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Microbiological Assessment of Used Kitchen Towels in some Refectories in a Tertiary Institution in Nigeria

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ABSTRACT

Microorganisms are ubiquitous in nature and can readily colonize surfaces and materials, creating a high risk of environmental transmission and infection. This study assessed microbial contamination of used kitchen towels from the Rivers State University campus refectories. Thirty (30) swab samples were collected from six refectories and transported to the Rivers State University Microbiological Laboratory for immediate analysis using aseptic techniques. Samples were cultured following standard microbiological procedures, and antibiotic susceptibility testing of both Gram-positive and Gram-negative bacteria was performed using the disc diffusion method. The heterotrophic bacteria isolated included *Bacillus* sp., *Shigella* sp., *Salmonella* sp., *Enterobacter* sp., *Escherichia coli*, and *Staphylococcus* sp., while fungi isolated included *Candida* sp., *Penicillium* sp., *Aspergillus flavus*, and *Aspergillus niger*. Biofilm formation was assessed for all bacterial isolates, and all were positive, indicating their ability to persist on surfaces and resist environmental stresses. Antibiotic susceptibility testing revealed high levels of resistance among the isolates. Among Gram-negative bacteria, *E. coli* was resistant to Augmentin (AU), Ciprofloxacin (CPX), Septrin (SXT), Streptomycin (S), Ampicillin (PN), and Nalidixic acid (NA). *Enterobacter* sp. was resistant to Reflacine (PEF), Augmentin (AU), Ciprofloxacin (CPX), Septrin (SXT), Streptomycin (S), Ampicillin (PN), and Nalidixic acid (NA). Both *Salmonella* sp. and *Shigella* sp. showed resistance to all tested antibiotics. Among Gram-positive bacteria, *Staphylococcus* sp. exhibited resistance to all antibiotics tested. The presence of pathogenic and opportunistic microorganisms on kitchen towels indicates that these materials can serve as vehicles for cross-contamination of food, fomites, and hands. It is therefore recommended that kitchen towels be regularly replaced, thoroughly washed, and properly disinfected before use to reduce the risk of microbial transmission and foodborne infections.

Keywords: Refectories, Used Hand Towel, Hygiene Practices, Public Health, Biofilm, Microbes, Antibiotics Resistance.

Introduction

Microorganisms are ubiquitous in nature and are present in virtually all environments inhabited by humans, including households, institutional settings, and refectories. Refectories are designated areas within schools where students gather to eat their meals. In food-handling environments such as refectories, the risk of microbial transmission is particularly high due to constant human contact, food preparation activities, and the repeated use of shared utensils and cleaning materials. These conditions create multiple opportunities for the spread of microorganisms that may compromise food safety and public health (Lopez-Galvez et al., 2020). Among the commonly overlooked items in food service environments are kitchen towels, which can serve as reservoirs and vectors for microbial contamination. Kitchen towels are frequently exposed to moisture, warmth, food residues, and organic debris during routine use. These conditions provide an ideal environment for the survival and proliferation of microorganisms, including bacteria and fungi (Scott & Bloomfield, 1990). When contaminated towels come into contact with hands, food, utensils, or food preparation surfaces, they facilitate cross-contamination and increase the risk of foodborne infections (Fijan et al., 2020).

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Although laundering with detergents and disinfectants can significantly reduce microbial load, the effectiveness of these practices depends on factors such as washing frequency, water temperature, type of detergent used, and post-washing handling and storage practices (Muthiani et al., 2010; Bloomfield et al., 2018).

In Nigeria and many other developing countries, kitchen towels are routinely used in refectories for multiple purposes, including wiping dishes and utensils, drying hands, and cleaning spills from food preparation surfaces. This multipurpose use often leaves towels damp and visibly soiled, creating favorable conditions for microbial growth. Studies have shown that damp kitchen textiles rapidly become colonized by diverse microbial populations, some of which are capable of causing foodborne illnesses (Anna & Ashley, 2009; Fijan & Šostar-Turk, 2019). As a result, contaminated kitchen towels represent a significant but often underestimated risk factor in food service environments. During routine use, skin cells and organic matter are deposited onto kitchen towels, providing nutrients that further support microbial multiplication (Scott & Bloomfield, 1990). The warm and humid conditions typical of refectories enhance microbial persistence and survival. Research has demonstrated that reusable kitchen cloths, including dishcloths and towels, can harbor high microbial loads within a short period of use (Bloomfield et al., 2011; López-Gálvez et al., 2020). Pathogenic microorganisms commonly associated with contaminated kitchen towels include *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus* spp., *Campylobacter* spp., and various fungal species (Arch Chemicals, 2012; Fijan et al., 2020). Most kitchen towels used in refectories across Africa are made from natural fibers such as cotton. Cotton textiles readily absorb moisture and organic matter, making them particularly susceptible to microbial colonization. Microorganisms generally thrive under warm, moist, and oxygen-rich conditions, with optimal growth occurring at temperatures between 25–37 °C and pH values ranging from 5–9 (Liam & Hudson, 2004; Fijan & Šostar-Turk, 2019). These conditions are commonly encountered in food preparation and service areas. Microbes isolated from contaminated towels have been associated with various infections, including gastrointestinal illnesses and skin conditions such as acne, ringworm, and dermatitis.

Poor hygiene practices, particularly inadequate hand hygiene among food handlers, further contribute to microbial contamination of kitchen towels. Contaminated hands play a major role in the transmission of microorganisms within food-handling environments (Curtis & Cairncross, 2003; Todd et al., 2010). The human skin harbors both resident and transient microbial flora, some of which are pathogenic. Transient microorganisms are easily transferred from hands to towels, utensils, and food-contact surfaces and include species such as *Staphylococcus aureus*, *Enterococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., and *Acinetobacter* spp. (Dorothy & Noble, 1974; Fijan et al., 2020). Hand hygiene is therefore recognized as a critical control measure in reducing the spread of pathogenic microorganisms in food service environments. Several studies have emphasized the importance of proper hand-washing practices, effective hand-washing agents, and hygiene compliance among food handlers (Smith, 2009; Erasmus et al., 2010; López-Galvez et al., 2020). Microbial transmission is known to occur more readily in moist environments than in dry conditions, making shared kitchen towels in refectories a significant public health concern.

Therefore, this study was designed to assess the microbiological quality of used kitchen towels in selected refectories at Rivers State University which is a tertiary institution in Nigeria by determining the presence, types, and level of microbial contamination, as well as evaluating their potential health risks to food handlers and consumers in these refectories**.**

Materials and Methods

Study Area and Location

This study was carried out on selected refectories within the campus of Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria. Rivers State University is located at Nkpolu-Oroworukwo in Port Harcourt, the capital of Rivers State, Nigeria, with the main campus situated within the geographical coordinates of approximately latitude 4°47′54″ N to 4°48′55″ N and longitude 6°58′57″ E to 6°59′23″ E, and is bordered by the Nigerian Agip Oil Company area to the west of the campus (Pepple et al., 2024).

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Sample Collection

Thirty (30) swab samples were collected from six different refectories within the campus of Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria. The refectories were designated as Refectories A, B, C, D, E, and F to ensure anonymity and clarity. Sterile swab sticks were used to swab the surfaces of kitchen towels that had been used in the refectories (Cheesbrough, 2010). From each refectory, five (5) used kitchen towels were swabbed during each visit, giving a total of thirty samples. Sampling was conducted during two separate visits, spaced one week apart, to account for variation in towel usage and hygiene within the refectories. Immediately after swabbing, the samples were transported under aseptic conditions to the Microbiology Laboratory, Rivers State University, for subsequent microbiological analysis.

Sterilization of Glassware and Materials

All glassware, including beakers, conical flasks, measuring cylinders, and test tubes, were thoroughly washed with detergent, rinsed with running tap water, followed by distilled water, and air-dried. The glassware was sterilized in a hot air oven at 160 °C for 1–2 hours. Culture media were sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. Work benches were disinfected with ethanol prior to analysis, and disposable gloves were worn and changed between procedures to ensure aseptic conditions (William & David, 2013).

Culture Media and Reagents

The media and reagents used in this study included Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), Eosin Methylene Blue Agar, Salmonella-Shigella Agar (SSA), Mannitol Salt Agar, Mueller-Hinton Agar, distilled water, ethanol, normal saline, lactophenol cotton blue stain, and Gram staining reagents.

Preparation of Culture Media

All media were prepared according to the manufacturers' instructions. Appropriate quantities of dehydrated media were weighed and dissolved in 1000 mL of distilled water in Erlenmeyer flasks, mixed thoroughly, heated to boiling, and sterilized by autoclaving at 121 °C for 15 minutes at 15 psi. Media were allowed to cool to approximately 45 °C before being aseptically poured into sterile Petri dishes and allowed to solidify. However, chloramphenicol (280 mg/L) was added to Sabouraud Dextrose Agar (SDA) to suppress bacterial growth, allowing selective isolation of fungi (Aleruchi & Obire, 2022). Mueller-Hinton Agar employed for antibiotic susceptibility testing was prepared according to the manufacturers' instructions, with the pH adjusted to 7.2. Plates were poured to a uniform depth of 4 mm using 90 mm Petri dishes and allowed to solidify on a level surface to ensure accurate antibiotic susceptibility testing.

Preparation of McFarland Turbidity Standard (0.5)

The 0.5 McFarland standard was prepared following CLSI (2017) guidelines. A 1% (v/v) sulphuric acid solution was prepared by adding 1 mL of concentrated sulphuric acid to 99 mL of distilled water. Separately, a 1% (w/v) barium chloride solution was prepared by dissolving 0.5 g of $BaCl_2 \cdot 2H_2O$ in 50 mL of distilled water. A volume of 0.6 mL of the barium chloride solution was added to 99.4 mL of the sulphuric acid solution, mixed thoroughly, and stored in a well-sealed container in the dark at room temperature (20–28 °C). The standard was agitated before use and compared visually with test inocula (Zapata & Ramirez-Arcos, 2015).

Preparation of Normal Saline

Normal saline was prepared by dissolving 8.9 g of sodium chloride in 1000 mL of distilled water. Aliquots of 9 mL were dispensed into test tubes, plugged with cotton wool, and sterilized by autoclaving at 121 °C for 15 minutes (Jin et al., 2024).

Microbiological Analysis

Isolation and Culturing of Microorganisms

Serial dilution was carried out by adding 1 mL of each kitchen towel sample to 9 mL of sterile normal saline and diluting serially from 10^{-1} to 10^{-6}. An aliquot of 0.1 mL from appropriate dilutions was spread-plated on Nutrient Agar (total heterotrophic bacterial count), Mannitol Salt Agar (Staphylococcus spp.), EMB Agar, SSA, and CLED Agar using a sterile bent glass rod. Plates were incubated aerobically at 37 °C for 24 hours (Cheesbrough, 2010).

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Identification and Characterization of Isolates

Distinct colonies were subcultured on Nutrient Agar and incubated at 37 °C for 24 hours. Identification was based on colony morphology, Gram staining (Norris & Swain, 1971), and biochemical tests including catalase, oxidase, motility, indole, methyl red, Voges-Proskauer, citrate utilization, and sugar fermentation tests (Nevena & Joy, 2014; Reiner, 2010; Shields & Cathcart, 2010).

Storage and Maintenance of Isolates

Pure isolates were preserved in 10% (v/v) glycerol as a cryoprotectant and stored in duplicate McCartney bottles at 4°C for further analysis (Baust et al., 2009).

Biofilm Formation Assay

Biofilm production was assessed using the Congo Red Agar (CRA) method (Rodney & Donlan, 2001). CRA was prepared using brain heart infusion agar supplemented with sucrose and Congo red dye. Plates were inoculated with test organisms and incubated at 37 °C for 24 hours. Black crystalline colonies indicated biofilm production (Freeman et al., 1989).

Antibiotic Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar according to CLSI (2017) guidelines. Bacterial suspensions were standardized to 0.5 McFarland turbidity, inoculated onto agar plates, and antibiotic discs were applied aseptically. Plates were incubated at 35 °C for 24 hours, and zones of inhibition were measured in millimeters.

Interpretation of AST Results

Zones of inhibition were measured from the underside of the plates using a meter rule. Results were interpreted as susceptible, intermediate, or resistant according to CLSI (2017) criteria.

Determination of Multiple Antibiotic Resistance Index

The Multiple Antibiotic Resistance (MAR) index was calculated using the formula:

MAR = a/b.

Where, *a* is the number of antibiotics to which an isolate showed resistance and *b* is the total number of antibiotics tested (Sandhu et al., 2016; Davis & Brown, 2016).

Results

The bacterial isolates recovered from used kitchen towels revealed the presence of *Shigella* spp., *Bacillus* spp., *Salmonella* spp., *Enterobacter* spp., *Escherichia coli*, and *Staphylococcus* spp. across the designated refectories. *Escherichia coli* was identified in Refectories A and C, while *Enterobacter* spp. occurred in Refectories A, B, and C. *Staphylococcus* spp. was present in all the refectories (A–F). *Bacillus* spp. was detected in Refectories B, C, E, and F, whereas *Shigella* spp. was found in Refectories A and B. *Salmonella* spp. was isolated only from Refectory B.

The fungal isolates recovered from used kitchen towels revealed the presence of *Candida* spp., *Penicillium* spp., *Aspergillus niger*, and *Aspergillus flavus* across the designated refectories. *Penicillium* spp. and *Candida* spp. were identified in Refectory A.

Refectory B harbored *Aspergillus flavus*, *Aspergillus niger*, and *Candida* spp., while Refectory C showed the presence of *Aspergillus flavus* and *Candida spp. Aspergillus niger* and *Penicillium* spp. were detected in Refectory D, whereas only *Candida* spp. was isolated from Refectory E. No fungal isolates were recovered from Refectory F.

The biofilm formation ability of the bacterial isolates is presented in Table 1 and varied among the organisms. Both *Escherichia coli* isolates were positive for biofilm formation. The three *Enterobacter* spp. isolates also demonstrated positive biofilm production, while the *Salmonella* spp. isolate was negative for biofilm formation. Similarly, the two *Shigella* spp. isolates were negative. Among the *Staphylococcus* spp., four isolates were positive for biofilm formation, whereas three isolates tested negative. In addition, all six *Bacillus* spp. isolates exhibited positive biofilm formation (Table 1).

Results of the antibiotic susceptibility profile for Gram -ve and Gram +ve bacteria isolates are presented in Tables 1 and 2 respectively.

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Table 1: Biofilm Formation by isolates

Bacteria Isolate	Biofilm Formation
E. coli	+ve
E. coli	+ve
Enterobacter sp	+ve
Enterobacter sp	+ve
Enterobacter sp	+ve
Salmonella sp	+ve
Shigella sp	-ve
Shigella sp	-ve
Staphylococcus sp	-ve
Staphylococcus sp	+ve
Staphylococcus sp	+ve
Staphylococcus sp	+ve
Staphylococcus sp	+ve
Staphylococcus sp	+ve
Bacillus sp	+ve
Bacillus sp	+ve
Bacillus sp	+ve
Bacillus sp	+ve
Bacillus sp	+ve
Bacillus sp	+ve

The results showed that most of the Gram-negative bacteria isolated from used kitchen towels were highly resistant to many of the antibiotics tested. All the isolates were resistant to Augmentin, Ciproflox, Septrin, Ampicillin, and Nalidixic acid. Reflacine and Streptomycin also showed high resistance among the isolates.

However, Gentamycin and Ceporex were more effective, with many of the bacteria showing susceptibility to these antibiotics. Tarivid was not very effective because most isolates were resistant, while a few showed intermediate responses. The results showed that the *Staphylococcus* species were resistant to most of the antibiotics tested, including Streptomycin, Rifampicin, Erythromycin, Gentamycin, Ciproflox, Norfloxacin, Chloramphenicol, Amoxil, and Ampiclox. In contrast, *Bacillus* species were susceptible to these antibiotics. Overall, 50% of the isolates were resistant while the other 50% were susceptible, indicating a mixed response among the Gram-positive bacteria. Levofloxacin showed a different pattern, with 50% of the isolates displaying intermediate reactions and 50% susceptible, while none were resistant.

The MAR index values for the Gram-negative bacteria and for Gram-negative bacteria are presented in Table 4 and Table 5 respectively. The MAR index values for the Gram-negative bacteria ranged from 0.6 to 1.0, indicating a high level of resistance among the isolates. *Shigella* sp. recorded the highest MAR index (1.0), showing resistance to all antibiotics tested, while *Salmonella* sp. (0.9) and *Enterobacter* sp. (0.8) also exhibited very high resistance levels. *Escherichia coli* showed a MAR index of 0.6, which still reflects significant exposure to antibiotics.

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Table 2: Antibiotic Susceptibility Profile for Gram -ve Isolates

Antibiotic	Gram -ve Bacteria Isolated from kitchen Towels				Total Antibiotic Reaction		
	E. coli (n=2)	*Enterobacter* sp (n=3)	*Salmonella* sp (n=1)	*Shigella* sp (n=2)	R (%)	I (%)	S (%)
Reflacine	S (2)	R (3)	R (1)	R (2)	6 (75)	0(0)	2 (25)
Gentamycin	S (2)	S (3)	R (1)	R (2)	3(37.5)	0(0)	5 (62.5)
Augmentin	R (2)	R (3)	R (1)	R (2)	8 (100)	0 (0)	0 (0)
Ciproflox	R (2)	R (3)	R (1)	R (2)	8 (100)	0 (0)	0 (0)
Septrin	R (2)	R (3)	R (1)	R (2)	8 (100)	0 (0)	0 (0)
Streptomycin	R (2)	R (3)	I (1)	R (2)	7 (87.5)	1 (12.5)	0 (0)
Ampicillin	R (2)	R (3)	R (1)	R (2)	8 (100)	0 (0)	0 (0)
Ceporex	S (2)	S (3)	R (1)	R (2)	3 (37.5)	0 (0)	5 (62.5)
Tarivid	I (1)	R (3)	R (1)	R (2)	6 (75)	2 (25)	0 (0)
Nalixidic acid	R (2)	R (3)	R (1)	R (2)	8 (100)	0 (0)	0 (0)

Key: R: Resistance, I: Intermediate, S: Susceptible.

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Table 3: Antibiotic Susceptibility Profile for Gram +ve Isolates

Antibiotic	Gram +ve Bacteria Isolated from kitchen Towels				Total Antibiotic Reaction		
	Staphylococcus sp	*Staphylococcus* sp	*Staphylococcus* sp	*Bacillus* sp	R (%)	I (%)	S (%)
Streptomycin	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Rifampicin	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Erthromycin	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Gentamycin	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Ciproflox	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Norfloxacin	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Levofloxacin	I (4)	I (1)	I (1)	S (6)	0 (0)	6 (50)	6 (50)
Chloramphenicol	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Amoxil	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)
Ampiclox	R (4)	R (1)	R (1)	S (6)	6 (50)	0	6 (50)

Key: R: Resistance, I: Intermediate, S: Susceptible

Table 4: Multiple Antibiotic Resistance of Gram Negative Bacteria Isolated from Kitchen Towels

Bacteria Isolate	Total Number of Antibiotics Tested (b)	Number of Antibiotics Resistant (a)	MAR Index
E. coli (n=2)	10	6	0.6
Enterobacter sp. (n=3)	10	8	0.8
Salmonella sp. (n=1)	10	9	0.9
Shigella sp. (n=2)	10	10	1.0

Table 5 showed that all *Staphylococcus* isolates had a value of 0.9, demonstrating a very high level of multidrug resistance. In contrast, *Bacillus* sp. recorded a MAR index of 0.0, indicating complete susceptibility to the antibiotics tested and suggesting minimal or no prior exposure to antimicrobial agents.

Table 5: Multiple Antibiotic Resistance of Gram Positive Bacteria Isolated from Kitchen Towels

Bacteria Isolate	Total Number of Antibiotics Tested (b)	Number of Antibiotics Resistant (a)	MAR Index
Staph sp.	10	9	0.9
Staph sp.	10	9	0.9
Staph sp.	10	9	0.9
Bacillus sp.	10	0	0.0

Discussion

This study evaluated the microbiological quality of thirty (30) used kitchen towels collected from refectories at Rivers State University, Nigeria, revealing that such textiles can harbor a wide array of potentially pathogenic microorganisms, including both bacteria and fungi, thereby posing a considerable risk for cross-contamination and foodborne infections.

The bacterial isolates, *Shigella* spp., *Bacillus* spp., *Salmonella* spp., *Enterobacter* spp., *Escherichia coli*, and *Staphylococcus* spp. are indicative of poor hygiene and possible fecal contamination. The detection of *E. coli*, a widely recognized indicator of fecal pollution, emphasizes the sanitation risks associated with reused kitchen towels. The presence of enteric pathogens such as *Salmonella* and *Shigella* further highlights the potential of these textiles to act as vectors for foodborne disease transmission, consistent with previous reports demonstrating that contaminated towels can facilitate microbial transfer to food and utensils (Gerba et al., 2014; Scott & Bloomfield, 2011; Maltick et al., 2012).

Fungal isolates, including *Candida* spp., *Penicillium* spp., *Aspergillus niger*, and *Aspergillus flavus*, were also recovered. The presence of *Aspergillus* species is of public health concern, given their ability to cause respiratory infections such as aspergillosis, particularly in immunocompromised individuals. These fungi thrive in damp conditions, emphasizing the role of moisture retention in towels as a driver of fungal colonization (Fijan & Šostar Turk, 2019).

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Assessment of biofilm formation revealed that the majority of bacterial isolates, including *E. coli*, *Enterobacter* spp., and *Bacillus* spp., were capable of forming biofilms. Biofilms enhance microbial persistence by increasing adherence to surfaces and providing protection against environmental stresses and disinfectants (Costerton et al., 1999; Flemming & Wingender, 2010). The high prevalence of biofilm-forming bacteria suggests that microorganisms present on kitchen towels may survive routine cleaning, thereby increasing their potential to contaminate food and utensils.

Antibiotic susceptibility testing demonstrated a high level of resistance among Gram-negative bacteria. While *E. coli* showed susceptibility to gentamicin and certain other antibiotics, *Shigella* spp. displayed resistance to all tested drugs. These findings reflect global trends in antimicrobial resistance among environmental enteric pathogens and suggest that these bacteria may have been frequently exposed to antibiotics or other selective pressures, (Krumperman, 1983; WHO, 2021; Sandhu et al., 2016). Gram-positive isolates showed variable resistance patterns: *Staphylococcus* spp. were resistant to several antibiotics, including erythromycin and amoxil, whereas *Bacillus* spp. remained fully susceptible.

Collectively, these findings demonstrate that used kitchen towels from institutional food service environments harbor significant populations of pathogenic, biofilm-forming, and antibiotic-resistant microorganisms. This highlights the potential for these textiles to serve as reservoirs for cross-contamination, posing a tangible public health risk. Effective mitigation strategies should include frequent laundering with hot water and disinfectants, strict hand hygiene among food handlers, and consideration of alternative disposable drying materials to reduce microbial transmission and prevent foodborne illnesses (Bloomfield et al., 2018; Todd et al., 2010).

Conclusion

This study demonstrates that used kitchen towels can harbor pathogenic and opportunistic microorganisms, providing conditions that promote microbial growth and facilitating cross-contamination of hands, surfaces, and food. The frequent dampness and repeated use of towels increase the risk of transmitting foodborne pathogens.

Regular washing with warm water, thorough air- or sun-drying, and frequent replacement of towels are essential to minimize microbial contamination. Implementing multiple towels for regular rotation further reduces contamination risks and enhances food safety in both domestic and institutional kitchen settings.

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