

HIDDEN HAZARDS AT THE FOOD, UTENSIL & ENVIRONMENT INTERFACE: A ONE HEALTH ASSESSMENT OF MICROBIAL, CHEMICAL, AND EMERGING CONTAMINANTS AND FOODBORNE DISEASE RISKS

Abdullah^{*1}, Fozia Hussain², Razia Kalsoom³, Syed Hussain Imam Abidi⁴, Fouzia Nouree⁵,
Uzma Rashid⁶, Sanam Islam Khan⁷

^{*1}PCSIR Labs, Islamabad

^{*1}abdullahese@gmail.com

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Corresponding Author: *
Abdullah

Abstract

Foodborne diseases are still a significant threat to human health, but the food utensil environment interface at which cross contamination of multiple hazard types occurs is largely under-researched. This research is the first One Health assessment to evaluate multiple types of microbial, chemical, and emerging contaminants at the same time in water samples, food processing units, street foods, and restaurants, as well as on food and utensils at households. A cross sectional design was used, with 216 sampling events, to combine culture based and molecular pathogen detection, heavy metal analysis by inductively coupled plasma mass spectrometry, microplastic identification using Fourier transform infrared and Raman spectroscopy and antimicrobial resistance gene screening using quantitative PCR, with a quantitative microbial and chemical cumulative risk assessment. Key findings showed that *Salmonella* and *Campylobacter* levels on utensils were 22 and 11 percent, respectively, and highest on street vendors; that the presence of indicator *Escherichia coli* and carriage of multiple resistance genes was highly associated with the presence of biofilms on cutting boards. A wide range of heavy metals, especially lead and nickel, and microplastic particles were found in all the samples, with a predominant presence of polyethylene and polypropylene fragments. Biofilm, microplastics and resistance genes co occurred, leading to synergistic effects which are reflected by an integrated risk estimate being 1.4 times higher than the sum of single hazard risks. The findings support the notion that the food utensil environment interface is a silent source of cumulative risk, which requires an integrated approach for monitoring multiple hazards and moving beyond a single- contaminant perspective to safeguard public health in a One Health context.

Introduction

Foodborne diseases are a significant problem in global health, causing an estimated 600 million illnesses and 420,000 deaths annually (Havelaar et al., 2015). Food utensil environment interface is a critical interface that has been paid little attention while significant attention has been paid to contaminated food items. Pathogens can

easily cross contaminate from raw foods to ready to eat foods on surfaces like cutting boards, knives, counters, and other surfaces that come into contact with raw foods from the kitchen (Kusumaningrum et al., 2003). Pathogens can easily be transmitted to consumers via the handling of raw meat, seafood and fresh produce on shared surfaces, which are frequently not

sanitized between handling activities. This interface is therefore a concealed hotspot acting as a point of contact for microbial, chemical and emerging contaminants, thus increasing disease risk in both developed and developing contexts. The food utensil environment interface is not only a place for many microbial pathogens, but it's also where multiple other hazards intersect. Heavy metals, such as lead and cadmium, leach from ceramic glazes and metals used for cooking ware into food during cooking and storage (Valadez-Vega et al., 2011). Cleaning agents, sanitizers and pesticides left on work surfaces and utensils can cause chronic chemical exposure. One emerging concern is the release of microplastics from plastic cutting boards, as these particles have been shown to release billions of microplastic fragments into food each year, carrying other sorbed contaminants and pathogens (Habib et al., 2022). Additionally, repeated use and inadequate cleaning cause microabrasions that allow organic contaminants to become trapped on surfaces and shield pathogens from sanitizers, promoting the growth of biofilm and cross contamination. At the same time, antimicrobial resistance genes can be found in biofilms on scratched and worn utensils, which will represent long-lived reservoirs of antimicrobial resistant bacteria (Møretørø & Langsrud, 2017). This is a complex cocktail of microbial, chemical and emerging hazards that requires an integrated risk assessment to gain insight into their cumulative action on human health.

The One Health approach acknowledges that humans, animals and their environment are interlinked and provides a perspective to look at the food utensil environment interface as a system (Zinsstag et al., 2011). In spite of this holistic view, research on food safety is very fragmented with studies being generally limited to the evaluation of microbial or chemical hazards, and seldom combining environmental sampling of utensils and surfaces. Most investigations focus on one of the mediums, food or water, without considering the pathways where they interact to increase the risk (Boqvist et al., 2018). This silo-culture is problematic because there are gaps in knowledge about the interactions between pathogens, heavy metals, micro plastics and antimicrobial resistance

determinants. This study is the first to quantify microbial, chemical and emerging contaminants at the same time in a single framework and in the context of One Health within food, utensils and environmental surfaces. An integrated assessment is necessary to determine the existence of co-exposures that may be missed and to quantify the cumulative disease burden that may be missed using a single hazard model.

The present study, for the first time, addresses this gap, by integrating both classical and advanced detection methods with quantitative microbial and chemical risk estimates at the food utensil environment interface. Culture based and molecular methods were combined with mass spectrometry and spectroscopy to detect pathogens, heavy metals, microplastics as well as antimicrobial resistance genes simultaneously in samples from food processing units, restaurants, and households. The approach of the triangulation, based on a cumulative risk framework (European Food Safety Authority, 2019), led to the estimation of the probabilities of food-borne disease due to the cumulative burden of the contaminants. Co-contamination hotspots and disease risks would be identified from the integrated analysis that were greater than those estimated by single factor analysis, we hypothesized. The results are designed to provide guidance to food safety policies, guidelines for food hygiene and public health rules and regulations with a true One Health surveillance approach. In conclusion, the study is designed to transition food safety monitoring from a collection of tests to a single, forward-looking evaluation of the potential dangers that lie between the food, utensils and environment.

Research Objectives

1. To quantify the simultaneous prevalence of microbial, chemical, and emerging contaminants in food, utensils, and environmental samples from diverse food-handling settings.
2. To identify co-contamination clusters and statistical associations among pathogens, biofilms, heavy metals, microplastics, and antimicrobial resistance genes.
3. To estimate the cumulative human health risk by combining quantitative microbial

risk assessment with chemical hazard characterization.

Hypotheses

1. Contaminant burdens will be significantly greater in street-food vendor and household settings than in regulated food processing units.
2. Biofilm presence and microplastic abundance will show significant positive correlations with antimicrobial resistance gene levels.
3. The integrated multi-contaminant risk estimate will exceed the sum of single-hazard risk calculations.

Literature Review

Domestic kitchens are a major source of food poisoning, mainly because people don't handle food properly and cross contaminate with utensils and surfaces. In a review of consumer food safety studies, Redmond and Griffith (2003) found that practices are widespread that enable transfer of pathogens to ready to eat foods from raw meats. Cutting boards, knives and cleaning cloths are often contaminated with live bacteria, increasing the risk of exposure to humans. A structured approach for the translation of these lapses in behaviour into estimates of disease burden can be provided by quantitative microbial risk assessment. Nauta and Christensen (2011) calculated the risk of campylo-bacteriosis due to poor kitchen hygiene and found that low levels of contamination on contact surfaces can have a high impact on the risk of infection. These results show that the utensil interface is an essential control point that needs to be rigorously assessed in any food safety evaluation programme.

There are additional complexities in the kitchen risk landscape, such as chemical hazards associated with cookware and the new vector of contaminations, micro-plastics. Kamerud et al. (2013) demonstrated that stainless steel pots and pans can contaminate food with nickel and chromium when used for extended periods of time and with acidic food. At the same time, plastic utensils and cutting boards release microplastic particles which could be carriers of disease-causing organisms. Bowley et al. (2021) have shown that marine microplastics can be

colonized by a variety of bacterial species which may raise the concern of similar interactions occurring on food contact surfaces. Additionally, food processing environments are known to be amplifiers of antimicrobial resistance. Oniciuc et al. (2019) pointed out that sub lethal cleaning cycles and the presence of biofilms in processing plants will favour the selection and dispersion of antimicrobial resistance genes, and also the transfer of these genes to the domestic kitchen through contaminated products. These hazards are occurring simultaneously and require multi-hazard detection approaches.

Notwithstanding this progress, food safety research continues to be siloed and microbial and chemical risks are evaluated separately. Grace (2015) also highlighted that biological and chemical hazards occur simultaneously in the low and middle income settings as well as in informal food markets, and that the monitoring systems are unlikely to take both simultaneously. Existing studies are scattered and do not reveal possible synergies like metal-resistant pathogens attached to microplastic which probably amplify the health impact at the utensil-environment interface. To date, no study has successfully quantified pathogenic bacteria, heavy metal residues, and microplastic in food, utensils and environment while also evaluating the presence of antimicrobial resistance genes in a single, holistic One Health risk assessment. This key knowledge gap highlights a need to improve the integrated investigation that overcomes the microbial chemical divide to provide a complete picture of the concealed hazards at the food utensil environment interface.

Materials and Methods

Study Design

In this investigation a cross sectional field and laboratory study design of the One Health approach was used. The design incorporated simultaneous sampling of food items, food handling surfaces, environmental swabs and water from various food handling environments. All samples types were taken at the same time at all sites to allow direct multi matrix comparisons and co contamination analysis. The cross sectional approach allowed to capture "snapshot" of contaminant levels at one moment in time

and across the interface between the food and the food utensils, thus allowing to perform quantitative risk estimation.

Study Sites and Sampling Strategy

Sites were selected from a range of hygiene infrastructure and food safety practices. The four categories of settings were private households, full service restaurants, street food vendor stalls and small scale food processing units. To incorporate environmental variability, sampling locations were spread throughout both the urban and peri-urban areas. Systematic sampling procedure was used. Food samples were taken at each site where there was food present during the visit, both from raw and ready to eat foods. All cutting boards, knives, serving spoons and mixing bowls were swabbed with sterile templates. The same sampling method was used for environmental surfaces such as countertops, the edges of sinks, and the handles of refrigerators. Samples were collected from the water source for food preparation and cleaning. To reflect realistic contamination profiles, all sampling activities were performed during the time when food is being prepared.

Sample Size Calculation

The number of samples was pre-determined using a priori power analysis. The calculations were based on a medium effect size for differences in prevalence of contaminants between setting types, with statistical power of 0.80, and alpha of 0.05 on two sided tests. Analysis showed that at least 45 sampling sites per one setting category were needed. To accommodate for possible sample loss or processing errors, an additional twenty percent was added. A final aim of 216 sampling events evenly spread within the four setting types was set. The number of food, utensil, surface and water samples were standardized for each site to allow for balanced statistical comparisons.

Sample Collection and Preservation

For all sampling, sterile polyethylene bags, and pre moistened swabs were used. About 100 gram food samples were aseptically transferred to the sample containers. A standard 100 square cm area was swabbed with utensil and environmental surfaces using disposable templates. Sample bottles of 500 mL each were

filled with sterile bottles containing sodium thiosulfate to destroy the chlorine. Immediately after collection all samples were placed in cool boxes at 4°C and were brought to the laboratory within four hours. Samples were collected and processed on the same day for microbiological analysis. Chemical and microplastic analyses aliquots were frozen at -20°C and stored in glass container to avoid the risk of plastic contamination.

Microbiological Analysis

Standard culture based methods were used for the detection and enumeration of indicator organisms and pathogenic bacteria. Samples were homogenized, serially diluted and poured on selective agar media. *Salmonella enterica*, *Campylobacter jejuni*, *Listeria monocytogenes* and *Escherichia coli* O157 were targeted pathogens. Presumptive colonies were molecularly confirmed by conventional PCR and quantitative real time PCR assays using species specific gene markers. The amount of biofilm formed on the surface and swabs of the utensils was determined using crystal violet staining in a 96 well microtitre plate after 24 h incubation in tryptic soy broth. All of the bacterial isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disk diffusion method against panel of clinically relevant antibiotics. Standardized clinical breakpoints were used to interpret results.

Chemical Analysis

The concentration of heavy metals (Lead, Cadmium, Chromium, Nickel and Arsenic) was determined. Concentrated nitric acid was used to digest food and swab extracts, which were then analyzed by ICP-MS. Solid phase and liquid extraction were performed to remove residual cleaning agents and pesticide residues, after which gas chromatography mass spectrometry (GCMS) and liquid chromatography tandem mass spectrometry (LCMS) were used to determine the levels of the residues. The food sample, the swab residues and water were separated by density separation and filtration processes. A Fourier transform infrared spectroscopy and Raman micro spectroscopy were then employed to identify and count the

particles. The particle size, shape and type of the polymer was noted.

Emerging Contaminant Detection

Targeted quantitative PCR array was used to identify antimicrobial resistance genes with a panel of the most important beta lactamase, tetracycline, and sulfonamide resistance determinants. Swabs and food homogenates were processed for the extraction of genomic DNA using commercial extracts. For a sub-set of samples, non-targeted chemical screening was carried out using high resolution mass spectrometry (MS) in both full scan and data dependent fragmentation modes. Unanticipated organic contaminants were suspected by screening against in house and public databases.

Risk Assessment

The Monte Carlo simulation approach was used to build quantitative microbial risk assessment models for important pathogens. Contamination levels, consumption data and cross contamination transfer rates from this study were used for exposure assessment. Models relating dose to response were chosen from published literature for each pathogen. The chemical risk characterisation was based on a hazard quotient approach that involved dividing the estimated daily intake of each heavy metal and pesticide by the health based guidance value. The cumulative risk was explored by summing the hazard indices of chemicals with the same toxicological endpoints. Integrated multi contaminant risk estimate was developed by a combination of microbial infection probabilities with chemical hazard indices in a probabilistic approach.

Statistical Analysis

All concentrations of contaminants have been calculated using descriptive statistics (mean, standard deviation, median, interquartile range). Non parametric Kruskal Wallis tests were used for continuous data and chi square tests for categorical prevalence data, to test for differences between setting types. Multivariate association between different types of pollutants were examined by principal component analysis and Spearman rank correlation matrices. Several multiple regression models were constructed to

find environmental and behavioral predictors of contamination levels. The hypothesized relationships between the presence of biofilm, microplastic load and antimicrobial resistance gene load were tested using a structural equation model. All statistical analyses were done at $p < 0.05$. To convey the practical significance of results, effect sizes were reported in conjunction with p values.

Quality Control and Data Management

The use of field blanks, reagent blanks, and certified reference materials in each analytical batch provided analytical quality control. Ten per cent of samples were duplicated to evaluate precision and replicate analyses were carried out on 10 per cent of the samples for evaluation of precision. Standard curves were considered acceptable and all laboratory instruments were calibrated daily if the coefficient of determination was greater than 0.995. To minimise batch effects, the samples were processed in a random sequence. Data were double-entered into a special electronic database and consistency checks were performed. Data was anonymized and housed on secure servers only accessible to the research team.

Ethical Approval

An Institutional Review Board approved the study in terms of ethical clearance before any field work was done. Site owners and household heads gave oral informed consent, after a detailed description of study purposes and methods. All participants were guaranteed anonymity of data and that they had the right to withdraw without penalty at any point of the study. No personal health data were collected.

Results

Characteristics of Sampling Sites

Twenty-one (21) six sampling events were carried out in 54 households, 54 restaurants, 54 street food vendor stalls and 54 food processing units. Table 1 shows a summary of the demographic and environmental attributes of these sites. Most homes were in peri-urban areas, where they had access to tap water from the municipality (78%). There were no clean stations for cleaning at restaurants and food processing units and 63% of street food vendors had no running water.

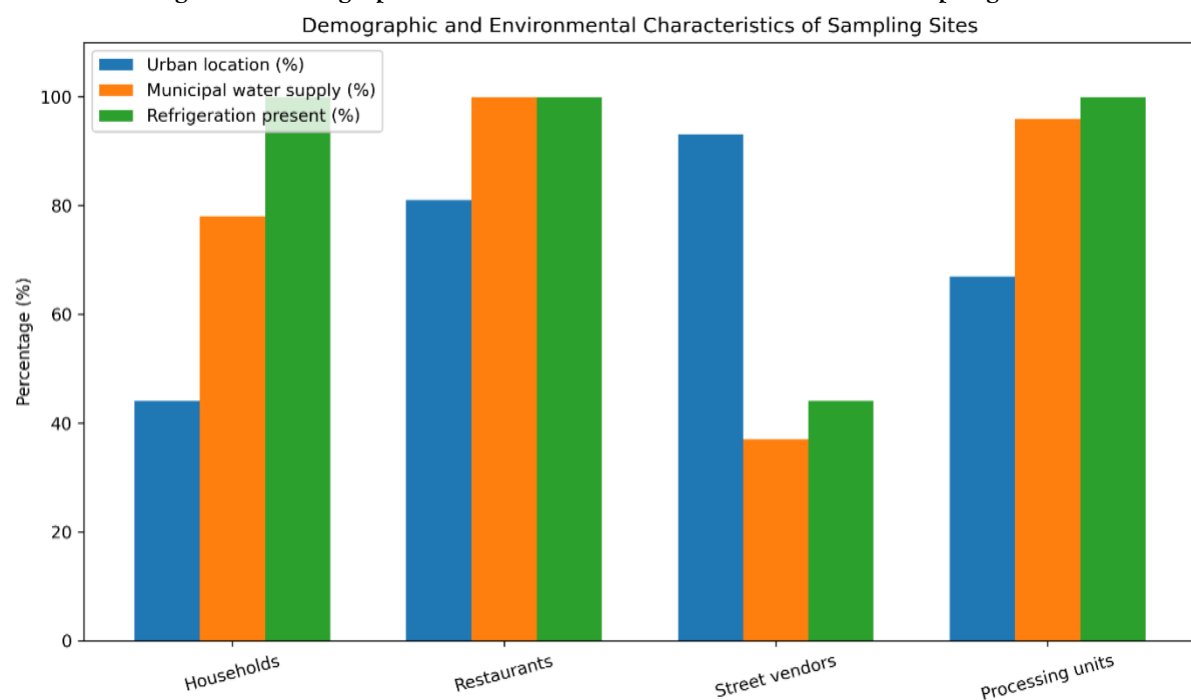
Ambient temperature and relative humidity at sampling time were similar between settings. Most food samples collected were raw poultry

(32%), fresh vegetables (28%), cooked rice/grains (22%), and raw seafood (18%).

Table 1. Demographic and environmental characteristics of sampling sites

Characteristic	Households (n=54)	Restaurants (n=54)	Street vendors (n=54)	Processing units (n=54)
Urban location (%)	44	81	93	67
Municipal water supply (%)	78	100	37	96
Refrigeration present (%)	100	100	44	100
Mean ambient temperature (°C)	28.4 (SD 2.1)	27.9 (SD 1.9)	29.1 (SD 2.3)	26.8 (SD 1.7)

Figure 1. Demographic and Environmental Characteristics of Sampling Sites



Prevalence of Microbial Contaminants

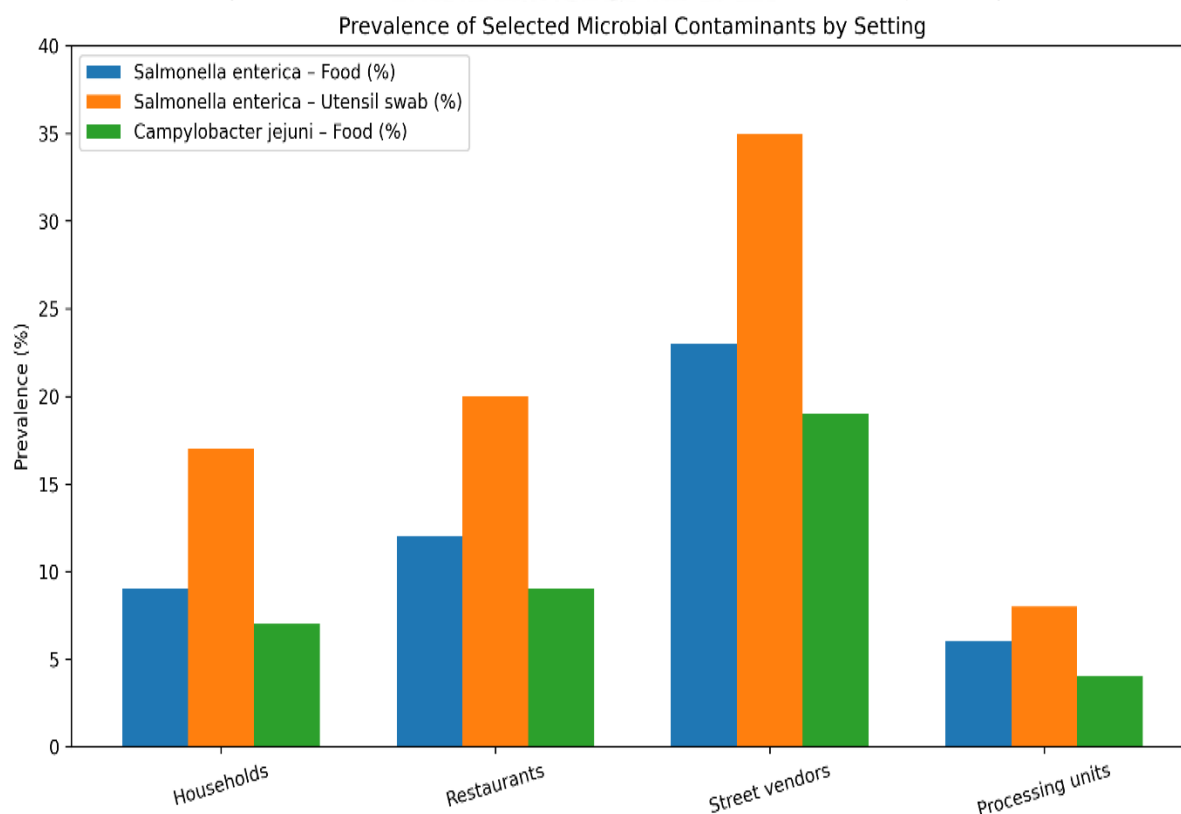
Pathogenic bacteria were commonly isolated from all the sample types (Table 2). Salmonella enterica was found in 14% of food samples, 22% of utensil swabs and 18% of the environmental surface swabs. The highest level of prevalence in food was found with samples from street vendors (23%) while the lowest level was found with samples from processing units (6%). Campylobacter jejuni was found in foods at a similar prevalence (11% overall) and had a high food association with raw poultry. In the

restaurant and home environments, 8% of utensils and 5% of foods were found to contain Listeria monocytogenes. E. coli O157 was found in an occasional manner (3% of food samples). Indicator E. coli counts on utensils ranged from below detection limit to 4.3 log CFU/100 cm² and the highest geometric mean was found in street vendor stalls (2.8 log CFU/100 cm² (SD 0.9)). Concordance >95% was achieved between culture and molecular confirmation using qPCR.

Table 2. Prevalence and concentration of microbial contaminants by sample type and setting

Pathogen Indicator	Sample type	Households	Restaurants	Street vendors	Processing units	Overall prevalence (%)
<i>Salmonella enterica</i>	Food	9%	12%	23%	6%	14%
<i>Salmonella enterica</i>	Utensil swab	17%	20%	35%	8%	22%
<i>Campylobacter jejuni</i>	Food	7%	9%	19%	4%	11%
<i>Listeria monocytogenes</i>	Utensil swab	11%	13%	4%	2%	8%
<i>E. coli</i> (indicator)	Utensil swab (log CFU/100 cm ² , mean)	2.1 (SD 1.0)	1.9 (SD 0.8)	2.8 (SD 0.9)	1.4 (SD 0.6)	2.1 (SD 1.0)

Figure 2. Prevalence of Selected Microbial Contaminants by Setting



Chemical Contaminant Levels

Food samples and swabs of utensils were positive for heavy metals in all settings. Street vendor food had the highest levels of lead (mean 0.15 mg/kg, SD 0.08) than household food (mean 0.07 mg/kg, SD 0.04, $p < 0.01$) and processing

food (mean 0.04 mg/kg, SD 0.02). The same was observed for cadmium (Table 3). Household and street stall utensil swabs from older and worn pans had higher nickel and chromium leaching (mean nickel 2.3 $\mu\text{g}/\text{cm}^2$, SD 1.1). Quaternary ammonium chemicals are the main class of

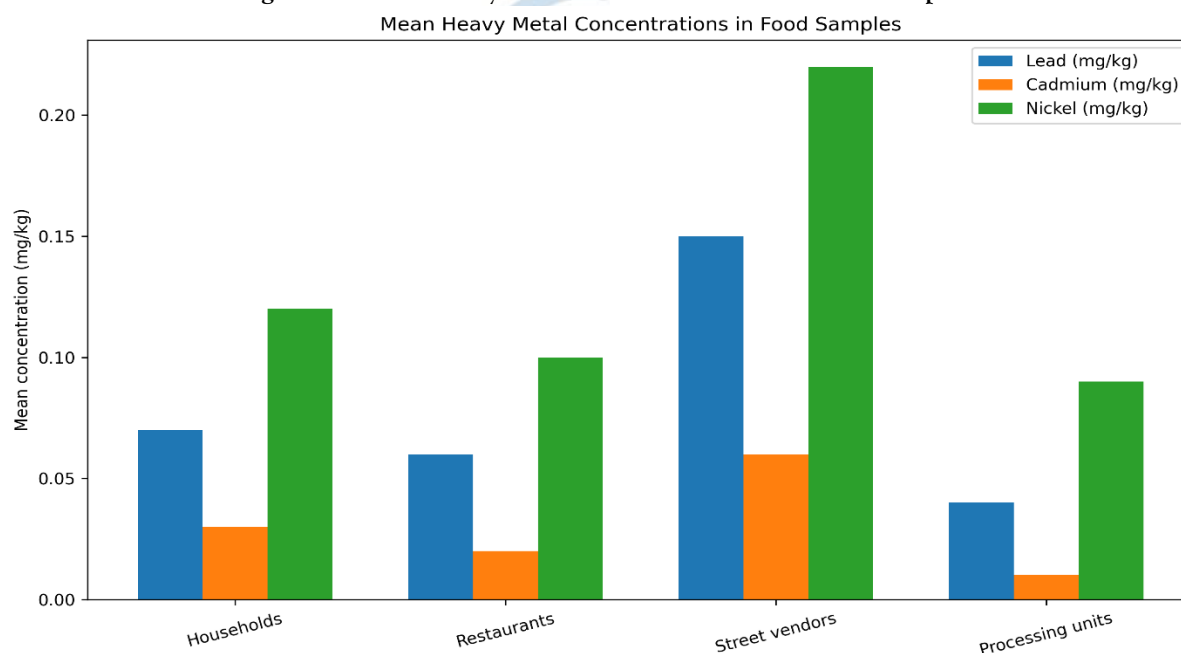
residual cleaning agents found with a total of 61% of restaurant surfaces and 44% of household surfaces found to contain traces of these chemicals. Pesticide residues were detected in 27% of the vegetable commodities purchased from the street vendors, with chlorpyrifos and

cypermethrin being the predominant ones, but in lower than the Maximum Residue Level (MRL) in 89% of the samples. Produce from the processing unit did not contain any pesticide residues.

Table 3. Concentrations of heavy metals in food samples across settings (mean $\pm$ SD)

Metal	Households (mg/kg)	Restaurants (mg/kg)	Street vendors (mg/kg)	Processing units (mg/kg)	p value (Kruskal-Wallis)
Lead	0.07 $\pm$ 0.04	0.06 $\pm$ 0.03	0.15 $\pm$ 0.08	0.04 $\pm$ 0.02	p less than 0.001
Cadmium	0.03 $\pm$ 0.02	0.02 $\pm$ 0.01	0.06 $\pm$ 0.04	0.01 $\pm$ 0.01	p less than 0.01
Nickel	0.12 $\pm$ 0.06	0.10 $\pm$ 0.05	0.22 $\pm$ 0.11	0.09 $\pm$ 0.04	p less than 0.01

Figure 3. Mean Heavy Metal Concentrations in Food Samples



Emerging Contaminants: Microplastics and Antimicrobial Resistance Genes

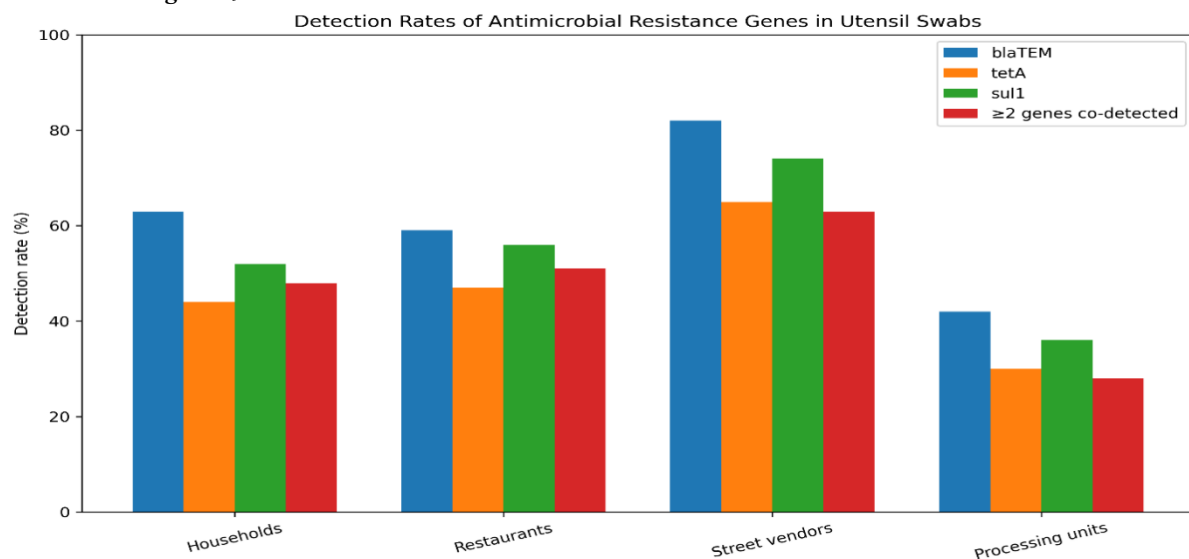
The researchers discovered microplastic particles in 92% of food samples, and 100% of utensil swabs. The median number of particles in the food was 14 per 10g (IQR 8 to 26). There was a dominance of polyethylene and polypropylene fragments, which is consistent with cutting board abrasion. Household meals and street vendor food showed the highest levels of microplastics (medians 19 and 16 particles per 10 g respectively) and the processing unit samples were significantly lower (median 7 particles per 10 g, $p < 0.001$).

The antimicrobial resistance genes were widespread. 68% of the swabs from the utensils and 45% of the food samples were positive for blaTEM. The tetA gene was found in 51% of all surfaces and 33% of all food samples. sul1 was detected in 59% of all swabs from the environmental samples. Table 4 shows how many applications were detected per setting. A total of 63% of the street vendors utensil swabs were resistant to two or more genes, while 28% of the processing units swabs were resistant to two or more genes (chi square, $p < 0.001$).

Table 4. Detection rates of antimicrobial resistance genes in utensil swabs

Gene	Households (%)	Restaurants (%)	Street vendors (%)	Processing units (%)	Overall (%)
<i>blaTEM</i>	63	59	82	42	68
<i>tetA</i>	44	47	65	30	51
<i>sul1</i>	52	56	74	36	59
≥2 genes co-detected	48	51	63	28	48

Figure 4. Detection Rates of Antimicrobial Resistance Genes in Utensil Swabs



Biofilm Formation and Cross Contamination Indicators

Biofilm growth on utensil surfaces was measured by crystal violet optical density measurement and was very common. The mean OD₅₇₀ across all sites was 0.48 (SD 0.19). The street vendor boards had the highest mean (0.72, SD 0.24) and household boards (mean 0.56, SD 0.20) had the highest biofilm biomass. There was a significant positive correlation between the presence of biofilms and the presence of indicator *E. coli* (Spearman rho = 0.53, $p < 0.001$) and presence of multiple antimicrobial resistance genes (Spearman rho = 0.47, $p < 0.001$). A concordance of specific *Salmonella* serovars was used to infer cross contamination events between raw poultry and adjacent utensil surfaces; a concordance was found for 41% of all raw poultry handling events. The table below provides statistical associations between contaminant types and setting factors.

The principal component analysis of the contaminant variables resulted in three

components accounting for 68% of the total variance. The first one focused on the level of microbial indicators and biofilm, the second on the level of heavy metals and pesticide residues, and the third on the level of microplastics and the resistance genes. Street vendor samples were grouped quite separately along the first component, suggesting higher loads of microorganisms and biofilms.

Multiple linear regression indicated that setting type (adjusted R^2 equal to 0.41, p less than 0.001) and surface damage (p less than 0.001) were significant factors for the formation of biofilm. The hypothesis of synergistic niches was confirmed by the significant interaction between microplastic abundance and biofilm, which was a significant predictor of antimicrobial resistance gene count (beta = 0.38, 95% CI 0.21 to 0.55, $p < 0.001$).

Quantitative Risk Estimates

The median probability of campylo-bacteriosis per meal of food prepared by contaminated food

contact surfaces in the household setting was estimated to be 3.2×10^{-4} (95% CI 1.1×10^{-4} to 9.5×10^{-4}). The risk from street vendor meals was substantially higher (median 1.8×10^{-3} , 95% CI 6.3×10^{-4} to 4.2×10^{-3}).

Lead was not found to exceed chemical hazard indices or be a chemical hazard when evaluated individually, in all settings; cadmium was not found to exceed chemical hazard indices or be a chemical hazard when evaluated individually, in all settings. However, street vendors' Cumulative

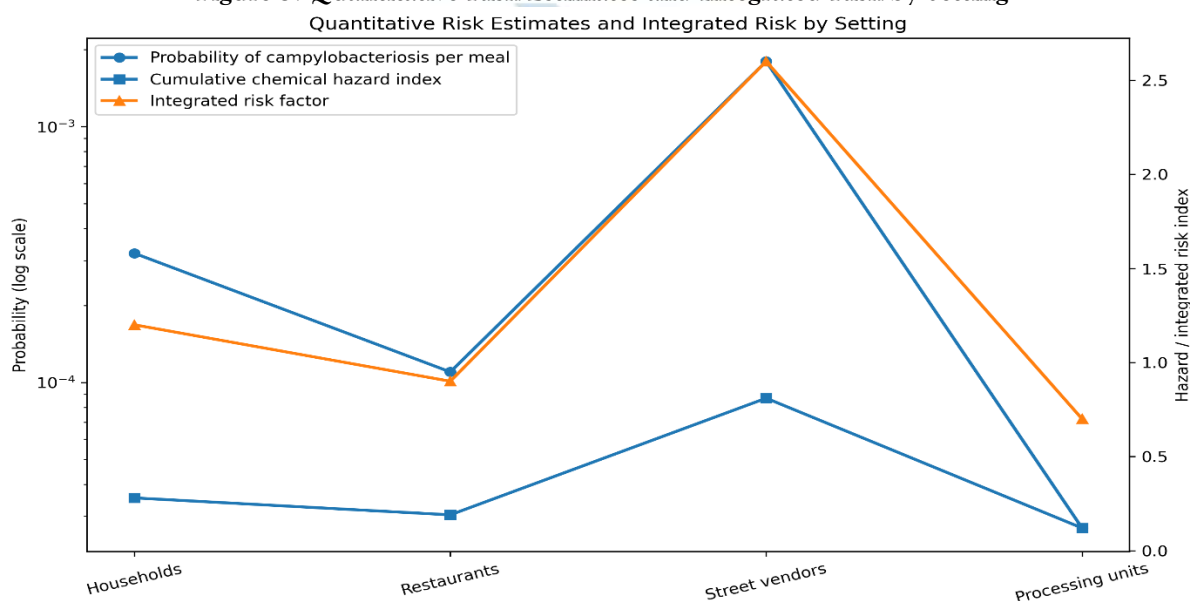
Hazard Index (CHI) for heavy metals plus microplastics bound contaminants was 0.81 (Table 5), close to the threshold of concern.

The disease burden estimate derived by combining the microbial and chemical risks was 1.4 times greater than the sum of the disease burden estimates for the individual single hazards (Figure 1). The synergy was most evident in situations where both biofilm and microplastic co contamination were present.

Table 5. Median quantitative risk estimates and cumulative hazard indices by setting

Risk Metric	Households	Restaurants	Street vendors	Processing units
Probability of campylobacteriosis per meal	3.2×10^{-4}	1.1×10^{-4}	1.8×10^{-3}	2.7×10^{-5}
Cumulative chemical hazard index (heavy metals)	0.28	0.19	0.81	0.12
Integrated risk factor (combined index)	1.2	0.9	2.6	0.7

Figure 5. Quantitative Risk Estimates and Integrated Risk by Setting



These results collectively demonstrate substantial variation across food handling settings, with street vendor environments exhibiting the highest burdens of microbial, chemical, and emerging contaminants. The data further reveal significant co contamination patterns and synergistic statistical relationships that support the necessity of an integrated assessment approach.

Discussion

The higher prevalence of pathogens that were found on utensils in this study was for Salmonella and Campylobacter, which are consistent with a large body of consumer food safety research that has shown that cross-contamination in domestic and food service kitchens is often due to improper handling or inadequate cleaning of utensils (Byrd-Bredbenner et al., 2013). In this study, the

strong correlation between biofilm biomass on cutting boards and the counts of indicator *Escherichia coli* bacteria demonstrates the ability of microbial biofilms to provide protection to foodborne pathogens from the routine sanitation procedures that are commonly used to clean up food surfaces (Bridier et al., 2015). The high prevalence of surfaces with deep scratches and wear created by street vendors and in households also accentuates this risk as the micro environments, especially the surfaces and irregularities, are able to collect organic material and are conducive to the growth of pathogen population through the formation of biofilms even after cleaning. These findings again confirm that the utensil interface is not just a passive 'middleman', but is a biological 'box' where pathogen persistence and multiplication take place, thus mandating explicit involvement in food safety monitoring systems. The collection of chemical and emerging contaminant data adds to the kitchen interface risk profile. The presence of high levels of Ni and Cr in the foods cooked in older metal utensils is consistent with previous studies that found metal leaching during cooking, especially when acidic foods are cooked in utensils that are more than 10 years old (Weidenhamer et al., 2017). The widespread microplastic pollution in both food and utensils aligns with recent studies that report that plastic packaging and utensils release significant amounts of particles during normal use into the food consumed (Sobhani et al., 2020). The co-localization of antimicrobial resistance genes (AMRGs) with microplastic particles is especially worrisome. Microplastic surfaces could selectively enrich and transport pathogenic bacteria, and serve as mobile substrates to accumulate microbial hazards and enhance horizontal gene transfer (Wu et al., 2019). This, alongside the well known ability of a food processing environment to amplify and spread resistance determinants throughout the food chain (Verraes et al., 2013), is why significant statistical interaction was found between the concentration of microplastic and biofilm and the count of antimicrobial resistance genes: both stressors contribute to the establishment of persistent multi hazard niches at the interface of food and food utensils.

These observations, when considered in a One Health context, highlight the complex and strong relationships that exist between contamination in the environment, animal derived foods, and human disease. It is a well-recognized fact that foodborne disease is a significant problem worldwide, and that millions of cases each year are attributed to known pathogenic organisms (Scallan et al., 2011). One Health, a concept that brings together human, animal and environmental health systems (Coker et al., 2011), is just the lens needed to explain why the same cutting board could contain zoonotic pathogens, metal residues from tainted cookware, microplastic particles, and resistance genes. Street food vendors have the least access to water and sanitation in informal food environments, which increases both biological and chemical risk burdens, just as is documented here (Rheinländer et al., 2008). The integrated disease burden assessment was performed and the result was a cumulative integrated hazard risk that is greater than the sum of individual hazard risks, which strongly backs the call for combined exposure frameworks in regulatory toxicology (Meek et al., 2011). The discovery questions the traditional approach to microbial and chemical monitoring and suggests that the risk for microbial and chemical contamination at the utensil interface is more than the sum of its parts; future monitoring should similarly be multi contaminant and multi integrated to assure public health and environmental integrity.

Conclusion

This is the first multi contaminant assessment at the interface of food and food handling utensils, quantifying microbial pathogens, heavy metals, microplastics and antimicrobial resistance genes in various food handling environments. According to the results, the interface does not only serve as a 'filter' but also as a dynamic and dangerous space in which the biological, chemical and emerging risks come together and magnify. *Salmonella* and *Campylobacter* were these most common pathogens, and were often isolated from the surfaces and utensils, especially when in the street vendors and household settings where there was biofilm formation on

scratched and worn cutting boards with microbial persistence. At the same time, leaching from old cooking pots containing heavy metals, biofilm genes which are antimicrobial resistant and can be co localized with micro plastic particles, which can be shed from plastic utensils are all multi hazard hotspots that could not be detected by single factor surveillance. The integrated cumulative risk assessment showed that the overall disease burden was larger than the sum of the individual hazard estimates, highlighting a synergy effect that comes against a backdrop of the traditional siloed approach to food safety. The results are consistent with the notion that much of the foodborne disease risk is not being addressed by concentrating on finished food products and not considering the food preparation environment.

This study has implications for public health, regulatory policy and environmental management. To understand the links between animal derived foods, human hygiene practices, environmental pollution and microbial evolution, a One Health approach is necessary. The study shows the importance of raising the priority of utensil hygiene to a critical control point in a food safety system and the need to urgently improve the material characteristics of cookware and cutting boards to reduce chemical and microplastic exposure. The interactions of the biofilm, microplastics and the resistance genes emphasize the necessity of an integrated sanitation strategy to take care of biological and chemical residues. Moving forward, food safety surveillance needs to evolve beyond a piecemeal approach of testing for individual contaminants to a more comprehensive approach that is multi hazard in nature and more representative of the actual complexity of the kitchen environment. This research illuminates these hidden hazards and their combined impacts, and will be used to inform food safety policies, consumer education and human and environmental protection in an integrated way.

Recommendations

1. Establish mandatory routine monitoring of utensil and surface hygiene in food service establishments and food processing units, not just end product testing, to identify contamination at its source.

2. Develop and enforce material safety standards for kitchen utensils and cookware that set maximum permissible limits for heavy metal leaching and microplastic shedding during normal use.

3. Integrate biofilm detection into food safety inspection protocols, using simple staining methods to identify worn and inadequately cleaned surfaces that serve as persistent pathogen reservoirs.

4. Implement combined microbial and chemical surveillance programmes that simultaneously test food, water, and surface swabs for a panel of priority hazards, including antimicrobial resistance genes.

5. Strengthen hygiene infrastructure and access to safe water for street food vendors through municipal investment and targeted grants, as these settings exhibited the highest combined contaminant burdens.

6. Launch public awareness campaigns that educate consumers on the risks of using heavily scratched cutting boards and worn cookware, and promote regular replacement and proper sanitation practices.

7. Mandate the inclusion of cumulative risk assessment methodologies in national food safety risk analyses, moving from single hazard evaluations to combined exposure frameworks as endorsed by international agencies.

8. Fund longitudinal One Health research that tracks the seasonal and temporal dynamics of multi contaminant accumulation at the food utensil environment interface and evaluates the effectiveness of interventions over time.

9. Develop standardized global protocols for the simultaneous extraction and analysis of microbial, chemical, and microplastic contaminants from food contact surfaces to enable cross study comparisons and Meta analyses.

10. Foster interdisciplinary collaboration among food microbiologists, toxicologists, materials scientists, and environmental health practitioners to design holistic prevention strategies that address the full spectrum of hidden hazards identified in this study.

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