorded the results. To identify bacterial isolates, we carried out biochemical tests including Catalase Test. For this, a small portion

of colony was picked and placed on a glass slide, and 3% H_2O_2 was added so that we can observe the reaction. Bubble formation indicated a catalase-positive result, helping us to differentiate between Staphylococci (positive) from Streptococci (negative). Afterwards, we performed with Oxidase Test in which a colony was smeared on filter paper moistened with oxidase reagent. As a result, we observed a dark purple/blue color within 30 seconds indicating an oxidase-positive result, used to differentiate non-enteric (e.g., Pseudomonas) from enteric (e.g., *E. coli*,). Later we conducted an Indole Test in which a colony was inoculated into tryptone broth and incubated there at 37°C for 24 to 48 hours. After incubation, we added a Kovac's reagent resulting in the appearance of a red ring at the surface which indicated a positive result. This confirmed the ability to break down tryptophan into indole (e.g., *E. coli* positive, Klebsiella negative). Then we performed Coagulase Test in which a bacterial colony was mixed with the plasma of rabbit and later it was incubated at 37°C for up to 4 hours. Formation of clot indicated a coagulase-positive result hence, confirming the presence of *Staphylococcus aureus* (positive), while other Staphylococcus species were negative.

Before collecting the samples, ethical principles were strictly considered and followed. Informing consent was taken from all participating vendors. We also developed a standardized consent form which clearly stated that the food and utensil samples were being collected only for educational and research purposes, and that the data would not be used for any legal or regulatory actions.

Participation was completely voluntary. Vendors who agreed to take part by their will signed the consent form, showing us their understanding and cooperation. Vendors not giving consent were excluded from this study.[16] To maintain the confidentiality and encouragement to participate openly, we didn't record any personal information.

We took the Ethical approval for this study from the National Skills University before initiating the fieldwork.

Our all collected data were entered into IBM SPSS Statistics (version 26) for analyzing of microbial contamination levels with maintaining hygiene factors. To summarize the bacterial counts (CFU/mL), we calculated the descriptive statistics. We performed frequency analysis to identify and quantify the prevalence of various bacterial isolates. Subsequently, We used multiple linear regression analysis which was then used to assess the impact of hygiene practices for example: washing the hands, wearing the gloves . We evaluated the model performance using the coefficient of determination (R^2), F-statistics, and p-values, with statistical significance value set at $p < 0.05$. Results were presented with SPSS-generated charts and tables for interpretation.

RESULTS

Hygiene practices among selected vendors

A total of 20 samples were collected from 10 different vendors selling street-food in various sectors of Islamabad. Initially vendor practices were observed and recorded appropriately by filling out observational survey. Among the 10 selected vendors, the mean hygiene score was 3.8 out of 6 that is equivalent to 62.5% hygiene compliance. 3 vendors out of 10 showed compliance with the Good Hygiene Practices (GHP) which involve basic sanitation, cleanliness, and safe food-handling procedures, whereas one of the vendors adhered to these practices partially (50%). The remaining vendors showed a variable compliance with the hygiene standards. 50% of the vendors were working in a suitable environment that was appropriate in terms of hygiene, while the other 50% were working in environments prone to contamination because they were ambulant, exposed or were surrounded by unsanitary environments.

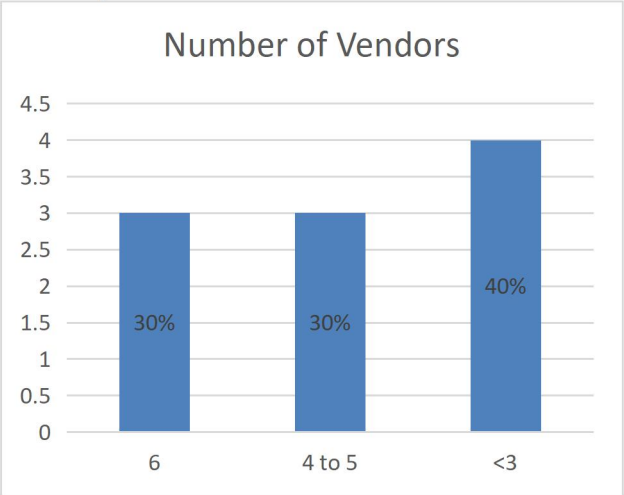


Figure 1: Graphical Representation of Hygiene Score Frequency

Microbial Contamination load in samples

Among all the food and utensil samples, contamination load was found to be variable. The maximum colony count among the 20 samples that were analyzed was recorded as (4.3*10^5 cfu/ml) in a food sample, whereas the minimum being (1*10^0 cfu/ml) found on utensil swabs. Secondly, various microbes were isolated after incubation; out of which 30% was found to be *E.coli*, followed by *Staphylococcus aureus* 20% and *Bacillus cereus* being 15%. Salmonella was isolated only in 5% of these samples. On the contrary, Opportunistic pathogens such as pseudomonas aeruginosa, enterococci and klebsiella pneumonia was found in 10% of samples.

Microbial Identification along with Biochemical Results

The microbial isolates obtained from nutrient agar plates included E.coli being the most abundant contaminant found in 30% of the samples (6/20), followed by *Staphylococcus aureus* found in 20% of the samples (4/20). Pseudomonas aeruginosa was found in 10% (2/20) of the samples, *Bacillus cereus* in 15% (3/20), enterococci also being in 10% (2/20) and klebsiella pneumoniae being the same (2/20). Whereas Salmonella was found to be in 5% of the samples tested (1/20). Subsequently, further identification was done by performing biochemical testing that revealed 60% and 40% distribution among the samples tested. 40% (8/20) of the samples were identified to be gram positive whereas 60% (12/20) were found to be gram negative microorganisms. Biochemical tests showed significant results. 100% of the samples tested were catalase positive. Oxidase test results revealed 15% oxidase positive microorganisms including pseudomonas aeruginosa and *Bacillus cereus*. 20% of the microbes were coagulase positive that consisted of *Staphylococcus aureus* found. Indole positive microbes were found to be 30% including all E.coli. The samples analyzed showed all the three hemolysis patterns including alpha, beta and gamma hemolysis. Alpha: 10% (enterococci), Beta: 45% (S.Aureus, Pseudomonas Aeruginosa, *Bacillus cereus*, klebsiella pneumonia, salmonella, E.coli) and Gamma: 45% (E.coli, S.Aureus, pseudomonas aeruginosa, *Bacillus cereus*)

Table 1: Colony Counts of Samples Obtained from Nutrient Agar Plates

Vendor ID	Sample Type	Location	CFU/ML	Unacceptable level
1	FOOD	h8	300	$\geq 10^2$ (indicates fecal contamination)
100	UTENSIL	h8	2	≥ 1 CFU/cm² (fecal contamination risk)
2	FOOD	h8	3000	$\geq 10^3$ (toxin risk)
200	UTENSIL	h8	1	≥ 1 CFU/cm² (toxigenic risk)
3	FOOD	g6	185000	$\geq 10^3$ (contamination risk)
300	UTENSIL	g6	1	≥ 1 CFU/cm² (biofilm/resistance risk)
4	FOOD	g9	9000	$\geq 10^4$ (food poisoning risk)
400	UTENSIL	g9	2	≥ 1 CFU/cm² (fecal contamination risk)
5	FOOD	f6	5000	$\geq 10^4$ (food poisoning risk)
500	UTENSIL	f6	2	≥ 1 CFU/cm² (toxigenic risk)
6	FOOD	f6	300	$\geq 10^2$ (indicates fecal contamination)
600	UTENSIL	f6	2	≥ 1 CFU/cm² (fecal contamination risk)
7	FOOD	i8	800	$\geq 10^2$ (indicates fecal contamination)
700	UTENSIL	i8	1	≥ 1 CFU/cm² (toxigenic risk)
8	FOOD	i8	430000	$\geq 10^4$ (poor sanitation)
800	UTENSIL	i8	6	≥ 10 CFU/cm² (indicator of fecal contamination)
9	FOOD	g9	7000	$\geq 10^3$ (indicates unsafe handling)
900	UTENSIL	g9	4	≥ 10 CFU/cm² (nosocomial infection risk)
10	FOOD	f7	50000	$\geq 10^4$ (food poisoning risk)
1000	UTENSIL	f7	2	Detected in any sample (immediate action required)

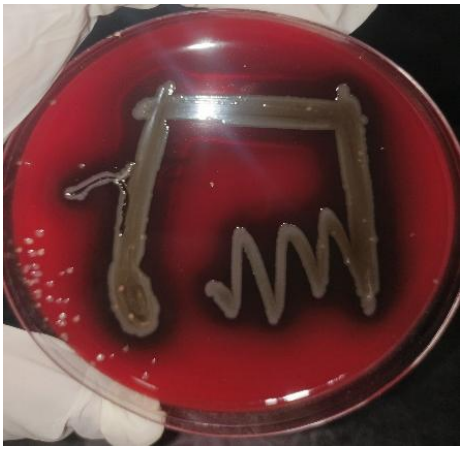
Table 2: Blood Agar Colony Characteristics & Hemolysis Patterns

Bacterium	Size	Shape	Margin	Elevation	Surface	Opacity	Color Pigment	Hemolysis	Additional Notes
Escherichia coli	M	Circular	Entire	Convex	Smooth	Opaque	Grayish-white	Beta (β)	Double zone hemolysis (some strains)
Staphylococcus aureus	M	Circular	Entire	Convex	Smooth	Opaque	Golden-yellow	Beta (β)	Clear zone around colonies
Bacillus cereus	L	Irregular	Undulate	Flat	Rough	Opaque	White-gray	Beta (β)	Spreading growth
Enterococcus faecalis	S	Circular	Entire	Convex	Smooth	Opaque	Grayish-white	Alpha (α) or Gamma (γ)	Variable hemolysis
Salmonella spp.	M	Circular	Entire	Convex	Smooth	Opaque	Grayish-white	Gamma (γ)	No hemolysis
Pseudomonas aeruginosa	L	Irregular	Undulate	Flat	Rough	Opaque	Greenish-gray	Beta (β)	Fruity odor, may show metallic sheen
Klebsiella pneumoniae	L	Mucoid	Entire	Convex	Glossy	Opaque	Grayish-mucoid	Gamma (γ)	Very mucoid colonies

Table 3: MacConkey Agar Colony Characteristics

Bacterium	Growth	Size	Shape	Color	Lactose Fermentation	Additional Notes
Escherichia coli	Good	Medium	Circular	Bright Pink	LF (Positive)	Metallic sheen possible
Staphylococcus aureus	No growth	-	-	-	-	Gram-positive (inhibited)
Bacillus cereus	Poor*	Small	Irregular	Pale Colorless	NLF (Negative)	Faint growth (some strains)
Enterococcus faecalis	No growth	-	-	-	-	Gram-positive (inhibited)
Salmonella spp.	Good	Medium	Circular	Colorless	NLF (Negative)	Transparent colonies
Pseudomonas aeruginosa	Good	Large	Irregular	Colorless	NLF (Negative)	Green pigment (pyocyanin)
Klebsiella pneumoniae	Good	Large	Mucoid	Pink to Dark Red	LF (Positive)	Very mucoid, "sticky"

Gram Negative Bacteria on BA



Bacillus cereus with Alpha Hemolysis on BA

DISCUSSION

This study presents critical insights into the microbial risks linked with street-vended foods in Islamabad. Over . We found out that the microbial contamination were strongly related to species and food specific. Due to temperature fluctuations during handling, meat based dishes such as shawarma and keema were often prominently contaminated by *E. coli*. *Staphylococcus aureus* was predominately found in hand assembled foods like chaat and samosas, resulting in direct transmission through improper food handling. In starch rich foods like gold gappay, *Bacillus cereus* was frequently detected due to spore survival post cooking and poor storage conditions. The specie of Pseudomonas was mostly present in beverages like mango shakes which indicates high moisture driven microbial loads. Apart from water, milk as well is likely to serve as a source of *E-coli* in any milk based fruit blends, such as milkshakes, the widespread use of unpasteurized milk among street vendors may further promote proliferation of such pathogens.[17] In contrast, our analysis revealed no statistically significant relation with hygiene scores and microbial loads ($R^2 = 0.093$, $p = 0.192$). To our notice, among vendors who practiced good hygiene, high B.cereus loads were detected. This indicated that microbial ecology and post cooking storage conditions outweighs the surface level cleanliness. The factor which was often overlooked in standard hygiene practices was the contamination of the Pseudomonas specie in beverages highlighting the importance of environmental moisture. Most of the street vendors prepare food without a proper shop, with carts often located above drain holes, the main source of contamination hence, the high levels of microbial contaminations were observed. These carts are also located along the busy roads which exposes the food to dust, dung and other pollutants.[18] These vending sites were also observed in unsanitary conditions such as the accumulation of garbage attracting insects . Most of the carts did not have any protective coverings which left the food uncovered and vulnerable to the exposure of environment. Occasionally, some vendors did clean their carts and surroundings however, utensil washing practices were not sufficient enough while many relying only on water rather than using detergent. These conditions favors the frequent transmission of pathogenic microorganisms, implementing significant public health risks, mainly for students, laborers, and other economically vulnerable groups.[19] *E. coli*, S. aureus, and Salmonella are linked with gastrointestinal illnesses and systemic infections which can trigger outbreaks, particularly in busy urban zones and lack of sanitation.[20] Culture techniques which are essential for bacterial detection, may have missed viruses, protozoa, and non-culturable pathogens. Furthermore, the environmental, temporal variables and time of sampling were not recorded.

CONCLUSION

The conclusion of this study reveal greater levels of microbial contamination in street vended foods around Islamabad. We found that the contamination was varied from microbial species to the food type. *E. coli* was most found in meat dishes, S. aureus in manually handled and assembled snacks, B. cereus in starch rich food items, and Pseudomonas in mostly unrefrigerated beverages. This study is consistent with the research that has been done before posing that microbial contamination in street foods is affecting public health concern across the country. We expected proper hygiene to reduce contamination however, the results showed us that the risk of contamination still remained. Since hygiene findings were not clearly linked to microbial load, this shows that the basic hygiene alone may not be enough. Food safety should also consider other factors such as environment and specific microorganisms present. Reducing microbial risk does not only depends on vendors behavior but also requires specific targeted actions into the account, the environment, the type of microorganisms present and specific risks linked to certain food types. Immediate actions including vendor education, regulatory forms, and improved overall sanitation infrastructure are important in busy populated areas such as H-8 and F-6. Our findings and results suggest that the food safety protocols in Pakistan may need to improve to better match the real actual conditions and actual microbial risk found in street food.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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