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DETECTION, IDENTIFICATION, AND ANTIBIOTIC RESISTANCE PATTERNS OF FOODBORNE BACTERIAL AND FUNGAL PATHOGENS

DETEKSI, IDENTIFIKASI, DAN POLA RESISTENSI ANTIBIOTIK PADA PATOGEN BAKTERI DAN JAMUR YANG DITULARKAN MELALUI MAKANAN

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A B S T R A C T

Background: *Foodborne diseases are verry common and easily spread, among strains of Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, and Clostridium spp.* ***Purpose:*** *To isolate and characterize foodborne pathogenic bacteria and fungi in various foodstuffs.* ***Method:*** *A total of 260 samples (130 each from Peshawar and Mardan) were collected and analyzed. Only 61 tested positive for various types of bacterial and fungal pathogens. Then evaluated for their antibiotic/anti-fungal sensitivity patterns towards a panel of selected antibiotics and anti-fungal.* ***Result:*** *The Gram-positive isolates showed the highest resistance to methicillin (79%) and amoxicillin (63%), most sensitive to ceftriaxone (88%), levofloxacin (86%), and cefotaxime (77%). Intermediate activities were exhibited by azithromycin (50%) and vancomycin (55%). In terms of the Gram-negative bacteria, the best activities were shown by ciprofloxacin (100%), cefoxitin (100%), chloramphenicol (100%), and ceclor (100%). Intermediate activity was discovered for cefixime (50%), cefuroxime (50%), and linezolid (50%). Three anti-fungal drugs (fluconazole, voriconazole, and nystatin) were used to assess their potency against the fungal pathogens. Mucor spp. proved relatively more susceptible to all anti-fungal drugs. The only Fusarium spp. isolate was highly resistant to all anti-fungal in this research.* ***Conclusion:*** *The prevalence of Gram-positive bacteria is greater than Gram-negative bacteria in the current study. The frequency of fungal pathogens was relatively high in both raw and ready-to-eat foods, while packaged foods were free from fungal contamination. We recommend that appropriate safety when handling and cooking food. Moreover, food products should be screened for different pathogenic microbes.*

A R T I C L E I N F O

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A B S T R A K

Latar belakang: Penyakit bawaan pangan sangat umum terjadi dan dapat dengan mudah ditularkan, diantaranya, strain *taphylococcus aureus, Bacillus cereus, Listeria monocytogenes,* dan *Clostridium* spp. **Tujuan:** Mengisolasi dan mengetahui karakter bakteri dan jamur patogen bawaan pangan diberbagai jenis makanan. **Metode:** Sebanyak 260 sampel (masing-masing 130 dari Peshawar dan Mardan) dikumpulkan dan dianalisis, hanya 61 sampel yang dinyatakan positif mengandung berbagai jenis patogen bakteri dan jamur. Kemudian pola sensitivitasnya dievaluasi menggunakan panel antibiotik dan anti-jamur yang dipilih. **Hasil:** Isolat Gram-positif menunjukkan resistensi tertinggi terhadap *methicillin* (79%) dan *amoxicillin* (63%), serta paling sensitif terhadap *ceftriaxone* (88%), *levofloxacin* (86%), *cefotaxime* (77%). Aktivitas intermediet ditemukan pada *azithromycin* (50%) dan *vancomycin* (55%). Pada bakteri Gram-negatif, aktivitas terbaik ditunjukkan oleh *ciprofloxacin* (100%), *cefoxitin* (100%), *chloramphenicol* (100%), dan *ceclor* (100%). Aktivitas intermediet ditemukan pada *cefixime* (50%), *cefuroxime* (50%), dan *linezolid* (50%). Tiga obat anti-jamur (*fluconazole*, *voriconazole*, dan *nystatin*) digunakan untuk menilai potensinya terhadap patogen jamur. *Mucor* spp. terbukti lebih rentan terhadap semua obat anti-jamur. Namun, isolat *Fusarium* spp. satu-satunya jamur yang menunjukkan resistensi tinggi terhadap semua anti-jamur dalam penelitian ini. **Kesimpulan:** Prevalensi bakteri Gram-positif lebih tinggi dibandingkan bakteri Gram-negatif di penelitian ini. Frekuensi patogen jamur relatif tinggi pada makanan mentah dan siap saji, sementara makanan kemasan bebas dari kontaminasi jamur. Kami merekomendasikan agar langkah-langkah keamanan yang tepat saat menangani dan memasak makanan. Selain itu, produk makanan harus diperiksa terlebih dahulu untuk berbagai mikroba pathogen.

Kata kunci:
Bakteri, Kontaminasi, Bahan makanan, Patogen

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INTRODUCTION

The occurrence of a substantial international public health threat. These organisms have been found in a variety of food sources ingested by humans. The importance of the information acquired on bacteria's resistance to antibiotics lies in comprehending the scope of the problem and setting benchmarks for implementing suitable measures. Microbiological analysis is a crucial tool for performing tests in accordance with the established microbiological criteria for each type of food. It is also vital for assessing the effectiveness of various management strategies based on the *Hazard Analysis and Critical Control Points* (HACCP) system (Lateef *et al*., 2004).

Foodborne diseases are becoming more apparent as a significant issue, encompassing a diverse range of illnesses resulting from parasitic, viral, bacterial, chemical contamination of food. Even though viruses are responsible for half of all foodborne diseases, it is bacterial agents that lead to the majority of hospitalizations and deaths. Diarrheal diseases are identified as the primary manifestation of food poisoning, which, in certain instances, may culminate in fatality (Nyenje *et al*., 2012). In the human food chain, pathogenic bacteria found in poultry can result in human illness, typically originating from microorganisms that have contaminated the bird. *Aeromonas* spp. is a rod-shaped, Gram-negative, facultative anaerobic bacterium, widely reported as an isolated species from various mammals, water surfaces, sewage, fish, shellfish, and birds. The pathogenicity of *Aeromonas* spp. is associated with the liberation of virulence factors and cell-associated endotoxins (Cao *et al*., 2023).

The microbiological testing and food safety are improved by the food management to take part in an imperative responsibility to assess how the food security objectives are achieved. However, conventional microbiological culture-based methods have limitations, particularly in their ability to provide accurate records (Hoorfar, 2011; Jasson *et al*., 2010; López-Campos, 2012). Mostly lactic acid bacteria are generally known to be harmless and play a significant role in antagonizing microbial pathogens that cause diseases and deterioration. The inhibitory effect exerted by *Lactobacillus* is primarily due to the production of signaling and defensive molecules, such as organic acids, hydrogen peroxide, acetaldehyde, diacetyl, D-isomers of amino acids, bacteriocins, and reuterin (Lijon *et al*., 2015; Zendo, 2013).

In comparison to bacteria, fungal isolates were found to be extremely potent in the rotten tomato fruit. *Bacillus* spp. and *Mucor* spp. exhibited the highest levels of fungal and bacterial infections. The best antibiotic for controlling all bacteria's microflora was chloramphenicol, showing varying degrees of antibiotic sensitivity and resistance, except for *Bacillus* (Bello *et al*., 2023; Naeem *et al*., 2012). Fungal contamination is extremely hazardous because food often does not appear spoiled even when it is severely infected. The presence of highly dangerous toxins and fungal spores is often detected, potentially leading to outbreaks of food poisoning, with laboratory examinations identifying the infectious agents (Ferdes and Ungureanu, 2012; Rawat, 2015).

A familiar cause of foodborne illness is *Bacillus cereus*. However, infections caused by this organism are usually not reported due to their mild symptoms. A fatal case of liver failure following the consumption of pasta salad highlights the potential severity of the emetic syndrome. Fried rice contaminated with a high quantity of *B. cereus* requires adequate handling and caution. *B. cereus* is considered to have been the cause of the illnesses, with the symptoms not aligning with those typically associated with food poisoning as reported in other studies. *B. cereus* spores, which are capable of surviving steaming, underwent proliferation when uncooked rice was left at room temperature overnight and subsequently fried. Different batches of rice were frequently blended. However, upon cessation of these practices, elevated levels of B (Kandeepan, 2014). Therefore, in this research, the researcher aims to isolate and characterize foodborne pathogenic bacteria and fungi in various foodstuffs.

MATERIAL AND METHOD

The present research study was carried out at the *Microbiology Research Laboratory* (MRL), Abasyn University Peshawar, from April 2022 to March 2023. A total of 260 samples were collected from Mardan and Peshawar city. The project focused on the isolation, identification, and antibiotic profiling of pathogenic microbes from various food items.

Collection and transportation of samples

Food samples were collected aseptically using sterilized test tubes containing transport media (peptone water). Raw, ready-made, and packaged food samples were gathered. All gathered food samples were transported to the laboratory within 1 hour and preserved at 4 °C in the refrigerator. The collected food samples were processed immediately.

Sample processing

Twenty-five grams of each meat, chicken, and fish sample were excised using a sterile scalpel. Each sample was placed in a sterile polyethylene zip bag and then transported to the laboratory within 1 hour and cultured on nutrient agar according to the method of 1 mL of raw milk being diluted from the sample. The sample quantities are 20 to 25 mL raw milk. The milk sample was diluted prior to being plated. The dilution was prepared in sterilized distilled water. One mL of milk from each sample was add to 9 mL of sterilized distilled water in

a test tube to achieve the desired dilution. The samples were then streaked on nutrient agar plates. The petri dishes were incubated for 24 hours at 37 °C. Vegetable samples were directly applied to nutrient agar and juice samples were also directly streaked onto the agar. The plates were incubated for 24 hours at 37 °C to allow for the growth of bacterial species (Khan *et al*., 2019).

Culture media

MacConkey agar, Salmonella Shigella agar, Nutrient agar, and Blood agar were used for bacterial growth. Nutrient agar is a basic media so the samples were first streaked on it. MacConkey agar, a selective and differential medium, was used for the growth of Gram-negative bacteria.

Gram staining

The Gram staining method is employed for categorizing bacterial species into two major groups: Gram-positive and Gram-negative bacteria, according to the physical and chemical characteristics of their cell walls. Crystal violet, gram's iodine, decolorizer (ethyl alcohol), and safranin reagents were used in the procedure. The gram staining procedure involved preparing a smear by mixing bacterial colonies from a petri dish with a drop of distilled water on a glass slide. The slide with the specimen was passed over a heat source several times to heat-fix the smear. The smear was subsequently covered with a few drops of crystal violet for a duration of 1 minute. After 1 minute, the stain was gently washed off with tap water. Gram's iodine was applied to the smear for 1 minute and then washed off with running tap water. A few drops of decolorizer (ethyl alcohol) were used for 5 to 10 seconds, followed by washing with running tap water. Finally, safranin was used for 45 seconds and then washed off with tap water. The prepared slide was dried in the air and examined under the compound microscope at 40x and 100x magnification power. A drop of immersion oil was used at 100x magnification for better visualization.

Biochemical tests

The biochemical tests used for the identification of bacterial isolates were *Triple Sugar Iron* (TSI) test, urease test, citrate utilization test, and indole test.

Isolation of fungi

A total of 100 randomly selected spoiled fruits and another 100 healthy-looking fruits were examined. The fruits were sliced into small (3 mm in diameter) with a sterilized blade, disinfected on the surface with 1% hypochlorite for 2 minutes, and subsequently transferred aseptically onto *Potato Dextrose Agar* (PDA). The plates were incubated at 28 °C for a duration of 5 days. A pure culture was acquired and maintained through by process subculturing each distinct colony that arose onto the PDA plates and subsequently incubating them at 28 °C for an additional period of 5 days. Each of the healthy fruits was sterilized with 75% ethanol, as a control. The healthy fruits were slice into small (3 mm in diameter) using a sterile blade, positioned on PDA, and subsequently incubated at 28 °C for 5 days.

Identification of isolated fungi

The identification of the fungal isolates was carried out through the analysis of morphological and cultural features such as conidial morphology and colony growth patterns. The technique described by Oyeleke and Manga was also adopted for identifying the isolated fungi using cotton blue in lactophenol stain. Identification was achieved by placing a drop of the stain on a clean slide with the assistance of a mounting needle. A small portion of the aerial mycelium from the representative fungal cultures was then extracted and placed in the lactophenol drop. The mycelium was well spread on the slide with the needle. A cover slip was delicately positioned with a gentle applied pressure to exclude any air bubbles. The slide was subsequently mounted and observed under the light microscope using ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms observed were identified in accordance with the frequency distribution of positive samples among various packed foods.

Anti-fungal assay

The agar disc diffusion method was used to screen for the anti-fungal activities of each antibiotic. A yeast inoculum in 0.85% NaCl solution was spread over the surface of the yeast extract–peptone–glycerol agar plate. Sterile filter paper discs (6 mm in diameter) containing 50 µg of nystatin, 25 µg of fluconazole, 1 µg of voriconazole, and 10 µL of caspofungin acetate at a concentration of 5 µg/mL were placed on the inoculated plates. Water was used instead of antibiotics as a positive control, while uninoculated plates were used as the negative control.

RESULT

The present study was conducted from April 2022 to March 2023 to identify foodborne pathogenic bacteria and fungi in various foodstuffs. A total of 260 samples (130 each from Peshawar and Mardan) were collected and analyzed. Out of these 260 samples, only 61 tested positive for various types of bacterial and fungal pathogens, as shown in Figures 1 and 2. The types of food items, sources, number of positive samples, and identified microbes are presented in Table 1, while the frequency of different bacterial pathogens identified in various foodstuffs is shown in Figure 3.

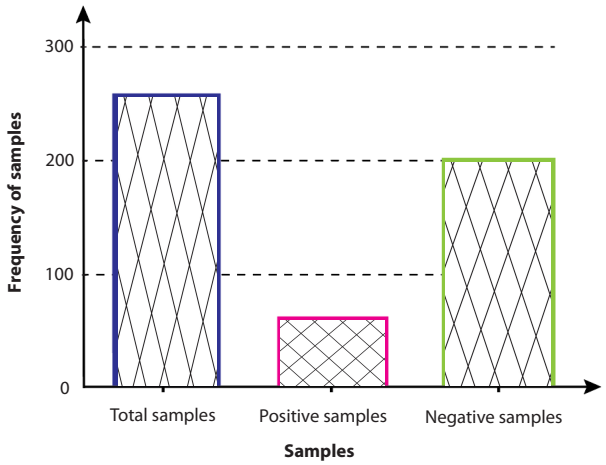

Figure 1. Frequency distribution of positive and negative samples of various foodstuffs

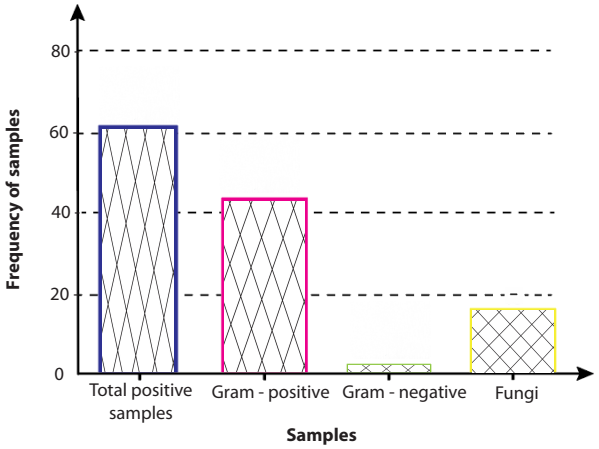

Figure 2. Frequency distribution of Gram-positive, Gram-negative, and fungi

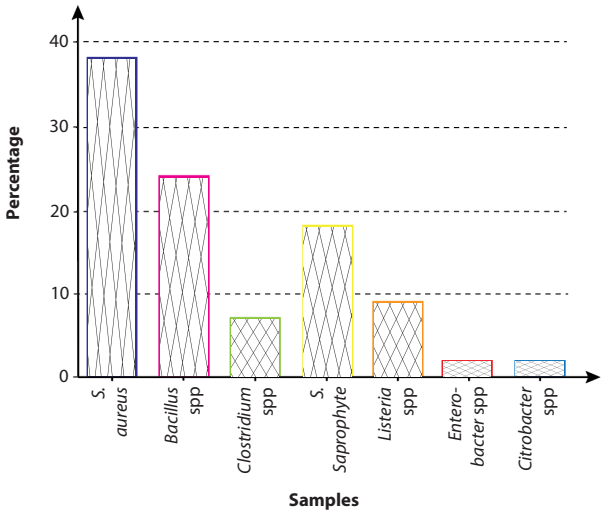

Figure 3. Percent frequency of different types of bacterial pathogens found in foodstuffs

Packed food

Various types of packed foods collected from different markets in Mardan and Peshawar were also analyzed for microbial pathogens. Out of a total of 260 samples, 40 were various types of packed foodstuffs. The percentage distribution of positive and negative food samples is shown in Figure 4.

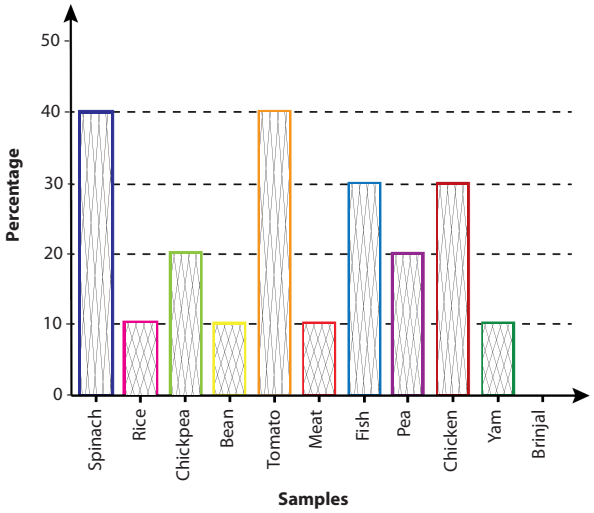

Figure 4. Frequency distribution of positive and negative samples among various packed foods

Raw food

Among the total food samples, 110 were raw food items that were included in the study for the analysis of microbial pathogens. Figure 5 shows the frequency distribution of positive and negative samples for bacterial and fungal contamination in raw food.

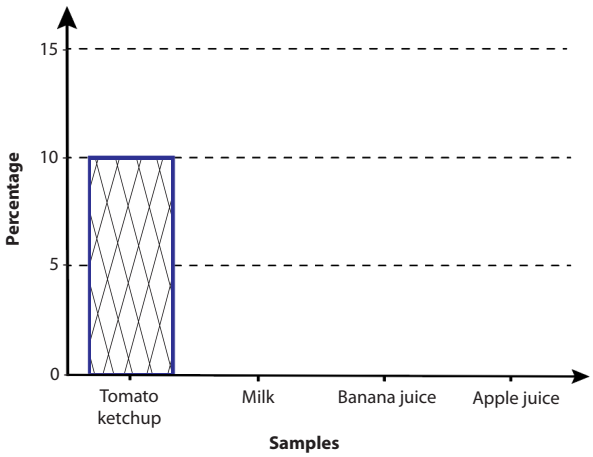

Figure 5. Frequency distribution of positive and negative samples among various raw food items

Ready-to-eat food

Similarly, 110 samples from the total were different types of ready-to-eat food. These samples were processed for bacterial and fungal analysis. The percentage frequency of positive samples for ready-to-eat foods is shown in Figure 6.

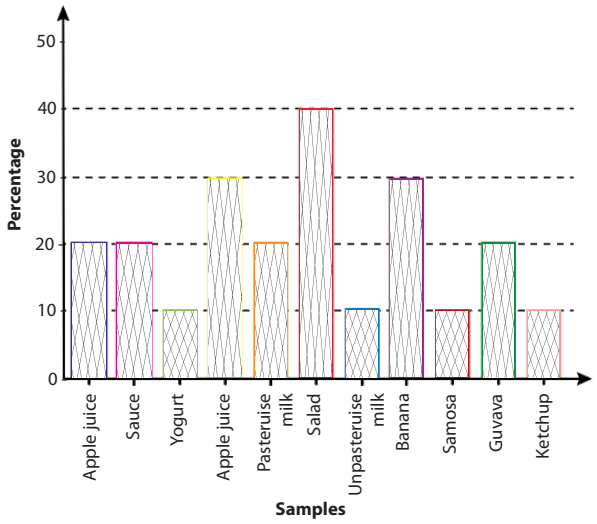

Figure 6. Frequency distribution of positive samples among various types of ready-to-eat foods

All food samples were analyzed for fungal pathogens. Out of 260 food samples, only 16 samples were found positive for fungal pathogens. The types of fungal pathogens and their percentage frequencies are shown in Figure 7.

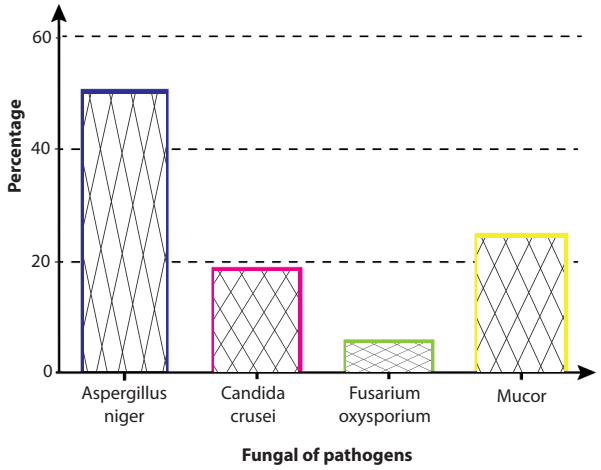

Figure 7. Percentage frequency of different types of fungal pathogens found in various foodstuffs

Culture sensitivity of Gram-negative bacteria

All the Gram-positive bacterial isolates were evaluated for their sensitivity and resistance to a panel of selected antibiotics. The efficacy of each antibiotic is shown in terms of the zone of inhibition along with standard deviation of triplicates in Table 2. *Ceftriaxone*, *cefotaxime*, *levofloxacine*, and *amoxicillin* were the most effective antibiotic against *S. aureus* which revealed 15(88%), 15(88%), 14(82%), and 12(71%) sensitivity. The resistance of *S. aureus* towards *methicillin*, *vancomycin*, and *azithromycin* was 16(94%), 10 (59%), and 9(53%) respectively. *Cefotaxime*, *levofloxacine*, *vancomycin*, *amoxicillin*, and *azithromycin* were the most effective antibiotics against *Bacillus* spp. which showing sensitivities 9(82%), 9(82%), 8(73%), 7(64%), 7(64%) respectively. The resistance of the *Bacillus* spp. towards *ceftriaxone* and *methicillin* was 7(64%) and 7(64%). *Ceftriaxone*, *levofloxacin*, *vancomycin*, and *azithromycin* were 100% effective, while the resistance of *Clostridium* spp. towards *amoxicillin*, *methicillin*, and *cefotaxime* was 2(67%), 2(67%), and 1(33%) respectively.

Antibiotic sensitivity of Gram-negative bacteria

In raw and in ready-made food, two Gram–negative bacterial isolates, *Enterobacter* spp. and *Citrobacter* spp. were found. Seven antibiotics were used against Gram–negative bacteria, including *cefixime*, *cefuroxime*, *ciprofloxacin*, *cefoxitin*, *chloramphenicol*, *ceclor*, and linezolid (Table 3). The best activity was shown by *ciprofloxacin* (100%), *cefoxitin* (100%), *chloramphenicol* (100%), and *ceclor* (100%), while intermediate activity was shown by *cefixime* (50%), *cefuroxime* (50%), and *linezolid* (50%).

Anti-fungal drugs for fungi

In the current study, four fungal pathogens were identified in both raw and ready-made food. The fungal pathogens identified were *Aspergillus* spp. (50%), *Candida* spp. (19%), *Fusarium* spp. (6%), and *Mucor* spp. (25%) respectively. Three anti-fungal drugs used were *fluconazole*, *voriconazole*, and *nystatin* (Table 4). There is o activity was shown by *fluconazole*, *voriconazole*, and nystatin against *Fusarium* spp., intermediate activity was shown by *voriconazole* (50%) against *Aspergillus nigar*, *Candida* spp., and *Mucor* spp., while the highest resistance was shown to *fluconazole* (87%) and *nystatin* (70%).

Table 1. Isolated pathogenic bacteria from Mardan and Peshawar taken from various food stuffs

S. No	Food item	Number of positive samples	*Clostridium* spp.	*Bacillus* spp	*S. aurus*	*S. saprophyticus*	*Listeria* spp.	*Enterobacter* spp	*Citrobacter* spp
Packed food									
1	Spinach	4	1	3	0	0	0	0	0
2	Rice	1	1	0	0	0	0	0	0
3	Tomato	4	0	0	4	0	0	0	0
4	Meat	1	1	0	0	0	0	0	0
5	Fish	3	0	1	2	0	0	0	0
6	Pea	2	0	0	0	0	2	0	0
7	Chicken	3	0	0	2	0	1	0	0
8	Yam	2	0	1	1	0	0	0	0
9	Brinjal	0	0	0	0	0	0	0	0
10	Chickpea	2	0	0	1	1	0	0	0
11	Bean	1	0	0	1	0	0	0	0
Raw food									
12	Sauce (tomato ketchup)	2	0	0	0	1	0	1	0
13	Milk shake	0	0	0	0	0	0	0	0
14	Banana juice	0	0	0	0	0	0	0	0
15	Apple juice	0	0	0	0	0	0	0	0
Ready-to-eat food									
16	Apple	2	0	0	2	0	0	0	0
17	Yogurt	1	0	1	0	0	0	0	0
18	Apple juice	3	0	1	1	1	0	0	0
19	Pasteurized milk	2	0	1	0	1	0	0	0
20	Salad	4	0	3	1	0	0	0	0
21	Unpasteurized-milk	1	0	0	0	1	0	0	0
22	Banana	3	0	0	1	2	0	0	0
23	Samosa	1	0	0	0	0	0	0	1
24	Guavas	2	0	0	0	1	1	0	0
25	Ketchup	1	0	0	1	0	0	0	0
26	Egg	0	0	0	0	0	0	0	0
Total		**45**	**3**	**11**	**17**	**8**	**4**	**1**	**1**

Table 2. Efficacy of selected antibiotics in term of zone inhibition against various Gram-positive bacteria isolates of various food stuffs

S. no	Organisms	*Ceftriaxone*	*Vancomycin*	*Azithromycin*	*Methicillin*	*Levofloxacin*	*Cefotaxime*	*Amoxicillin*
		Mean\SD	Mean\SD	Mean\SD	Mean\SD	Mean\SD	Mean\SD	Mean\SD
1	*Clostridium* spp.	14 ± 1.5	14 ± 1	19 ± 1	0 ± 0	18 ± 1.5	12 ± 0.5	10 ± 1.4
2	*Clostridium* spp.	14 ± 0.5	16 ± 0.5	21 ± 3.6	0 ± 0	19 ± 1.7	15 ± 0.5	12 ± 1
3	*S. aureus*	15 ± 0.5	7 ± 6	10 ± 5	5 ± 4	15 ± 1	15 ±1	12 ± 3
4	*S. saprophyticus*	16 ± 1.7	19 ± 3	9 ± 3	7 ± 6	24 ± 2	18 ± 2	14 ± 3
5	*S. aureus*	19 ± 3	18 ± 3	16 ± 6	5 ± 2	26 ± 1	17 ± 1.7	16 ± 6
6	*S. saprophyticus*	17 ± 2.5	17 ± 2.6	16 ± 1.7	8 ± 3	2 ± 1	16 ± 1	26 ± 2
7	*Bacillus* spp.	18 ± 1.5	6 ± 5.8	6 ± 4	8 ± 1.5	26 ± 1	18 ± 3	25 ± 1
8	*Bacillus* spp.	14 ± 2	19 ± 2	17± .4	3 ± 1	20 ± 1	15 ± 2	16 ± 1
9	*Bacillus* spp.	17 ± 1	11 ± 1.8	7 ± 6	15 ± 2	17 ± 2	26 ± 1	24 ± 0.5
10	*Bacillus* spp.	17 ± 1.5	10 ± 2	16 ± 1	13 ± 1.5	9 ± 0.5	22 ± 0.5	18 ± 2
11	*S. aureus*	18 ± 1.5	17 ± 1	16 ± 1	15 ± 2.5	20 ± 3	18 ± 2	22 ± 3
12	*S. aureus*	17 ± 3.6	16 ± 2	16 ± 2	8 ± 3	25 ± 0.5	17 ± 1	25 ± 0.5
13	*S. saprophyticus*	17 ± 1	14 ± 1	6 ± 0.5	6 ± 1	20 ± 1	17 ± 3	17 ± 1
14	*S. aureus*	12 ± 2	12 ± 3	14 ± 1	0 ± 2.5	23 ± 2	12 ± 1	14 ± 1.5
15	*Bacillus* spp.	17 ± 1.5	16 ± 2.5	19 ± 1	17 ± 0.5	23 ± 1	10 ± 0.5	26 ± 1
16	*Bacillus* spp.	18 ± 0.5	14 ± 0.5	15 ± 0.5	13 ± 1	16 ± 0.5	10 ± 2	28 ± 1
17	*Bacillus* spp.	18 ± 1	7 ± 1	21 ± 1	11 ± 0.5	18 ± 3	18 ± 1	27 ± 2
18	*S. aureus*	20 ± 1	4 ± 2	15 ± 2	0 ± 2	16 ± 1	18 ± 0.5	0 ± 1.5
19	*Bacillus* spp.	21 ± 1	20 ± 1	18 ± .2	12 ± 3	26 ± 2	20 ± 3	24 ± 1
20	*S. saprophyticus*	17 ± 3	17 ± 1	20 ± 2.5	12 ± 1	23 ± 1	17 ± 1	15 ± 3
21	*S aureus*	20 ± 1	20 ± 2	15 ± 2	17 ± 3	11 ± 3	24 ± 2	22 ± 2
22	*S. aureus*	18 ± 3	16 ± 2	16 ± 0	10 ± 4	14 ± 4	25 ± 1.5	17 ± 2
23	*S. aureus*	12 ± 2	14 ± 0.5	8 ± 3	12 ± 0	6 ± 2	23 ± 1	12 ± 1
24	*S. aureus*	22 ± 2	22 ± 1	17 ± 0	22 ± 0	19 ± 2	25 ± 3	16 ± 2
25	*S. saprophyticus*	20 ± 1	20 ± 1	19 ± 2	19 ± 2	20 ± 0	22 ± 3	16 ± 1.5
26	*Clostridium* spp.	19 ± 3	16 ± 2	18 ± 2	17 ± 1.5	16 ± 1.5	22 ± 1	13 ± 2
27	*S. aureus*	16 ± 3	16 ± 1	17 ± 3	19 ± 2	14 ± 1.5	19 ± 3	16 ± 2
28	*S. saprophyticus*	12± 1.5	15 ± 2	14 ± 0.5	12 ± 0.5	11 ± 2	22 ± 1.5	12 ± 3
30	*S. aureus*	12 ± 2	14 ± 0.5	8 ± 3	12 ± 0	6 ± 2	23 ± 1	12 ± 1
31	*S aureus*	18 ± 2	12 ± 2	16 ± 1	12 ± 2	22 ± 2	18 ± 1.5	20 ± 1.8
33	*Listeria* spp.	12 ± 1.5	12 ± 0.5	15 ± 1.5	12 ± 3	20 ± 2	24 ± 3	15 ± 1.5
34	*Listeria* spp.	8 ± 1.5	0 ± 1	13 ± 0.5	0 ± 0	14 ± 3	12 ± 1.5	14 ± 1.4
35	*S. aureus*	20 ± 1	0 ± 3	0 ± 0	0 ± 3	18 ± 1.5	24± 2.2	18 ± 1
36	*Listeria* spp.	18 ± 3	0 ± 2	14 ± 2	0 ± 3	20 ± 0.5	20 ± 1.8	0 ± 2
37	*Bacillus* spp.	15 ± 1	8 ± .2	13 ± 1.5	8 ± 2	22 ± 2	14 ± 1.4	14 ± 1.5
38	*S. aureus*	12 ± 1.5	12 ± 1	22 ± 2	0 ± 0	22 ± 2	10 ± 1	8 ± 0.5
39	*S. aureus*	22 ± 2	8 ± 1	22 ± 2	12 ± 1	22 ± 3	24 ± 2	12 ± 1
40	*Listeria* spp.	20 ± 2	16 ± 1	17 ± 1	15 ± 1.5	18 ± 2	19 ± 1.5	15 ± 1.6
41	*S. aureus*	20 ± 1	0 ± 0	16 ± 1	0 ± 0	20 ± 1.5	17 ± 1.6	10 ± 3
42	*Bacillus* spp.	21 ± 3	0 ± 2	16 ± 2	0 ± 0	19 ± 3	18 ± 1	11 ± 2
43	*S. saprophyticus*	20 ± 1	16 ± 1.5	30 ± 1	12 ± 3	15 ± 1	21 ± 2	0 ± 1
44	*S. aureus*	24 ± 1.5	17 ± 0	30 ± 3	17 ± 4	22 ± 1	25 ± 1	0 ± 0.5
45	*Bacillus* spp.	16 ± 1.5	14 ± 1.5	25 ± 2	0 ± 0	18 ± 2	14 ± 1.5	14 ± 2

Table 3. Efficacy of selected antibiotics in term of the mean zone of inhibition against various Gram-negative bacterial isolates of various foodstuffs.

S. no	Organisms	*Ceftriaxone*	*Vancomycin*	*Azithromycin*	*Methicillin*	*Levofloxacin*	*Cefotaxime*	*Amoxicillin*
		Mean\SD	Mean\SD	Mean\SD	Mean\SD	Mean\SD	Mean\SD	Mean\SD
1	*Enterobacter* spp.	0 ± 0	10 ± 2	18 ± 2	19 ± 2	19 ± 2	19 ± 2	23 ± 2
2	*Citrobacter* spp.	18 ± 3	22 ± 2	17 ± 1	20 ± 2	20 ± 1	22 ± 2	10 ± 3

Table 4. Efficacy of selected anti-fungals in term of zone of mean zone of inhibition against various fungal isolates of various food stuffs

S. no	Organisms	Fluconazole	Voriconazole	Nystatin
		Mean\SD	Mean\SD	Mean\SD
46	*Aspergillus* spp.	0 ± 0	0 ± 0	0 ± 0
47	*Aspergillus* spp.	0 ± 0	0 ± 0	0 ± 0
48	*Aspergillus* spp.	0 ± 0	16 ± 1	0 ± 0
49	*Candida* spp.	0 ± 0	0 ± 0	0 ± 0
50	*Fusarium* spp.	0 ± 0	0 ± 0	0 ± 0
51	*Candida* spp.	0 ± 0	14 ± 2	0 ± 0
52	*Aspergillus* spp.	0 ± 0	17 ± 13	0 ± 0
53	*Candida* spp.	0 ± 0	0 ± 0	0 ± 0
54	*Aspergillus* spp.	0 ± 0	0 ± 0	0 ± 0
55	*Aspergillus* spp.	0 ± 0	0 ± 0	0 ± 0
56	*Aspergillus* spp.	8 ± 2	8 ± 3	11 ± 2
57	*Mucor* spp.	10 ± 1	12 ± 1	11 ± 3
58	*Mucor* spp.	0 ± 0	0 ± 0	0 ± 0
59	*Mucor* spp.	13 ± 2	17 ± 1	0 ± 0
60	*Aspergillus* spp.	0 ± 0	14 ± 2	0 ± 0
61	*Mucor* spp.	15 ± 2	14 ± 1	0 ± 0

DISCUSSION

This research project aimed to isolate bacteria and fungi from various types of food sources available in the open markets of Peshawar and Mardan. A total of 17.30% of the food samples were found positive for the foodborne bacterial pathogens, including *S. aureus* (38%), *Bacillus* spp. (24%), *Clostridium* spp. (7%), *S. saprophyticus* (18%), *Listeria* spp. (9%), and only 4% for Gram-negative bacteria (*Enterobacter* spp. (2%) and *Citrobacter* spp. (2%)). In this research, the overall frequency of *S. aureus* species was 38% (packaged (6%), raw (41%), and ready-to-eat food (53%)) from Mardan and Peshawar. Various earlier reports have also described the isolation and antibiotic sensitivity pattern of *S. aureus* from different food sources (Nyenje *et al*., 2012; Rasheed *et al*., 2014; Tufail *et al*., 2011).

In their study on the microbiological analysis of different ready-made foods sold in South Africa, a total of 252 samples, consisting of rice, vegetables, pies, potatoes, chicken, and beef were examined. Biochemical tests, and bacterial growth were used to identify all isolates. The highest bacterial frequency was found in vegetables, followed by rice, while pies had the lowest count. The organisms isolated included *S. aureus* (32%), *Listeria* spp. (22%), *Aeromonas hydrophila* (12%), *Enterobacter* spp. (18%), *Klebsiella oxytoca* (8%), *Proteus mirabilis* (6%), and *Pseudomonas luteola* (2.5%) (Nyenje *et al*., 2012; Rasheed *et al*., 2014; Tufail *et al*., 2011).

The *B. cereus* species found in various food items from Mardan and Peshawar accounted for around 24% [raw food (41%) and ready-to-eat food (59%)]. Previously, *B. cereus* was also reported by Hillers *et al*. (2003) and Lateef *et al*. (2004), in which forty samples

from 20 different brands of sachet orange juice products were identified for microbiological analysis. All products were found to be infected with yeasts and bacteria. The pathogens identified included *Rhodotorula* sp., *Saccharomyces cerevisiae*, *B.cereus*, *Saccharomyces* sp., *Bacillus subtilis*, *E. coli*, *Streptococcus pyogenes*, *S. aureus*, and *Micrococcus* spp. The resistance of 30 bacterial strains, extracted from orange juice products, to commonly used antibiotics was examined. About 66.69% of the isolates were resistant to *amoxicillin* and *augmentin*, 63.34% to *cotrimoxazole*, 57% to *cloxacillin*, and 23.34% to *tetracycline*. Resistance rates of 3.33% were obtained for *erythromycin*, *gentamicin*, and *chloramphenicol* respectively. Out of these, six strains were found to have multiple drug resistance (Hillers *et al*., 2003; Lateef *et al*., 2004).

In the present study, frequencies of 9% and 2% were found for *Listeria* spp. and *Enterobacter* spp. in raw and ready-to-eat food items, respectively. *Listeria* spp. and *Enterobacter* spp. have also been reported in 252 samples of various foods, such as pies, potatoes, vegetables, rice, beef, and chicken, through microbiological analysis of different ready-made foods sold in South Africa (Nyenje *et al*., 2012). In the present work, *Clostridium* spp. (7%) was also discovered in various food items. Similarly, Granum (1990) found *Clostridium* spp. in different foodstuffs and discovered that *Clostridium* spp. was responsible for two specific types of food poisoning caused by toxins A and B. Mild classic food poisoning is caused by toxin A while a severe form of food poisoning known as necrotic enteritis in humans is caused by toxin B.

In the current study, *Staphylococcus saprophyticus* (18%) was also detected in raw and ready-made food. Contamination of food with *Staphylococcus saprophyticus* had also been described previously by Lee (2003). Furthermore, *Citrobacter* spp., another bacterial pathogen, was also discovered in this study. Specifically, during outbreaks, the same pathogen was found in various foodstuffs (Bennett *et al*., 2013). A total of 1229 foodborne illnesses caused by *S. aureus*, *Clostridium perfringens*, and *B. cereus* were reported in the United States, for instance illnesses were reported in 75% of *B. cereus* cases and 87% of *S. aureus* outbreaks, but were rare in *C. perfringens* outbreaks (9%) and *C. fruendii* (6%). Moreover, meat and poultry dishes were commonly involved in *C. perfringens* (64%) and *S. aureus* outbreaks (56%), while rice dishes were frequently occupied in *B.cereus* outbreaks (50%) (Bennett *et al*., 2013).

Besides bacterial pathogens, fungal pathogens were also isolated from various food items in the current study. The fungal pathogens isolated were *Aspergillus* spp. (50%), *Candida* spp. (19%), *Fusarium* spp. (6%), and *Mucor* spp. (25%). Similarly, the same fungal pathogens were also detected in various foods by Bello *et al*. (2023). The isolated fungal pathogens were further tested for their sensitivity to various selected anti-fungals. Notably, six fungal pathogens were detected in their study, including *Fusarium* spp., *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium* spp., *Mucor* spp., and *Saccharomyces cerevisiae*, all of which were isolated and characterized. The fungal isolates were found to be more virulent compared to bacterial isolates, such as *Mucor* spp. and *Bacillus subtilis*. *Chloramphenicol* was identified as the most effective antibiotic for targeting both microbial groups (Bello *et al*., 2023).

The antibiotic sensitivity pattern of isolated bacterial and fungal pathogens, a panel of selected antibiotics was used. These included *azithromycin*, *amoxicillin*, *vancomycin*, *cefotaxime*, *methicillin*, *ceftriaxone*, and *levofloxacin* for Gram-positive bacteria, and *cefixime*, *cefuroxime*, *linezolid*, *cefoxitin*, *ceclor*, *ciprofloxacin*, and *chloramphenicol* for Gram-negative bacteria. Anti-fungal activity, *fluconazole*, *voriconazole*, and *nystatin* were used.

In the present study, five Gram-positive bacteria were identified, including *S. aureus* (38%), *Bacillus* spp. (24%), *Clostridium* spp. (7%), *S. saprophyticus* (18%), and *Listeria* spp. (9%) (Table 2). Seven antibiotics were used for Gram-positive bacteria, including *vancomycin*, *azithromycin*, *amoxicillin*, *cefotaxime*, *methicillin*, *ceftriaxone*, and *levofloxacin*. The best activity was observed with *ceftriaxone* (88%), *levofloxacin* (86%), and *cefotaxime* (77%) against all species. Intermediate activity was shown by *azithromycin* (50%) and *vancomycin* (55%). The highest resistance rates were observed with *methicillin* (79%) and *amoxicillin* (63%) across all isolated bacterial pathogens. Similar research was conducted by Rasheed *et al*. (2014). Other research found that *S. aureus* and *S. saprophyticus* exhibited varying degrees of resistance to *methicillin*, *penicillin G*, *ampicillin*, and *amoxicillin*, but were found to be 100% and 97.4% susceptible to *ampicillin*/*sulbactam* and *amoxycillin*/*clavulanic acid*, respectively (Agwa *et al*., 2012). All *Bacillus* species isolates were susceptible to *chloramphenicol*, *rifampin*, *erythromycin*, *streptomycin*, *ciprofloxacin*, *gentamycin*, and *lincomycin*, but showed 100% resistance to *norfloxacin* and *ampiclox* (Moreno *et al*., 2014; Saleh and Wongwattana, 2019). *Clostridium* spp. demonstrated varying levels of resistance to antibiotics, such as *tetracycline* (56%), *imipenem* (24%), *metronidazole* (9%), *penicillin G* (9%), *vancomycin* (4%), *chloramphenicol* (3%), and *ceftriaxone* (1%) (Kandeepan, 2014). All *Listeria* spp. isolates were susceptible to *penicillin*, *ampicillin*, *tetracycline*, *erythromycin*, and *carbapenems*. However, some degree of resistance to *clindamycin*, *daptomycin*, *oxacillin*, and *fluoroquinolones* was observed in *Listeria* spp.

In the current research, only two types of Gram-negative bacteria, *Enterobacter* spp. and *Citrobacter* spp., were identified in different food items. These Gram-negative bacteria were evaluated for their antibiotic sensitivity patterns against a panel of seven selected antibiotics. The panel included *cefixime*, *cefuroxime*, *ciprofloxacin*, *cefoxitin*, *chloramphenicol*, *ceclor*, and *linezolid*. The highest activity was exhibited by

ciprofloxacin (100%), *cefoxitin* (100%), *chloramphenicol* (100%), and *ceclor* (100%), while intermediate activity was shown by *cefixime* (50%), *cefuroxime* (50%), and *linezolid* (50%) against all Gram-negative isolates (Table 3).

Similar types of Gram-negative bacteria were also isolated from various kinds of food in a previous study by Nawas *et al*. (2012). Out of 5695 Gram-negative isolates identified, 690 were *Citrobacter* spp. Among these, *Citrobacter freundii* (62.5%) and *Citrobacter koseri* (37.5%) were the most common species isolated. In line with the antibiogram, as per the CLSI guidelines, resistance to *fluoroquinolones*, *cephalosporins*, and *beta-lactamase* inhibitors has been noted, while carbapenems exhibit sensitivity. The resistance to *beta-lactamase* inhibitors increased with the presence of AmpC *beta-lactamase* (76%) and ESBL (50%). The antibiotic susceptibility pattern showed sensitivity to *carbapenems* (98%) and resistance to third-generation *cephalosporins* (70%).

In the present study, four fungal pathogens were identified in raw and ready-made food. Specifically, the fungal pathogens identified, along with their frequency percentages, were *Aspergillus* spp. (50%), *Candida* spp. (19%), *Fusarium* spp. (6%), and *Mucor* spp. (25%) to assess treatment options, three anti-fungal drugs (*fluconazole*, *voriconazole*, and *nystatin*) were used to test their potency against the isolated fungal pathogens. *Mucor* spp. was relatively more susceptible to all anti-fungals except nystatin. On the other hand, the *Fusarium* isolates were highly resistant to all anti-fungals used in this research. Moreover, similar fungal species were also reported from various foods in a study by Ramesh *et al*. (2013), where their susceptibility to anti-fungals such as *itraconazole*, *ketaconazole*, and *amphotericin B* was assessed. In that study, the fungal isolates showed varying degrees of susceptibility to the anti-fungals (Ramesh *et al*., 2013).

CONCLUSION

The prevalence of Gram-positive bacterial pathogens was significantly higher in various foodstuffs compared to Gram-negative bacteria. In terms of various types of foodstuffs, a single microbe was found in packed food, while the frequency of microbes was higher in raw and ready-to-eat food. The antibiotic sensitivity patterns of various antibiotics used in the study revealed that *ceftriaxone* (88%), *levofloxacin* (86%), and *cefotaxime* (77%) were the most effective against all bacterial isolates. Intermediate activity was observed with *azithromycin* (50%) and *vancomycin* (55%). The highest resistance was found with *methicillin* (79%) and *amoxicillin* (63%) among all isolated bacterial pathogens. *Listeria* spp. and *Staphylococcus saprophyticus* showed the highest resistance, while *S. aureus*, *Bacillus* spp., and *Clostridium* spp. showed the lowest susceptibility to the antibiotics tested.

Our research also concluded that the frequency of fungal pathogens was relatively high in raw and ready-to-eat food, whereas packed food was free from any fungal contamination. Among fungal pathogens, *Aspergillus* spp. and *Mucor* spp. had higher frequencies compared to *Candida* spp. and *Fusarium* spp. The drug sensitivity pattern of the anti-fungal drugs used in the study showed that *fluconazole*, *voriconazole*, and *nystatin* had varying levels of effectiveness against various types of fungal isolates.

We recommended for public awareness that (1) Food available in the open markets of Peshawar and Mardan is contaminated with pathogenic bacteria, so proper precautions must be taken when handling and cooking the food, (2) Food handlers should be screened for pathogenic bacteria to identify potential sources of contamination, (3) Strict regulations must be implemented on raw food shop owners to ensure the proper supply and distribution of food items, and (4) The use of appropriate drugs for foodborne bacterial and fungal diseases is recommended based on the findings of this study.

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