Volume 3, Issue 4, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA AND FUNGI ISOLATED FROM HOUSEHOLD REFRIGERATORS

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DOI: https://doi.org/10.5281/zenodo.15703557

Keywords
Household refrigerators, foodborne pathogens, microbial contamination, antibiotic resistance, E. coli, Staphylococcus aureus, Penicillium spp., disk diffusion method, CLSI guidelines, food safety

Article History
Received on 11 May 2025
Accepted on 11 June 2025
Published on 19 June 2025

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Abstract

Refrigerators, though designed to preserve food, can harbor diverse microbial populations that contribute to food spoilage and pose health risks. This study aimed to monitor and identify pathogenic microorganisms present in household refrigerators and to evaluate their antimicrobial resistance profiles. A total of 100 swab samples were collected from three compartments (shelves, vegetable boxes, and egg trays) of domestic refrigerators in Faisalabad. Samples were cultured on selective and differential media and identified through colony morphology, Gram staining, and biochemical tests including catalase, oxidase, TSI, motility, and IMViC. Antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method following CLSI 2014 guidelines. The bacterial isolates included Bacillus spp. (17.3%), Staphylococcus aureus (8%), Micrococcus spp. (7.3%), Listeria spp. (3.3%), E. coli (14.7%), Klebsiella spp. (11.3%), Enterobacter spp. (10.6%), Pseudomonas aeruginosa (6%), Salmonella spp. (5.3%), Shigella spp. (5.3%), Citrobacter spp. (3.3%), Pantoea spp. (3.3%), Proteus vulgaris (2.7%), and Campylobacter spp. (1.3%). Fungal isolates included Penicillium spp. (45%), Aspergillus spp. (25%), Rhizopus spp. (20%), and Saccharomyces cerevisiae (10%). Alarmingly, gram-negative bacteria exhibited high resistance to ampicillin (93%), amoxicillin/clavulanic acid (72%), ceftazidime (69%), and cefepime (64%). Staphylococcus aureus was 92% resistant to both penicillin and amoxicillin/clavulanic acid. Amikacin and ciprofloxacin were the most effective

Volume 3, Issue 4, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

antibiotics across all isolates. These findings emphasize the potential risk posed by household refrigerators as reservoirs of resistant pathogens, highlighting the need for better hygiene practices and public awareness.

INTRODUCTION

Microorganisms are ubiquitous in the environment and can thrive under a variety of conditions, including low temperatures(Gupta, Srivastava et al. 2014). Refrigerators are commonly used in households to preserve food and prevent microbial spoilage. However, certain psychrotrophic and psychrophilic microorganisms have the ability to survive and even proliferate at refrigeration temperatures (4–7 °C), posing a risk of food contamination and spoilage(Zhang, Wei et al. 2019). Pathogenic bacteria such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aeromonas* spp., *Pseudomonas* spp., *Plesiomonas shigelloides*, and fungi including *Penicillium* and *Cladosporium* spp., have been reported to persist in refrigerated environments(Cortés-Sánchez, Espinosa-Chaurand et al. 2021).

Household refrigerators, in particular, play a significant role in cross-contamination events leading to foodborne illnesses(Scott 2000). Improper handling practices—such as the storage of unwrapped raw meat, unwashed produce, or leaking packages—can result in microbial contamination of interior surfaces, which may subsequently contaminate other stored food items(Michaels 2003). According to USDA (2010), raw meat, poultry, and seafood should be securely wrapped and stored in sealed containers to minimize such risks(Reames 2012).

Despite the cold conditions, several fungi and bacteria are capable of surviving and multiplying in refrigerators. Psychrotrophic molds, including *Trichothecium*, *Rhizopus*, *Penicillium*, *Mucor*, *Monascus*, *Geotrichum*, *Fusarium*, *Cladosporium*, *Botrytis*, *Aspergillus*, and *Alternaria*, along with yeasts such as *Torulopsis*, *Saccharomyces*, *Debaryomyces*, and *Candida albicans*, are commonly associated with food spoilage at low temperatures(Hassan, Rafiq et al. 2016). Environmental factors such as high acidity, packaging conditions, and low water activity often favor fungal growth over bacterial proliferation in cold-stored foods(Abbas, Saleh et al. 2009).

Several studies have assessed the prevalence of foodborne pathogens in domestic refrigeration units. For example, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Escherichia coli* have all been isolated from the interior surfaces of refrigerators, with prevalence rates of 1.2%, 6.4%, and 0.6%, respectively(Kilonzo-Nthenge, Chen et al. 2008). In contrast, *Salmonella* and *Campylobacter* species were not detected. Since 1981, *L. monocytogenes* has been recognized as a foodborne pathogen capable of adhering to plastic, glass, and stainless steel surfaces materials commonly found in refrigerator interiors(Alsharif 2024). This bacterium is also noted for its resistance to disinfectants and cleaning agents, thereby posing a continual threat of recontamination(Tong, Hu et al. 2021).

Contamination of ready-to-eat foods such as vegetables, dairy, and meat stored in such environments presents significant health risks, especially to immunocompromised individuals, pregnant women, and the elderly(Makinde, Ayeni et al. 2020). The World Health Organization (WHO, 2008) has highlighted foodborne illnesses as a major global health concern, estimating that approximately 1.8 million deaths in 2005 were due to diarrheal diseases, many of which were linked to contaminated food. According to the European Food Safety Authority (EFSA), 32.7% of reported foodborne outbreaks in Europe originated in domestic kitchens(Authority, Prevention et al. 2018).

Improper cleaning, poor temperature control, and inadequate food handling practices are primary contributors to the spread of foodborne pathogens such as *E. coli*, *Salmonella spp.*, *Shigella spp.*, and *L. monocytogenes(Abebe, Gugsa et al. 2020)*. Regular cleaning of refrigerator interiors is essential to minimize microbial load. Surfaces should be sanitized with hot soapy water and spills should be cleaned immediately. Leftovers should be discarded after four days, and raw poultry or ground meat should be consumed within one to two days (USDA, 2010). Some studies suggest that sodium bicarbonate can effectively reduce mold growth on refrigerator surfaces, while other bacteria, such as *L. monocytogenes*,

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ISSN: (e) 3007-1607 (p) 3007-1593

are more susceptible to anionic detergents(Walton 2012).

Given the increasing reliance on refrigeration and the limited data available particularly from Pakistan, on the microbiological status of household refrigerators, this study was designed with the following objectives:

- To detect microbiological contaminants in household refrigerators.
- To isolate and identify bacterial and fungal pathogens from refrigerator interior surfaces.
- To determine the antimicrobial susceptibility patterns of the isolated pathogens.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of 100 swab samples were randomly collected from domestic refrigerators located in different households. From each refrigerator, three samples were obtained using sterile cotton swabs from three distinct internal locations: the shelves, the vegetable compartment, and the egg tray. After sampling, the swabs were immediately placed into sterile plastic tubes and transported to the Department of Microbiology, Government College University, Faisalabad. All samples were preserved at -20°C until further processing.

2.2. Sample Processing

For microbial analysis, each swab was immersed in sterile buffered peptone water and agitated thoroughly to release the microbial load. The resulting mixture was serially diluted in Tris-peptone buffer with a pH of 7.5, from 10^{-1} up to 10^{-6}. From each dilution, 1 mL was poured onto nutrient agar plates using the pour plate method to determine the Total Viable Count (TVC). The contents were evenly spread with a sterile glass spreader. All plates were incubated aerobically at 37°C for 18–20 hours, following protocols described in the FDA Bacteriological Analytical Manual (Jackson et al., 2001).

2.3. Microbial Load Confirmation

Post incubation, only those plates with colony counts ranging from 30 to 300 were selected for analysis. Colonies were counted using a colony counter, and the TVC was calculated and expressed in colony-forming units per milliliter (cfu/mL).

2.4. Isolation and Identification of Microorganisms

2.4.1. Bacterial Isolation

To obtain pure bacterial isolates, sub-culturing was performed on nutrient agar using the streak plate method, followed by incubation at 37°C for 18–24 hours. Pure colonies were then streaked on different media including:

- **Blood agar** (enriched medium)
- **Salmonella-Shigella agar** and **Staph 110 agar** (selective media)
- **MacConkey agar** (differential medium)

Bacterial identification was based on colony morphology, cultural characteristics, and further confirmed through biochemical testing, including coagulase, oxidase, catalase, IMViC, TSI, and motility tests (Ilyas et al., 2016).

2.4.2. Fungal Isolation

Swab samples were also inoculated on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) for fungal isolation and incubated at room temperature for 5–17 days. For microscopic observation, Lactophenol Cotton Blue staining was used. Both microscopic and macroscopic fungal features were analyzed based on identification keys by Peterson (2003).

2.5. Morphological Examination

2.5.1. Gram Staining

Bacterial isolates were initially differentiated through Gram staining (Cain et al., 2013):

1. Thin smear of bacteria prepared and heat-fixed.
2. Smear stained with crystal violet (1 min), followed by Gram's iodine (1 min).
3. Decolorized briefly and counterstained with safranin (30–45 sec).
4. Observed under 100X oil immersion lens.

Purple-colored cells indicated Gram-positive bacteria, while pink-colored cells represented Gram-negative bacteria.

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2.6. Biochemical Characterization

2.6.1. Catalase Test

A drop of hydrogen peroxide was added to a glass slide, and a bacterial colony was mixed in. The formation of oxygen bubbles confirmed a positive result.

2.6.2. Oxidase Test

A colony was rubbed on filter paper soaked with 1% oxidase reagent. Development of a purple color within 5–10 seconds indicated a positive test.

2.6.3. Triple Sugar Iron (TSI) Test

TSI agar was inoculated by stabbing the butt and streaking the slant. After 24 hours of incubation at 37°C:

- Yellow color and gas formation indicated sugar fermentation.
- Black precipitate signified hydrogen sulfide (H_2S) production.

2.6.4. Motility Test

Motility was checked via the hanging drop method using a 40X objective lens. Movement of bacteria in the drop indicated motility.

2.6.5. Indole Test

Isolates were grown in tryptone broth at 37°C for 24 hours, followed by the addition of Kovac's reagent. A red ring in the reagent layer confirmed a positive result.

2.6.6. Methyl Red Test

Cultures were incubated in MR-VP broth at 37°C for 48 hours. Addition of methyl red reagent turned the broth red in case of mixed acid fermentation (positive result).

2.6.7. Voges-Proskauer Test

Following incubation in MR-VP broth, 0.6 mL of 5% α-naphthol and 0.2 mL of 40% KOH were added. Development of a cherry red color indicated a positive result for acetoin production.

2.7. Antibacterial Susceptibility Testing

The Kirby-Bauer disc diffusion method was employed to assess antibiotic sensitivity. The method was carried out in accordance with CLSI guidelines (2014):

- Fresh colonies were suspended in 5 mL sterile saline and adjusted to 0.5 McFarland standard turbidity.
- Bacterial suspensions were streaked on Mueller-Hinton agar.
- Antibiotic discs were applied, and plates were incubated at 37°C for 18–24 hours.
- Zones of inhibition were measured and interpreted using CLSI 2014 breakpoints.

Antibiotics Used

Gram Positive Bacteria	Gram Negative Bacteria
Penicillin	Ampicillin
Linezolid	Cefepime
Vancomycin	Ciprofloxacin
Ciprofloxacin	Ceftriaxone
Clindamycin	Ceftazidime
Amoxicillin + Clavulanic Acid	Amoxicillin + Clavulanic Acid
Sulfamethoxazole + Trimethoprim	Amikacin

2.8. Statistical Analysis

The experimental data collected from microbial counts and antibiotic sensitivity tests were subjected to statistical analysis using **Minitab software**. Appropriate statistical procedures were applied to evaluate the significance of results.

3. Results

3.1 Microbial Load of Household Refrigerator Samples

All household refrigerator samples collected in this study were contaminated with various microorganisms. The microbial load varied across the three examined refrigerator compartments: shelves,

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ISSN: (e) 3007-1607 (p) 3007-1593

egg trays, and vegetable boxes. The microbial counts ranged from **7.3 × 10^2 to 1.06 × 10^4 CFU/ml** for shelves, **5.0 × 10^3 to 1.06 × 10^6 CFU/ml** for egg trays, and **7.2 × 10^2 to 1.5 × 10^6 CFU/ml** for vegetable boxes. The **egg tray** showed the highest microbial load (1.06 × 10^6 CFU/ml), while the **lowest load** was recorded in the vegetable box (0.7 × 10^3 CFU/ml).Notably, fungal contamination was most prevalent in the vegetable box (45%), followed by the egg trays (35%) and shelves (20%), as shown in Figure 3.1.

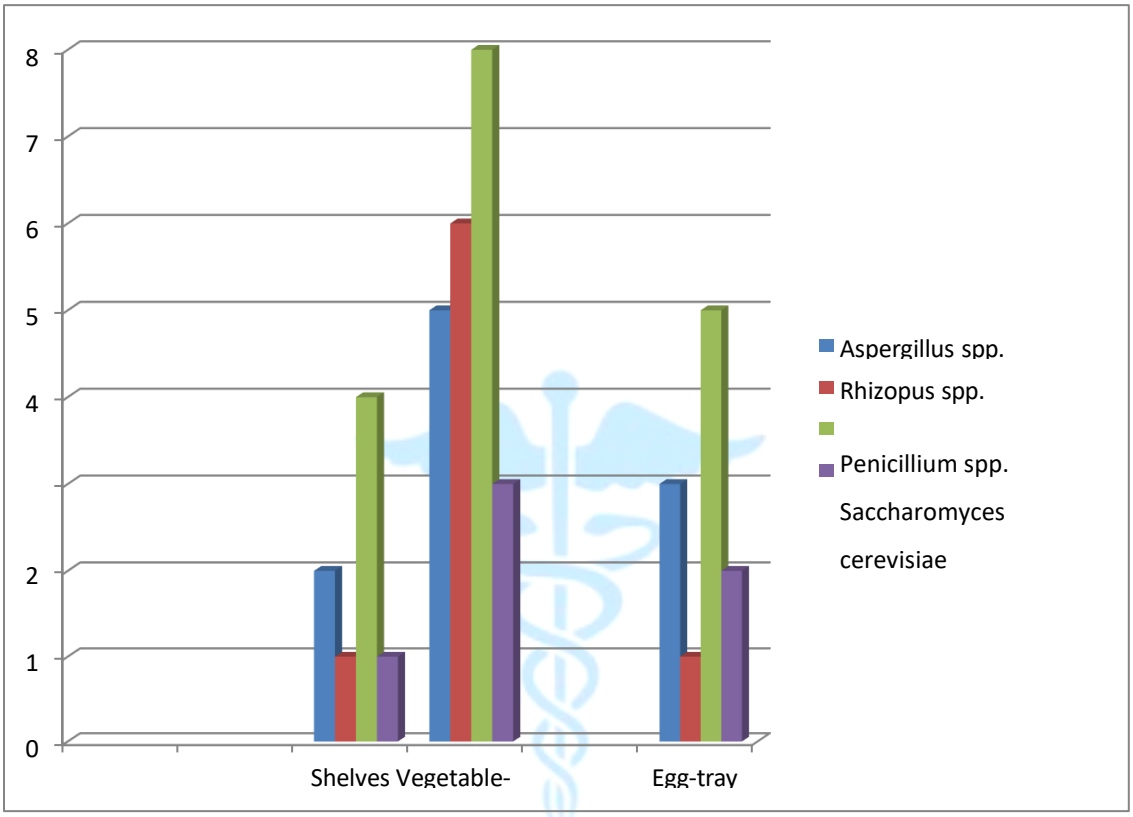

Figure 3.1: The total viable counts and types of microorganisms isolated from the respective compartments

3.2 Microorganisms Isolated from Household Refrigerator Samples

Out of **100 swab samples** collected from household refrigerators in Faisalabad, **150 bacterial** and **20 fungal** isolates were obtained. **Gram-negative bacteria** comprised **64%**, while **Gram-positive bacteria** made up **36%** of total isolates (Figure 3.2). Among Gram-positive bacteria, **15%** were cocci and **21%** were rods (Figure 3.3).

Table 3.1 Frequency of Micro-organisms Isolated from Household Refrigerator Samples

Micro-organisms (n= 150)	Number	Percentage
Gram negative bacteria (n=96)		
E.coli	22	14.7
***Klebsiella* spp.**	17	11.3
***Pantoea* spp.**	05	3.3
***Enterobacter* spp.**	16	10.6

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Citrobacter spp.	05	3.3
Shigella spp.	08	5.3
Salmonella	08	5.3
Pseudomonas aeruginosa	09	06
Proteus vulgaris	04	2.7
Compylobacter spp.	02	1.3
Gram positive cocci (n=23)		
Micrococcus spp.	11	7.3
Staphylococcus aureus	12	08
Gram positive rods (n=31)		
Bacillus spp.	26	17.3
Listeria spp.	05	3.3

Table 3.2. Frequency of Fungal isolates Isolated from Household Refrigerator Samples

Fungal Isolates (n= 20)	Number	Percentage
Aspergillus flavus	01	05
Aspergillus niger	02	10
Aspergillus fumigates	02	10
Rhizopus spp.	04	20
Penicillium spp.	09	45
Saccharomyces cerevisae	02	10

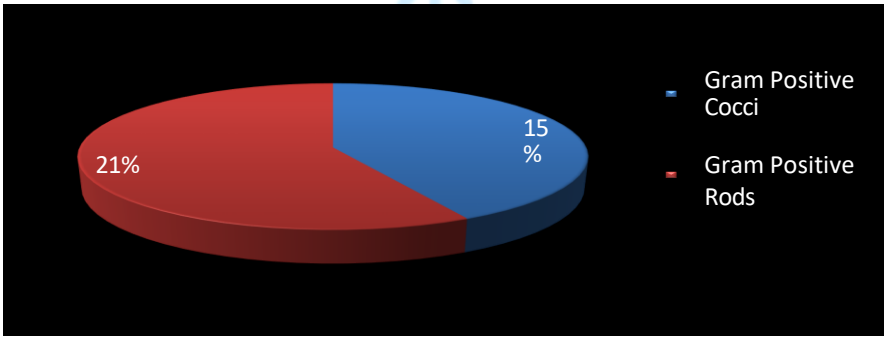

Figure.3.2. Frequency of Gram Positive Rods and Cocci Bacteria in Refrigerators

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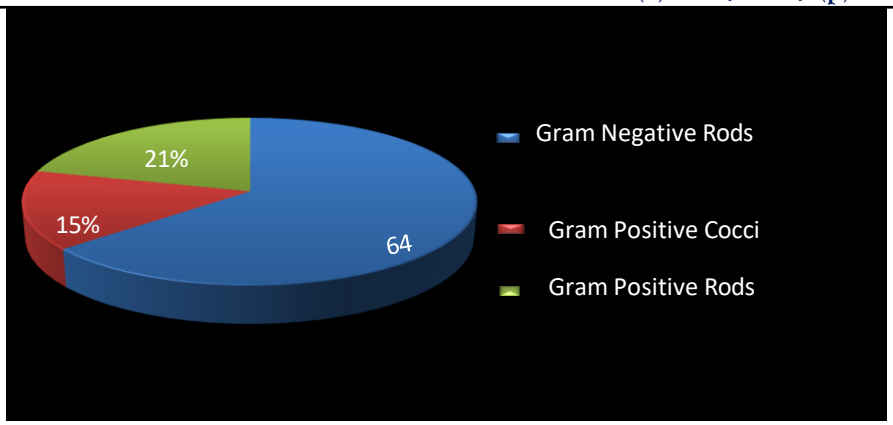

Figure.3.3. Frequency of Gram Positive Cocci, Gram Positive Rods and Gram Negative Rods in Refrigerator Samples

Predominant Gram-positive bacteria included:

- *Bacillus spp.* (17.3%)
- *Staphylococcus aureus* (8%)
- *Micrococcus spp.* (7.3%)
- *Listeria spp.* (3.3%)

Common Gram-negative bacteria were:

- *Escherichia coli* (14.7%)
- *Klebsiella spp.* (11.3%)
- *Enterobacter spp.* (10.6%)
- *Pseudomonas aeruginosa* (6%)
- *Salmonella spp.* (5.3%)
- *Shigella spp.* (5.3%)
- *Citrobacter spp.*, *Pantoea spp.* (3.3% each)
- *Proteus vulgaris* (2.7%)
- *Campylobacter spp.* (1.3%) (Table 4.4)

Among fungal isolates, *Penicillium spp.* was dominant (45%), followed by *Aspergillus spp.* (25%), *Rhizopus spp.* (20%), and *Saccharomyces cerevisiae* (10%) as detailed in Table 3.3.

3.3 Identification of Bacterial Isolates

3.3.1 Gram Staining and Colony Morphology

Bacterial isolates were identified using Gram staining and colony morphology (Table 3.3). **Gram-positive cocci** such as *S. aureus* and *Micrococcus spp.* were observed in grape-like clusters and diplococci forms (Figure 3.4), while **Gram-positive rods**, including *Bacillus spp.* and *Listeria spp.*, exhibited characteristic colony morphologies (Figure 3.5, 3.6).

Gram-negative bacteria exhibited pink-stained rods or coccobacilli (Figure 4.6) with distinguishable colony traits:

- *E. coli*: Smooth, pink, lactose-fermenting colonies on MacConkey agar (Figure 4.9)
- *Shigella spp.*: Colorless, translucent colonies (non-lactose fermenters)
- *Salmonella spp.*: Translucent colonies with black centers on SS agar
- *P. aeruginosa*: Green-pigmented colonies
- *Klebsiella spp.*: Mucoid pink colonies
- *Citrobacter spp.*: Light pink, odorous colonies
- *Proteus vulgaris*: Swarming colonies with fishy odor
- *Campylobacter spp.*: Droplet-like colonies on blood agar

Fungal isolates also showed distinctive morphologies on selective media. *Aspergillus flavus* (dark green), *A. fumigatus* (blue-grey), *A. niger* (brown), *Rhizopus spp.* (cotton-like), and *Penicillium spp.* (greenish-black) were observed on MEA and PDA.

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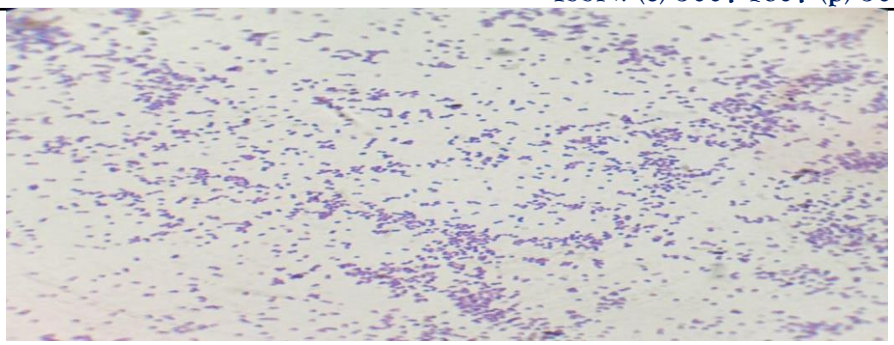

Figure.3.4. Shows Purple Color Gram Positive Bacteria arranged as Cocci, Diplococci and Grape like Clusters

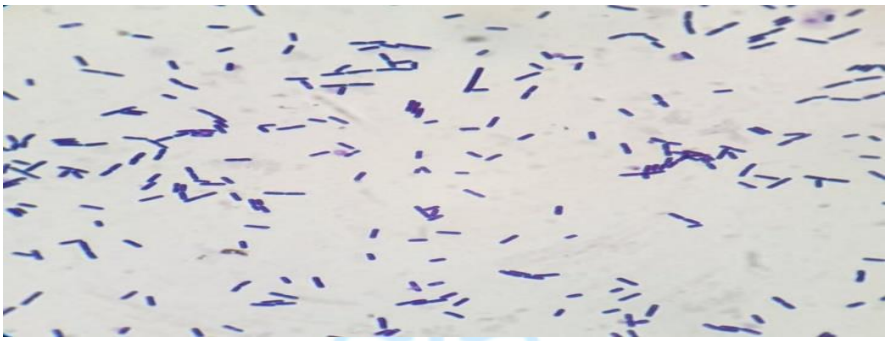

Figure.3.5. Shows Purple Color Gram Positive Rods Shaped Bacteria (*Bacillus* spp.)

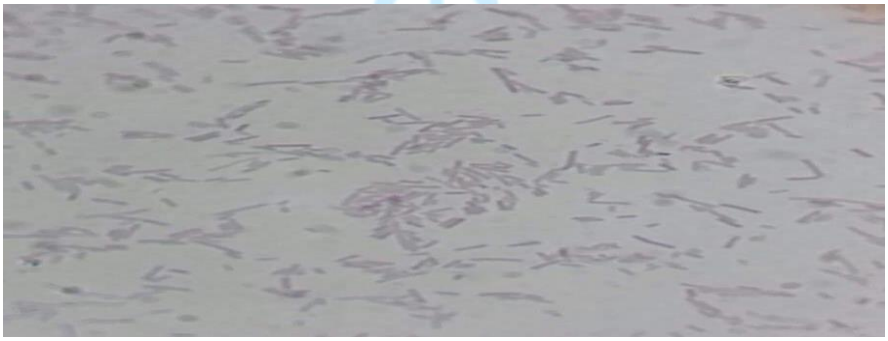

Figure.3.6. Shows Pink Color Gram Negative Rods Shaped Bacteria

Table 3.3. Staining Characters, Morphology and Arrangement of Isolated Bacterial Species

Bacterial isolates	Staining character	Morphology	Arrangement
Micrococcus	Gram positive	Cocci	Single, Diplococci, Tetrad
Staphylococcus aureus	Gram positive	Cocci	Grape like clusters
Bacillus spp.	Gram positive	Bacilli	Single
Listeria spp.	Gram positive	Cocco Bacilli	Single
E. coli	Gram negative	Bacilli	Single
Klebsiella spp.	Gram negative	Bacilli	Single
Salmonella spp.	Gram negative	Bacilli	Single
Pantoea spp.	Gram negative	Bacilli	Single
Enterobacter spp.	Gram negative	Bacilli	Single

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Shigella spp.	Gram negative	Bacilli	Single
Peudomonas aeruginosa	Gram negative	Bacilli	Single
Citrobacter spp.	Gram negative	Bacilli	Single
Proteus vulgaris	Gram negative	Bacilli	Single
Compylobacter spp.	Gram negative	Helical/Comma Sahped	Single

3.4 Biochemical Characterization of Bacterial Isolates

Biochemical tests were performed for species confirmation (Table 3.4). Key results including *S. aureus*: Positive for catalase (oxygen bubbles, Fig. 4.10) and coagulase tests, *P. aeruginosa*: Positive oxidase test (violet to purple), and, *E. coli*, *Klebsiella spp.*, *Enterobacter spp.*, and others: Confirmed using indole, methyl red, Voges-Proskauer, and triple sugar iron tests.

Table 3.4. Biochemical Test Results for Microorganisms Isolated from Household Refrigerator Samples

Bacterial isolates	Catalase test	Coagulase test	Indole test	Oxidase test	Methyl red test	Voges-proskauer test	Triple sugar iron test	Motility test
Staphylococcus aureus	Positive	Positive	Positive	Negative	Positive	Negative/ Positive	Not applicable	Not applicable
Bacillus spp.	Positive	Negative	Negative	Negative	Negative	Negative/ Positive	Not applicable	Motile/ Non-motile
E.coli	Positive	Not applicable	Positive	Negative	Positive	Negative	A/A with or without gas	Motile
Klebsiella spp.	Positive	Not applicable	Negative	Negative	Negative	Positive	A/A with abundant gas	Non-motile
Compylobacter spp.	Positive	Not applicable	Negative	Positive	Not applicable	Negative	Variable	Motile
Pantoea spp.	Positive	Not applicable	Variable	Negative	Variable	Variable	Variable or Alk/A	Motile
Enterobacter spp.	Positive	Not applicable	Negative	Negative	Negative	Positive	A/A with abundant gas	Motile
Citrobacter spp.	Positive	Not applicable	Positive	Negative	Positive	Negative	Alk/A	Motile
Salmonella spp.	Positive	Not applicable	Negative	Negative	Positive	Negative	Variable	Motile
Shigella spp.	Positive	Not applicable	Variable	Negative	Positive	Negative	Alk/A	Non- motile
Pseudomonas aeruginosa	Positive	Negative	Negative	Positive	Negative	Negative	Non-fermenter	Motile
Proteus vulgaris	Positive	Not applicable	Positive	Negative	Positive	Negative	Alk/A, H_2S Positive	Motile

3.5 Antimicrobial Susceptibility Testing

Antibiotic susceptibility tests revealed multidrug resistance among isolates. The highest resistance across most species was observed against **ampicillin**. *S. aureus* was highly resistant to **penicillin and amoxicillin/clavulanic acid (92%)**, followed by **SXT (83%)**, **linezolid (75%)**, and **vancomycin (50%)**. *E. coli* showed resistance to **ampicillin (77%)**, **cefepime (73%)**, and **amoxicillin/clavulanic acid (64%)**.

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ISSN: (e) 3007-1607 (p) 3007-1593

Klebsiella spp. exhibited **100% resistance** to ampicillin, and high resistance to **Ceftazidime** (88%), **amoxicillin/clavulanic acid** (76%), and **cefepime** (76%) (Figure 3.7).

Pantoea spp. and *Enterobacter spp.* showed **100% resistance** to ampicillin and amoxicillin/clavulanic acid.*Shigella spp.* had 100% resistance to ampicillin, and moderate resistance to ciprofloxacin, cefepime, and ceftazidime (63%).*Citrobacter spp.* and *P. aeruginosa* showed high resistance across multiple antibiotics including **100% resistance** to ampicillin and **amoxicillin/clavulanic acid** (Figure 3.8).The overall resistance pattern is shown in Tables 3.5-3.14.

Table 3.5. Antibiotic Susceptibility profile of *Staphylococcus aureus* isolated from Household Refrigerator Samples

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		P	LZD	CIP	AMC	VA	CN	SXT
S.aureus	1	R	I	S	R	I	S	I
	2	R	I	S	R	S	S	R
	3	I	R	S	I	S	I	R
	4	R	I	S	R	R	S	S
	5	R	S	S	R	S	I	R
	6	R	I	S	R	I	S	R
	7	S	I	S	I	R	S	I
	8	R	I	S	I	S	S	R
	9	R	I	S	S	R	S	R
	10	R	R	S	R	I	S	R
	11	R	S	S	I	S	S	S
	12	R	S	S	R	S	S	R

Key: P = 10 µg Penicillin, LZD = 30 µg Linezolid, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, VA = 30 µg Vancomycin, CN = 10 µg Clindamycin, SXT = 23.75/1.25 µg Sulfamethaxazole/Trimethoprim.
R = Resistance, S = Sensitive, I = intermediate

Table 3.6. Antibiotic Susceptibility profile of *E. coli* isolated from Household Refrigerators

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		AK	CRO	CIP	AMC	AMP	FEP	CZ
	1	S	R	S	S	I	S	S
	2	S	R	S	R	R	R	R
	3	I	R	S	I	R	R	R
	4	S	I	S	R	R	S	S
	5	S	S	S	R	R	I	R
	6	S	S	S	S	I	R	S
	7	S	S	S	I	R	I	I
	8	S	S	S	I	S	R	S
	9	S	I	S	S	R	R	S
	10	S	R	S	R	I	R	R
	11	S	S	S	I	R	I	S
	12	I	R	I	R	R	R	R

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E.coli	13	S	R	S	R	S	S	I
	14	I	S	S	S	R	I	S
	15	S	R	I	R	I	R	S
	16	S	S	I	S	R	I	I
	17	S	S	S	S	S	R	R
	18	S	R	S	R	I	R	S
	19	I	R	I	R	R	R	S
	20	S	I	S	S	S	S	I
	21	S	S	S	S	S	S	R
	22	S	S	S	R	R	S	I

Key: AK = 30 µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

Table 3.7. Antibiotic Susceptibility profile of *Klebsiella* spp. isolated from Household Refrigerator Samples

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		AK	CRO	CIP	AMC	AMP	FEP	CZ
Klebsiella spp.	1	S	I	S	I	R	R	R
	2	S	S	S	R	R	I	R
	3	S	S	S	R	R	R	R
	4	S	S	S	R	R	R	I
	5	S	S	S	S	R	R	I
	6	S	S	S	R	R	S	R
	7	S	R	S	R	R	R	I
	8	S	S	S	R	R	R	R
	9	S	S	S	I	R	S	S
	10	S	R	S	I	R	R	I
	11	S	S	S	S	R	I	I
	12	S	S	S	R	R	I	R
	13	S	R	S	S	R	S	S
	14	S	S	S	S	R	R	R
	15	S	S	S	R	R	R	R
	16	S	S	S	R	R	S	R
	17	S	S	S	R	R	R	R

Key: AK = 30 µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

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Table 3.8. Antibiotic Susceptibility profile of *Pantoea* spp. isolated from Household Refrigerator Samples

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		AK	CRO	CIP	AMC	AMP	FEP	CZ
Pantoea spp.	1	S	R	S	R	R	S	I
	2	S	I	I	R	R	R	R
	3	S	S	I	R	R	I	R
	4	S	R	I	R	R	S	R
	5	S	R	S	I	R	R	S

Key: AK = 30 µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

Table 3.9. Antibiotic Susceptibility profile of *Enterobacter* spp. isolated from Household Refrigerator Samples

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		AK	CRO	CIP	AMC	AMP	FEP	CZ
Enterobacter spp.	1	S	S	S	S	R	S	S
	2	S	S	S	R	R	S	R
	3	S	S	S	S	I	S	S
	4	S	S	S	R	R	S	R
	5	S	S	S	R	R	S	R
	6	S	S	S	R	R	R	R
	7	S	S	S	R	R	I	R
	8	S	R	S	R	R	I	R
	9	S	R	S	R	I	S	R
	10	S	S	S	S	R	S	I
	11	S	S	S	I	R	S	S
	12	S	S	S	R	R	S	R
	13	S	S	S	S	I	I	S
	14	S	S	S	R	I	R	I
	15	S	S	S	I	R	R	R
	16	S	S	S	R	R	S	R

Key: AK = 30 µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

Table 3.10. Antibiotic Susceptibility profile of *Shigella* spp. isolated from Household Refrigerator Samples

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		AK	CRO	CIP	AMC	AMP	FEP	CZ
	1	S	S	S	S	R	S	R
	2	S	R	I	R	R	S	S
	3	S	I	I	I	R	R	R
	4	S	S	S	S	I	R	R

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Shigella spp.	5	S	R	I	S	R	I	S
	6	S	S	S	I	I	R	R
	7	S	R	S	R	R	R	R
	8	S	I	S	S	R	S	S

Key: AK = 30 µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

Table 3.11. Antibiotic Susceptibility profile of *Citrobacter* spp. isolated from Household Refrigerator Samples

Bacterial isolate	Isolate number	Antibiotic Susceptibility Profile						
		AK	CRO	CIP	AMC	AMP	FEP	CZ
Citrobacter spp.	1	S	R	S	I	R	R	S
	2	S	R	S	R	R	S	R
	3	S	S	S	I	R	R	R
	4	S	I	S	R	R	R	S
	5	S	S	S	R	R	R	R

Key: AK = 30 µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

Table 3.12. Antibiotic Susceptibility profile of *Pseudomonas aeruginosa* isolated from Household Refrigerator Samples

Bacterial Isolate	Isolate number	Antibiotic Susceptibility Profile						
		TZP	CRO	CIP	AMC	AMP	FEP	CZ
Pseudomonas aeruginosa	1	I	R	S	R	R	S	S
	2	I	I	S	R	R	R	R
	3	S	R	S	I	R	S	S
	4	R	S	S	R	R	R	R
	5	S	S	S	R	R	S	S
	6	S	R	S	I	R	S	S
	7	S	R	S	I	R	R	I
	8	S	I	S	R	R	R	R
	9	I	R	S	I	R	S	R

Key: AK = 30µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime.
R = Resistance, S = Sensitive, I = intermediate

Table 3.13. Mean Antibiotic Susceptibility Profile of Isolated Bacteria from Household Refrigerator Samples

Bacterial isolates	Zone of inhibition (mm)												
	AK	CRO	CIP	AMC	AMP	FEP	CZ	P	LZD	VA	CN	SXT	TZP
S.aureus	NT	NT	S	R	NT	NT	NT	R	I	R	S	R	NT
E.coli	S	I	S	R	R	R	I	NT	NT	NT	NT	NT	NT
Klebsiella	S	S	S	R	R	R	S	NT	NT	NT	NT	NT	NT

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spp.													
Pantoea spp.	S	R	I	R	R	I	R	NT	NT	NT	NT	NT	NT
Enterobacter spp.	S	S	S	R	R	I	R	NT	NT	NT	NT	NT	NT
Shigella spp.	S	I	I	I	R	I	I	NT	NT	NT	NT	NT	NT
Citrobacter spp.	S	I	S	R	R	R	I	NT	NT	NT	NT	NT	NT
Pseudomonas aeruginosa	NT	R	S	R	R	I	I	NT	NT	NT	NT	NT	I

Key: AK = 30µg Amikacin, CRO = 30 µg Ceftriaxone, CIP = 5 µg Ciprofloxacin, AMC = 30 µg Amoxicillin/Clavulanic acid, AMP = 10 µg Ampicillin, FEP = 30 µg Cefepime, CZ = 30 µg Ceftazidime, P
= 10 µg Penicillin, LZD = 30 µg Linezolid, VA = 30 µg Vancomycin, CN = 10 µg Clindamycin, SXT =
23.75/1.25 µg Sulfamethaxazole/Trimethoprim, TZP = 100/10µg Piperacillin-Tazobactem.
R = Resistance, S = Sensitive, I = intermediate NT: Not Tested

Table 3.14. Percentage Resistant pattern of Gram Negative Microorganisms

Antibiotics	*E.coli*	*Klebsiela* spp.	*Pantoea* spp.	*Enterobacter* spp.	*Shigella* spp.	*Citrobacter* spp.	*Pseudo monas aerugin osa*
Amikacin	18.1%	0%	0%	0%	0%	0%	100%
Ceftriaxone	54.5%	23.5%	80%	12.5%	62.5%	60%	77.7%
Ciprofloxacin	18.1%	0%	60%	0%	37.5%	0%	0%
Amoxicillin/Clavulanic acid	63.6%	76.4%	100%	75%	50%	100%	100%
Ampicillin	77.2%	100%	100%	100%	100%	100%	100%
Cefepime	72.7%	76.4%	60%	37.5%	62.5%	80%	44.4%
Ceftazidime	54.5%	88.2%	80%	75%	62.5%	60%	55.5%
Piperacillin/Tazobactem	NT	NT	NT	NT	NT	NT	44.4%

NT: Not Tested

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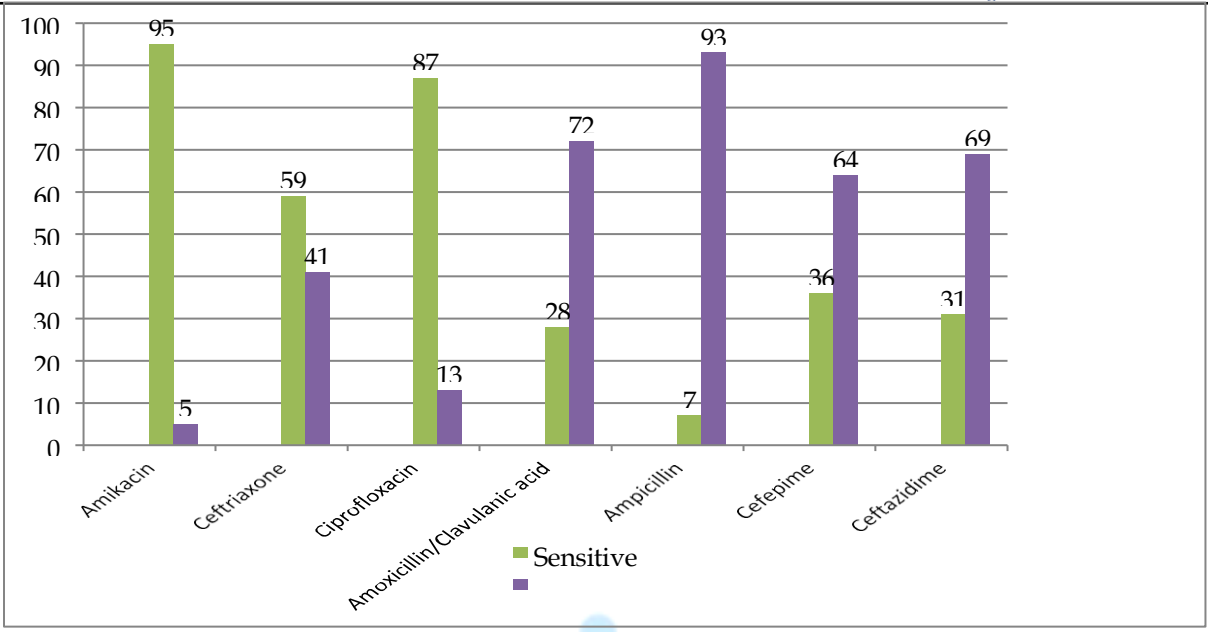

Figure 3.7. Overall Percentage Susceptibility Pattern of Gram Negative Microorganisms

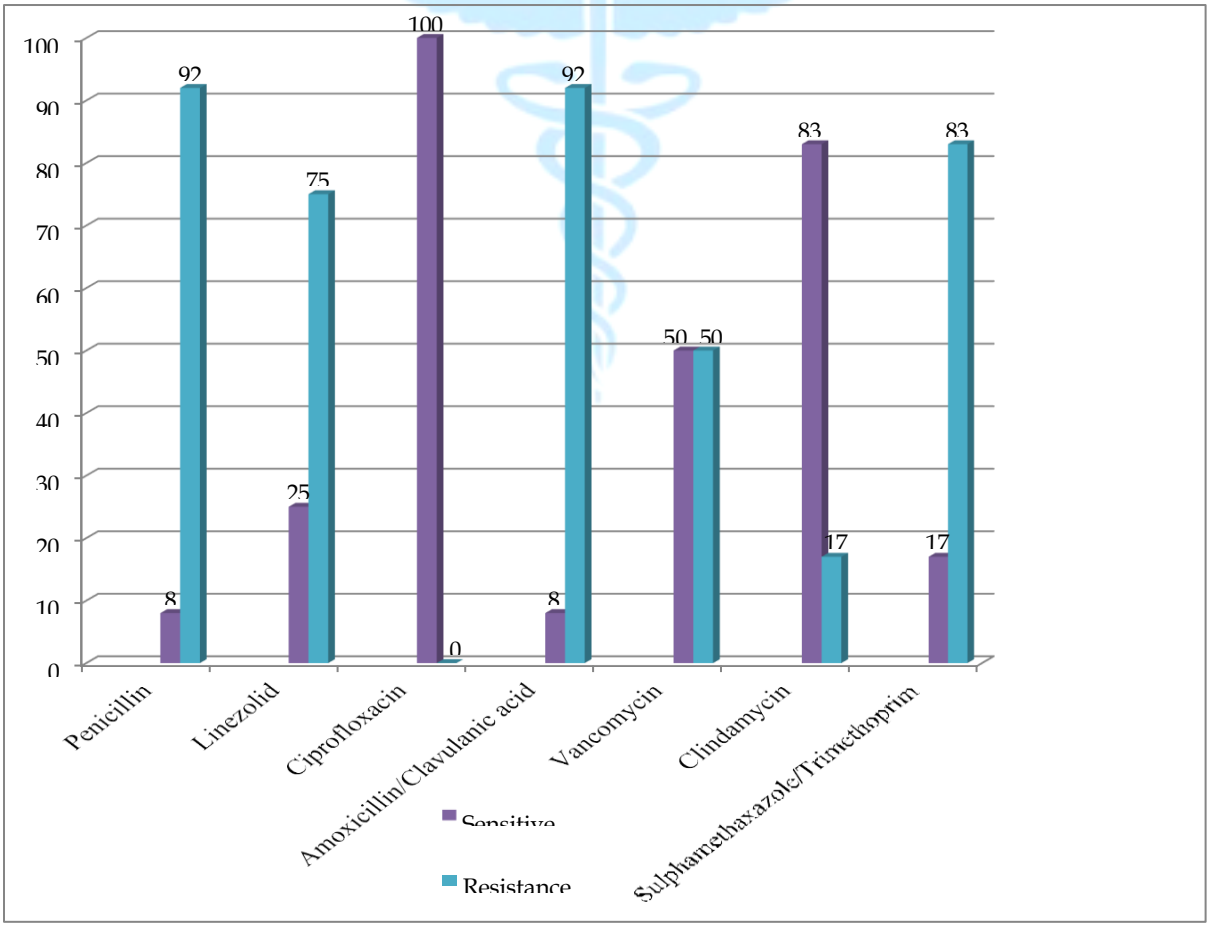

Figure 3.8. Percentage Susceptibility pattern of *Staphylococcus aureus*

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Discussion

According to the World Health Organization (WHO), foodborne illnesses affect millions of individuals globally each year, with substantial morbidity and mortality(Organization 2000). In the United States alone, foodborne diseases impact approximately 76 million people annually, leading to 325,000 hospitalizations and around 5,000 deaths(Almaary 2023). These alarming figures have driven national and international food safety programs to prioritize the prevention of foodborne illnesses. Notably reported that household refrigerators are more frequently associated with foodborne outbreaks than commercial refrigeration units(Jackson, Blair et al. 2007).

The findings of the present study highlight significant bacterial contamination levels in household refrigerator samples. Microbial loads varied across different refrigerator compartments, ranging from 7.3×10^2 to 1.5×10^7 CFU/mL. Similar studies have reported variable microbial burdens. For instance, Kennedy et al. (2005) documented total viable counts (TVC) of 7.1 log CFU/cm² and total coliform counts of 4.0 log CFU/cm² across 900 samples in Ireland. Altunatmaz et al. (2012) recorded mean airborne psychrotrophic bacterial and fungal loads of 82.3 CFU/m³ and 54.6 CFU/m³, respectively. In Italy, Catellani et al. (2014) reported a mean TVC of 1.2 log CFU/cm², while Bharathirajan et al. (2012) observed TVCs ranging from 3.8 to 9.7 $\log_{10}$ CFU/cm² in Saudi Arabian domestic refrigerators. Such variations can be attributed to differences in hygiene practices, temperature regulation, cleanliness of refrigerator interiors, and handling of perishable food items such as fruits, vegetables, and eggs(Aung and Chang 2014).

A total of 150 bacterial and 20 fungal isolates were identified in the current study. Among Gram-positive bacteria, *Bacillus* spp. were the most frequently isolated (17.3%), while *Escherichia coli* (14.7%) was predominant among Gram-negative bacteria. These findings align with previous studies by Kennedy et al. (2005), Catellani et al. (2014), and Bassey et al. (2017), who similarly identified these genera as dominant contaminants in domestic refrigerators. Regarding fungal contamination, *Penicillium* spp. were the most commonly isolated (45%), consistent with the findings of Altunatmaz et al. (2012). Together, these results emphasize that household refrigerators serve as reservoirs for microbial contamination, which can lead to gastrointestinal and other infections, posing significant public health risks. Routine cleaning and disinfection—ideally on a weekly basis—are essential, along with regular maintenance to ensure effective refrigeration and food preservation(Aste, Del Pero et al. 2017).

The study further revealed concerning patterns of antibiotic resistance among the bacterial isolates. *Staphylococcus aureus* exhibited high resistance to penicillin (92%), amoxicillin/clavulanic acid (92%), and sulfamethoxazole/trimethoprim (83%). In contrast, ciprofloxacin (92%), clindamycin (83%), and vancomycin (50%) were found to be the most effective agents. These findings are consistent with earlier research by Ilyas et al. (2016), who reported 83.3% resistance to amoxicillin/clavulanic acid and 75% to penicillin in *S. aureus*. Similar resistance trends were also noted by (Busani, Del Grosso et al. 2004).

Among Gram-negative isolates, the highest resistance was observed against ampicillin (93%), amoxicillin/clavulanic acid (72%), and ceftazidime (69%). These results corroborate findings from Trokenheim et al. (2006) and Nipa et al. (2011), who examined antimicrobial resistance in foodborne bacteria. The most effective antibiotics against Gram-negative organisms in this study were amikacin (95%) and ciprofloxacin (87%), supporting earlier observations by Ilyas et al. (2016).

The growing prevalence of antimicrobial resistance poses a major challenge in developing countries like Pakistan. The continuous and often unregulated use of antibiotics exerts selective pressure on bacterial populations, facilitating the emergence and persistence of resistant strains(Heuer and Smalla 2007). This alarming trend underscores the urgent need for regular surveillance of antimicrobial resistance phenotypes, particularly in household environments where food is stored and consumed(Samtiya, Matthews et al. 2022).

Conclusion

In conclusion, the current study demonstrates that household refrigerators can harbor a variety of potentially pathogenic bacteria and fungi, many of which show resistance to commonly used antibiotics. These findings stress the importance of

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ISSN: (e) 3007-1607 (p) 3007-1593

implementing proper hygiene practices, including thorough washing of produce and regular cleaning of refrigerator interiors. Additionally, public awareness regarding food safety, proper food storage, and the consequences of antimicrobial resistance must be increased to mitigate foodborne health risks(Odeyemi 2016).

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