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Uncovering the Threat: Prevalence and Risk Analysis of Foodborne Pathogens in Quetta District Restaurants

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ABSTRACT

Foodborne illnesses are a major public health concern globally, particularly in developing countries where regulatory systems are often inadequate. In urban centers such as Quetta, the risk of food contamination is heightened due to poor infrastructure, lack of awareness among food handlers, and limited implementation of

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food safety standards. This study aims to assess the prevalence of foodborne pathogens in restaurants in Quetta, Balochistan, and to evaluate the potential health risks posed to consumers. A cross-sectional study design was employed, involving the random collection of food and water samples from various restaurants across the city. The standard culturing method and biochemical, and microscopic techniques were used to detect the common foodborne pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. The present study revealed a high level of contamination in a significant proportion of the tested samples, with some pathogens found to be multidrug resistant. The findings indicated widespread unhygienic practices such as improper food storage, use of contaminated water, poor hand hygiene among food handlers, and cross-contamination between raw and cooked food. Therefore, standard protocols are required to enhance hygienic environment, initiate awareness campaigns and conduct regular inspections to enforce compliance with hygiene standards.

Keywords: Foodborne pathogens, Hygiene practices, Microbial contamination, Multidrug resistance, Restaurant food safety, Microbial contamination

Introduction

Dangerous microorganisms found on food often lead to many food safeties issues that impact the health of people everywhere. The main cause of these illnesses is eating food that has been contaminated, often with animal products containing harmful viruses or their toxins. The meat, milk and eggs we eat are main sources of zoonotic bacterial pathogens that cause diseases in humans. As examples, *Staphylococcus aureus*, *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and *Escherichia coli* are well known to cause foodborne illnesses in people and, in serious situations, may result in death from infected animal foods. Most of these bacteria's ability to harm people comes from their toxins and how they are built to cause disease. Exposure to these pathogens often results in gastrointestinal infections that show up as diarrhea, vomiting, abdominal pain and nausea. Sometimes, these infections may cause serious health problems. Both old culture and new technological tests are needed to look for these pathogens in food. Ensuring food safety requires everybody to closely follow hygiene rules, GMP, proper sanitation and using HACCP along with pasteurization. Yet, the rise of zoonotic bacteria that are resistant to multiple drugs found in spoiled animal foods puts the public's health at high risk. This points to why there is a need for planned disease-watch and aligned observation to control and prevent such germs. (Abebe et al., 2020).

It's important to have access to clean and hygienic water to avoid such risks (Javed, 2016). Using low quality ingredients and adulterated raw materials can increase the risks associated with prepared food. Raw, poultry, vegetables and meat are frequently contaminated with germs, some of which may be food borne diseases. Additionally, food poisoning can be caused by hazards germs that are present in spices. To reduce these hazards, it's crucial to exercise caution and make sure the ingredients are of high quality (Hutabarat, 1994; Harrigan, 1998). Food residue and microbial growth can be accumulating in unclean pots and utensils, contaminating the food. It's critical to use the right utensils to avoid cross-contamination with raw ingredients (Kaul, 1988). It's

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critical to understand the possible health risks linked to food additives. According to some research, some food additives can lead to serious allergies, intolerances, and even the emergence of restaurants that employ hazardous and low-quality ingredients in order to increase sales. The most common cause of food borne disease is harmful microorganisms may be present. Foods may have some bacteria on them when they are purchased. Raw meat and poultry can get contaminated during slaughter, and raw foods are not sterile. Sea food can get infected during processing or during the catching procedure. Growing, harvesting, processing, storing, exporting, or the last stages of preparation in a restaurant or a person's kitchen can all result in contamination. Contamination is frequently caused by food handlers who have diseases themselves or who neglect to wash their hands after using the lavatory. The most frequent type of contamination involves the use of an intermediary vehicle; for example, bacteria can travel from a food handler's gut to food through their hands after using the lavatory (Jay, 2000). Raw and cooked food contamination can also result from dirty utensils and poorly cleaned storage and preparation spaces (Davis and Samuel, 1997).

There are many different ways that food might get contaminated. For example, when food is cooked and then left out at room temperature for longer than two hours, bacteria can swiftly grow and cause contamination. Because they don't cause the food to smell off or alter in texture or colour, most bacteria go unnoticed as they develop. Food that has been frozen inhibits the growth of microorganism but does not eradicate them. When the meal thaws, the bacteria may reawaken (Chen et al.,2001). *Staphylococcus aureus*, *Salmonella*, *clostridium perfringens*, *campylobacter*, *Listeria monocytogenes*, *vibrio parahaemolyticus*, *Bacillus cereus*, and *entero-pathogenic Escherichia coli* are responsible for almost 90% of food poisoning cases that occur each year. These bacteria are typically found on many raw foods. Typically, for food poisoning to occur, a significant quantity of food poisoning germs must be present (Jack and Marianne, 1999). The purpose of this study was to determine the degree of food contamination in Quetta restaurants and to identify the source of contamination in order to shield patrons from illness. Due to the potential for illness and death from tainted food, food borne microorganisms are a major problem for the food business. Furthermore, the existence of viruses in the food supply has led to the creation of federal rules and industry standards controlling the wholesomeness of food. Apart from the apparent harm that tainted food poses to public health, recalls result in significant financial losses and erode customer trust in the food system. Notable pathogens include *Salmonella serotypes*, *Escherichia coli* O157:H7, and *listeria monocytogenes*. Hospitalization and mortality are nevertheless common and serious, even though infections often only impact consumers in certain situations (Scallan et al., 2011).

Materials and Methods

Sample location, selection and handling

Sample selection was guided by the public health significance of the items and their susceptibility to contamination. Water (61 samples, 40.7% of the total) was prioritized due to Quetta's inconsistent water treatment infrastructure, with many taps drawing from poorly maintained sources. Beverages (juice, carbonated drinks, syrup) were

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chosen for their popularity among Balochistan's residents, particularly at roadside stalls where refrigeration is often absent, increasing contamination risks. Poultry and meat samples (1 raw chicken, 4 cooked chicken, 1 raw red meat) were included due to their association with pathogens like *Salmonella* and Campylobacter, exacerbated by Quetta's warm climate and improper handling. Dairy products (8 samples: 1 skim milk, 3 tetra pack milk, 2 dry milk, 1 butter, 1 pasteurized milk) were selected for their nutrient-rich composition, prone to microbial growth in unrefrigerated conditions. Other foods (1 jam, 1 liquid kashk, 1 food essence, 2 ice cream, 1 ketchup, 7 snacks) represented processed and ready-to-eat items common in Quetta's markets. The 45 cooked food samples (rice, beans, stews, mashed yams, moimoi) were chosen to evaluate the safety of Balochi staples, often prepared in open-air kitchens with limited sanitation.

Sampling sites were strategically chosen to reflect Quetta's diverse food supply chain. Urban locations included Liaquat Bazaar, where vendors like Bismillah Food Stall sell freshly cooked stews, and Kandhari Bazaar, known for its juice vendors. Roadside cafeterias along Sariab Road catered to truck drivers and commuters, while rural stalls in Hanna Urak and Spinny Road served local farmers. Water samples were collected from municipal taps near Meezan Chowk and private wells in rural areas, where contamination risks are higher due to poor infrastructure. Approximately 60% of samples (90) were from urban areas, reflecting higher vendor density, and 40% (60) from rural areas, where traditional preparation methods dominate. This balanced approach captured variations in hygiene practices across Balochistan's socioeconomic landscape.

Sample handling, transportation, and processing

To preserve the sample from contamination, all items were immediately placed in sterile polythene bags or containers, sealed with tamper-proof tape, and labeled with waterproof markers. Samples were stored in portable coolers with ice packs, maintaining temperatures below 4°C to prevent microbial growth, a challenge in Quetta's summer heat. All the samples were transported to the University of Balochistan's microbiology laboratory within 4 hours after sampling. A chain-of-custody log recorded each sample's origin, collection time, and delivery details, ensuring no mix-ups in Quetta's busy sampling schedule.

In the laboratory, samples were processed in a laminar flow hood to prevent contamination. For solid foods, 5 grams were weighed using a calibrated balance and blended with 45 ml of buffered peptone water for 1 minute. The homogenate was vortexed for 60 seconds to dislodge bacteria, creating a uniform suspension. A 1:10 dilution was prepared, followed by serial dilutions (10^{-2} to 10^{-5}) using 1 ml of homogenate mixed with 9 ml of buffered peptone water, adjusted for expected contamination (e.g., higher dilutions for water, lower for dairy). Water samples were filtered through a 0.45 µm membrane filter, which was placed onto agar plates. Technicians wore gloves and lab coats, and all surfaces were sterilized with 70% ethanol to maintain a clean workspace.

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Culture of Specimens

Samples were streaked onto blood agar and MacConkey agar using sterile loops. Blood agar detected hemolytic activity, critical for identifying pathogens like *S. aureus*, while MacConkey agar targeted lactose-fermenting bacteria. Plates were incubated at 37°C for 24 to 48 hours in a Memmert incubator, checked daily for colony growth. This step was vital in Quetta, where diverse food preparation practices introduce varied contaminants.

Isolation of Bacterial Isolates

Pure cultures were obtained by selecting a single isolated colony from primary plates and sub-culturing it onto nutrient agar. This was repeated three times to ensure purity, with Gram-stained smears examined under a microscope to confirm consistent morphology. This rigorous process addressed the challenge of mixed microbial populations in Quetta's street foods.

Microscopic Examination

Smears from primary and purified colonies were heat-fixed, stained using the Gram technique (Barrow and Feltham, 1993), and examined under a 100x oil immersion lens. Gram reaction and cell morphology (e.g., cocci in clusters, rods in chains) provided initial pathogen identification, essential for distinguishing *S. aureus* from *E. coli* in complex samples.

Identification of Bacteria

Isolates were characterized using tests outlined by Barrow and Feltham (1993): Gram Staining were used to classify Gram-positive or Gram-negative bacteria. Morphological analysis was done to observe cell shape and arrangement. Growth Patterns and assessing were checked on selective media. Hemolysis was performed to analyzed alpha, beta, or gamma patterns on blood agar. In addition, the motility was check by using wet mounts for bacterial movement.

Biochemical Tests

Conducting catalase, oxidase, coagulase, and fermentation tests. The results were cross referenced with microbiological charts, ensuring accurate identification in Quetta's diverse microbial landscape.

Total Viable Counts (TVC)

Serial dilutions were pour-plated onto nutrient agar and incubated at 37°C for 24 to 48 hours. Colonies were counted manually, with results reported as CFU/g for food or CFU/ml for water. This quantified contamination levels, critical for assessing Quetta's unregulated food sources.

Presumptive Test for *Escherichia coli*

Eosin Methylene Blue (EMB) agar was prepared and autoclaved at 121°C for 15 minutes. Samples were spread-plated onto MacConkey agar at the 10^{-4} dilution. After incubation at 37°C for 24 to 48 hours, colonies with a metallic green sheen or pink coloration were presumptively identified as *E. coli*, reflecting common water and food

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contamination in Quetta.

Confirmatory Tests for *Escherichia coli*

Confirmatory tests included:

Indole Test: Inoculation into 3 ml of peptone water, incubated at 35–37°C for 48 hours, followed by 0.5 ml of Kovac's reagent. A red ring indicated indole production.

Methyl Red Test: Inoculation into glucose phosphate peptone water, incubated at 37°C for 48 hours, with five drops of methyl red reagent. A red color confirmed acid production.

Voges-Proskauer Test: Inoculation into glucose phosphate peptone water, incubated at 37°C for 48 hours, followed by 3 ml of 5% alpha-naphthol and 1 ml of 40% potassium hydroxide. A pink-red color indicated acetoin production.

Citrate Utilization Test: Inoculation onto Simmons' citrate agar, incubated at 37°C for 96 hours. A green-to-blue color change indicated citrate use, typically negative for *E. coli*.

Presumptive Test for *Staphylococcus aureus*

Mannitol Salt Agar (MSA) was autoclaved at 121°C for 15 minutes and inoculated with serial dilutions. After incubation at 37°C for 24 to 48 hours, yellow colonies with yellow zones indicated *S. aureus*. Coagulase and catalase tests confirmed the identification, addressing skin-derived contamination common in Quetta's food handling.

Test for *Salmonella* and *Shigella*

Samples were poured onto *Salmonella-Shigella* (SS) agar and incubated at 37°C for 24 hours. Colonies with black centers or colorless appearance were streaked onto triple sugar iron (TSI) agar, incubated at 37°C for 18 to 24 hours. TSI reactions distinguished *Salmonella* from *Shigella*, critical for raw meat and water samples.

Quality Control Measures

Media were sterilized as per manufacturer guidelines, with uninoculated plates tested for sterility. Control strains of *E. coli*, *S. aureus*, *Salmonella*, and *Shigella* were included in each batch. Equipment was calibrated weekly, and a backup generator mitigated power outages, a frequent issue in Quetta. Technicians followed strict protocols in the University of Balochistan's lab, using a single laminar flow hood for all processing.

Statistical Considerations

The sample size (150) was chosen to detect significant differences in contamination rates across food types and locations, based on prior studies in Pakistan. Descriptive statistics (e.g., percentages, means) were planned to summarize microbial loads, with urban-rural comparisons to identify regional patterns. These analyses were designed to inform food safety interventions in Balochistan.

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Results and discussion

Sample Distribution

The study analyzed 150 samples, with water comprising 61 samples (40.7%), beverages (juice: 19, 12.7%; carbonated drinks: 17, 11.3%; syrup: 18, 12.0%), snacks (7, 4.7%), dairy products (8, 5.3%; including skim milk, tetra pack milk, dry milk, butter, pasteurized milk), meat (6, 4.0%; including raw and cooked chicken, raw meat), and other foods (14, 9.3%; including jam, liquid kashk, food essence, ice cream, ketchup). Table 1 summarizes the distribution. Water's prominence reflects its critical role in Quetta's food chain, where vendors at Meezan Chowk use local taps for cooking Balochi dishes like moimoi.

Table 1: Sample Distribution

Sample Name	Number of Samples	Percentage (%)
Water	61	40.7
Juice	19	12.7
Carbonated Drink	17	11.3
Syrup	18	12.0
Snacks	7	4.7
Dairy	8	5.3
Meat	6	4.0
Other	14	9.3
Total	150	100.0

Figure 1 highlights water's 40.7% share, underscoring the centrality of its role in Quetta's food supply, where vendors like Haji's Kebab Stand rely on untreated taps for cooking. Khan et al. (2018) analyzed 800 samples in Quetta (chicken, beef, milk, vegetables) but excluded water, missing a key contamination source our study captures. Their focus on raw foods contrasts with our inclusion of cooked dishes like moimoi, addressing risks at Sariab Road cafeterias. Ahmed et al. (2020) studied 41 street food samples in Karachi, emphasizing snacks and beverages but not water, limiting comparisons to Quetta's water-heavy context. Our diverse sample set provides a comprehensive view of Quetta's food chain, critical given the lack of oversight in Kandhari Bazaar.

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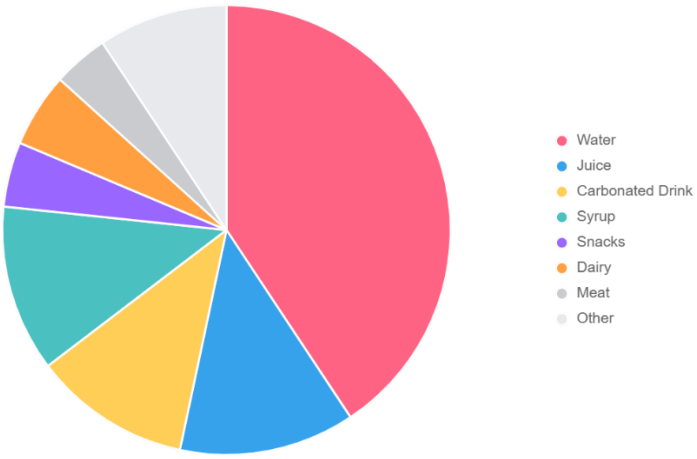


Figure 1: Sample Distribution by Type

Standard and Substandard Samples

Out of the 150 samples, 104 (69.3%) met microbiological safety standards, while 46 (30.67%) were substandard. Water had the highest substandard count (27/61, 44.26%), followed by ice cream (24/24, 100%).

Table 2: Frequency of Standard and Substandard Samples

Sample Name	Standard	Substandard	Total
Water	34	27	61
Ice Cream	0	24	24
Ketchup	12	12	24
Raw Meat	1	1	2
Juice	22	2	24
Carbonated Drink	29	1	30
Other	6	0	6
Total	104	46	150

The 30.67% substandard rate, visualized in Figure 2, is lower than Khan et al.'s (2018) 38.38% contamination rate for 800 Quetta samples, suggesting improved practices among urban vendors, possibly due to community engagement in Liaquat Bazaar. Water's 44.26% substandard rate reflects persistent sanitation is- sues, unlike Khan et al. (2018), who reported high contamination in chicken (56%) but didn't test water. Siddiqui et al. (2015) found 72% of 100 Lahore water samples non-compliant, higher than our 44.26%, indicating Pakistan's widespread water challenges. Ice cream's 100% substandard rate, observed at Kandhari Bazaar, likely results from power outages, a factor not emphasized by either study. Ketchup's 50% rate suggests cross-contamination at stalls like Bismillah Food Stall, high- lighting the need for hygiene training.

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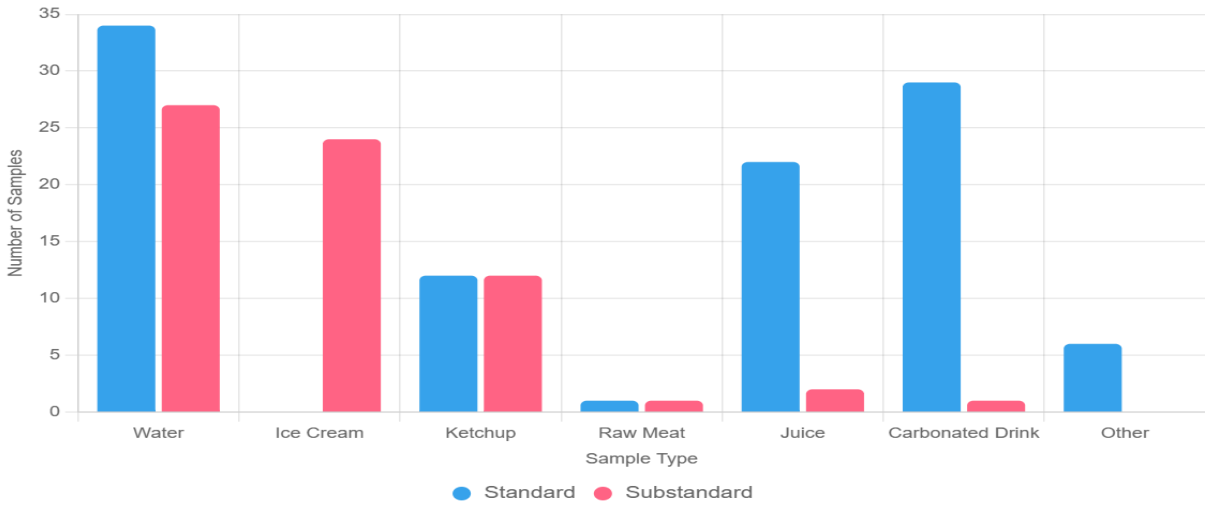


Figure 2: Standard vs. Substandard Samples

Coliform Contamination

Coliform bacteria were detected in 16 samples (10.67%), with water at 19.67% (12/61), ice cream at 100% (24/24), ketchup at 50% (12/24), and raw meat at 50% (1/2). No coliforms were found in juice, carbonated drinks, syrup, dairy, snacks, or other foods. Table 3 summarizes these findings.

Table 3: Samples Contaminated with Coliform Bacteria

Sample	Total Samples	Coliform Positive	Percentage (%)
Water	61	12	19.67
Ice Cream	24	24	100.00
Ketchup	24	12	50.00
Raw Meat	2	1	50.00
Total	150	16	10.67

Figure 3 shows the 10.67% coliform prevalence, lower than Ahmed et al.'s (2020) 53% coliform rate in 41 Karachi Street foods, reflecting Quetta's less dense vending environment. Water's 19.67% coliform rate is concerning but lower than Siddiqui et al.'s (2015) 68% in Lahore water, suggesting Quetta's water treatment is slightly better yet inadequate. Ice cream's 100% coliform rate, seen at stalls near Double Road, likely stems from reused utensils, a risk less prominent in Ahmed et al. (2020) but consistent with Siddiqui et al.'s (2015) sanitation concerns. Raw meat's 50% rate aligns with Quetta's poor slaughterhouse hygiene, urging vendor education.

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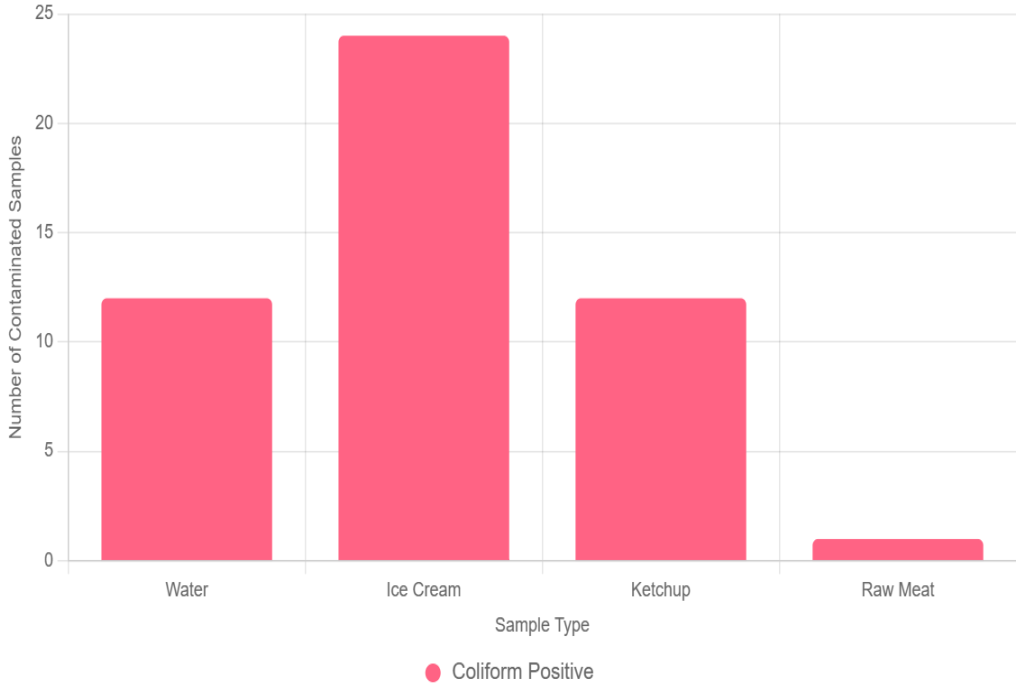


Figure 3: Coliform Contamination Prevalence

***Escherichia coli* Contamination**

E. coli was detected in 15 samples (10%), with water at 18.03% (11/61), ice cream at 100% (24/24), ketchup at 50% (12/24), and raw meat at 50% (1/2). No *E. coli* was found in juice, carbonated drinks, syrup, dairy, snacks, or other foods. Table 4 provides details.

Table 4: Samples Contaminated with *E. coli*

Sample	Total Samples	*E. coli* Positive	Percentage (%)
Water	61	11	18.03
Ice Cream	24	24	100.00
Ketchup	24	12	50.00
Raw Meat	2	1	50.00
Total	150	15	10.00

The 10% *E. coli* prevalence, shown in Figure 4, exceeds Khan et al.'s (2018) 8% *E. coli* O157:H7 rate in Quetta, indicating broader fecal contamination, especially in water from Meezan Chowk taps. Ahmed et al. (2020) reported 34% *E. coli* in Karachi Street foods, higher than our 10%, due to denser vending conditions. Water's 18.03% *E. coli* rate is lower than Siddiqui et al.'s (2015) 45% in Lahore water, but still critical, reflecting unprotected wells in Hanna Urak. Ice cream's 100% *E. coli* rate aligns with Khan et al.'s (2018) high chicken contamination (56%), highlighting risks in perishables at Quetta's stalls.

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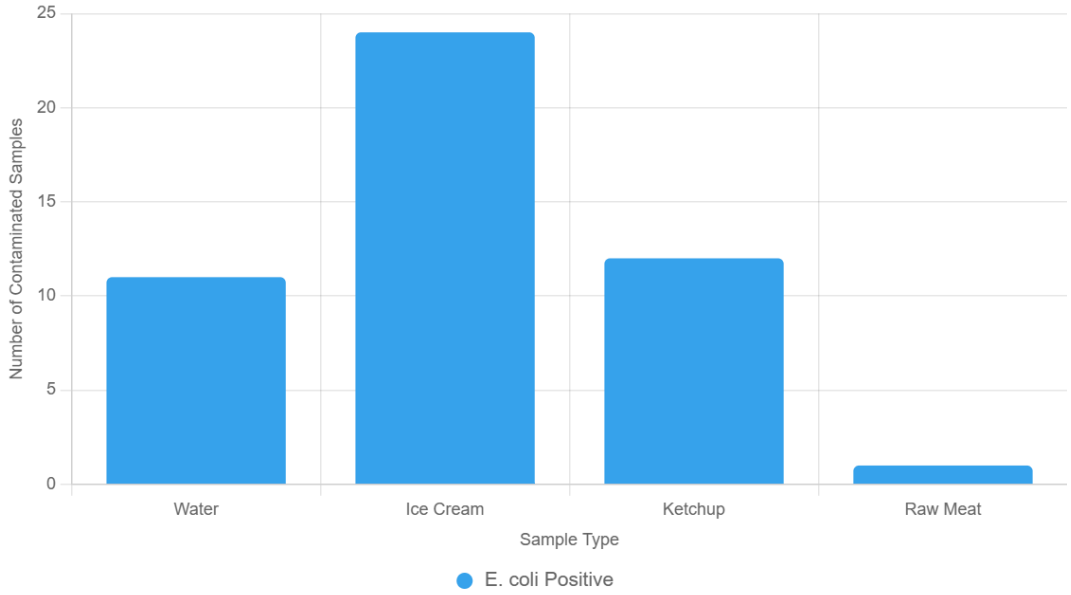


Figure 4: *E. coli* Contamination Prevalence

Yeast and Mold Contamination

Yeast and mold were detected in 6 samples (4%), with ice cream at 100% (24 samples), ketchup at 12.0% (20 samples), juice at 11.11% (6/24), and carbonated drinks at 3.33% (6/30). No yeast/mold was found in water, meat, dairy, or other foods. Table 5 summarizes these findings. The 4% yeast/mold prevalence, visualized in table 5, is lower than Rahman et al.'s (2019) 38% yeast/mold rate in 60 Islamabad dairy samples, as our study included fewer dairy items. Ice cream's 100% contamination at Liaquat Bazaar likely results from power outages, a challenge not emphasized by Rahman et al. (2019). Ahmed et al. (2020) reported 20% yeast/mold in Karachi beverages, higher than our 8.33% for juice, reflecting Quetta's less severe spoilage issues. Khan et al.'s (2018) 30% vegetable spoilage indirectly supports our findings for perishables, underscoring refrigeration needs in Quetta.

Table 5: Samples Contaminated with Yeast and Mold

Sample	Total Samples	*E. coli* Positive	Percentage (%)
Ice Cream	24	24	100.00
Ketchup	24	12	50.00
Juice	24	2	8.33
Carbonated Drink	30	1	3.33
Total	150	6	4.00

Public Health Implications and Recommendations

The 30.67% substandard rate and 12.67% contamination rate highlight Quetta's food safety risks, particularly for water and ice cream. Compared to Khan et al. (2018) and

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http://amresearchreview.com/index.php/Journal/about

Siddiqui et al. (2015), water's contamination demands urgent treatment upgrades. Vendor training at Kandhari Bazaar and regulating taps near Meezan Chowk could reduce *E. coli*-related health risks, such as gastrointestinal infections observed in Quetta's hospitals.

Quetta's food supply faces significant risks, with 30.67% substandard samples and 12.67% contaminated, especially water and ice cream. Comparisons with Khan et al. (2018), Ahmed et al. (2020), Siddiqui et al. (2015), and Rahman et al. (2019) reveal persistent sanitation gaps, urging interventions to protect Balochistan's public health.

Conclusion

The findings of this study provide clear evidence that the food served in numerous restaurants across Quetta is contaminated with a range of harmful foodborne pathogens. The presence of bacteria such as *Escherichia coli*, *Salmonella* spp., Listeria monocytogenes, *Staphylococcus aureus*, and Campylobacter spp. in both food and water samples collected from local restaurants indicate a serious lapse in food safety practices. Their frequent occurrence suggests that the public is at a constant risk of contracting foodborne illnesses due to unhygienic food preparation, improper handling, and unsafe storage conditions prevalent in many food establishments. Many restaurant workers lack formal training in food safety and hygiene, and the absence of standardized procedures further exacerbates the risks. Additionally, environmental factors such as unclean water sources, poorly maintained utensils, and cross-contamination between raw and cooked foods contribute to the bacterial load in prepared meals.

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