African Scientist Vol. 25, No. 1 March 31, 2024
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2024007/25104

Microbiological Analysis of Surfaces of Hospital Kitchen Equipment in Benin City, Nigeria

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(Received February 23, 2024; Accepted in revised form March 12, 2024)

ABSTRACT: Hospital kitchen equipment are the materials used in the kitchen in carrying out food preparation effectively in hospitals. This study aimed to analyse the surfaces of some hospital kitchen equipment for microbial quality. Samples were collected before and after food preparation from surfaces of tables, sinks, gas cookers and freezer handle of three major hospital kitchens in Benin City, Nigeria. The samples were transported to the laboratory and subjected to standard microbiological analysis. The total bacterial counts ranged from $5.00 \pm 0.33 \times 10^3$ - $4.40 \pm 0.26 \times 10^4$ cfu/cm^2 while the fungal counts ranged from $5.00 \pm 0.00 \times 10^3$ - $4.30 \pm 0.58 \times 10^4$ sfu/cm^2. Four (4) Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter* spp; and four (4) Gram-positive bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus luteus* were isolated. Fungi isolated were *Fusarium oxysporum, Aspergillus niger, Mucor mucedo, Saccharomyces* sp. and *Penicillium notatum. Staphylococcus aureus* was the most occurring bacterial isolate (28.6%) while the least occurring was *Enterobacter* spp (2.5%). *Mucor mucedo* was the most occurring fungal isolate (34.0%) and the least was *P. notatum* (11.3%). Therefore, proper cleaning of kitchen equipment and surfaces before and after food preparation should be observed.

Keywords: Hospital, Kitchen Equipment, Surfaces, Bacteria, Fungi.

Introduction

Hospital surfaces, including those in food preparation areas, are some of the major contributing factors to the spread of food-borne illnesses and hospital-acquired infections (Kir *et al*., 2006). In Nigeria, it has been reported that 48 million food-related illnesses occurred annually, with 128,000 people being admitted to hospital and 3,000 deaths occurring. In addition, evidence indicates that this is a worldwide problem affecting both developed and developing countries (CDC, 2011).

Food handlers play an important role especially in hospitals as they could be sources of contamination. They could also cross-contaminate food during its preparation and distribution within the hospital (Sala *et al*., 2005). Contamination from food handlers usually results due to inadequately washed hands, improper food preparation techniques as well as incorrect cleaning procedures of food preparation surfaces such as chopping boards and tables.

Although contaminated surfaces can serve as possible reservoirs for pathogenic microorganisms, studies have suggested that surfaces are not directly associated with transmission of hospital-acquired food-borne infections to patients and the source of transmission from kitchen surfaces to patients is mainly via hand contact of food handlers with the surface (Nkhebenyane, 2010).

In addition, Salo *et al*. (2000) reported that wet items such as dishcloths, hand towels and sponges, as well as sink drain areas with leaking pipes might also serve as continuous reservoirs that harbour potentially harmful microorganisms, which may end up settling on kitchen surfaces (Zhao *et al*., 1998). Improper food hygiene practices and unclean surfaces have been associated with opportunistic pathogenic

microorganisms such as *Staphylococcus aureus* (Andargie *et al*., 2008). The presence of *S. aureus* is often perturbing due to the possibility of antibiotic resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) (McCaughey, 2007).

The role of hospital kitchen areas in the transmission of healthcare-associated infections (HAIs) has long been recognized, however, the evidence that environmental surfaces play a role in the transmission of HAIs has been weak. Studies have demonstrated that pathogens can be transmitted from surfaces to personnel and patients, and that these pathogens are not adequately removed by routine room cleaning. This has led to an increased focus on the importance of cleaning and disinfecting hospital surfaces and equipment and efforts to assess and improve the effectiveness of these practices (Trail, 2007).

Residential kitchens have been reported to be heavily colonised by microbes originating from different sources, including kitchen equipment surfaces, cleaning utensils, food, and human contact (Møretrø *et al*., 2021; Moen *et al*., 2023). Few studies have reported the microbial contamination of hospital kitchen equipment. This study aimed to analyse the surfaces of some hospital kitchen equipment: tables, sinks, gas cookers and freezer handle of three major hospital kitchens in Benin City, Nigeria, for microbial quality.

Materials and methods

Sample collection: This study was carried out in three major hospitals in Benin City, Nigeria. Samples were collected from each kitchen from surfaces of tables, sinks, gas cookers and freezer handle before and after food preparation. The surfaces were swabbed, using sterile cotton swab sticks (pre-moistened in phosphate buffered saline) around 100cm^2 or the whole surface if the area was less than 100cm^2 as earlier described by Møretrø *et al*. (2021). All samples were placed in a cooling bag (at temperature of 4-6 ^{o}C) and transported to the laboratory for microbiological analysis.

Culture media: The media used in this study were nutrient agar (Oxoid) for purification and storage in slants, MacConkey agar (Oxoid) for isolation of Gram-negative bacteria, eosin-methylene blue (EMB) agar for selective isolation of *Escherichia coli*, blood agar and mannitol salt agar (MSA) for isolation of *Staphylococcus aureus*, and Sabouraud dextrose agar (SDA) for selection of fungi. The media were prepared according to the manufacturer's prescription and poured onto sterile petri plates.

Enumeration of bacteria and fungi: The method described by Holt *et al*. (2000) for estimating bacterial and fungal counts was used to enumerate the total viable counts of the isolates. The discrete colonies on the Nutrient agar and Sabouraud dextrose agar were selected and counted. The mean colony count on the nutrient agar and Sabouraud dextrose agar plates of each given dilution were used to estimate the total viable count for the samples in colony forming units per centimeter square (cfu/cm^2).

Isolation and identification of bacteria and fungi: A single colony of the bacteria was streaked on nutrient agar. The nutrient agar plates were incubated at 37 ^{o}C for 24 h. The isolated and purified bacterial strains were stored in slants at 4^{o}C. A pure colony on the surface of the petri dish was selected with the use of sterile wire loop and streaked onto MacConkey, EMB and MSA and SDA agar plates at 37 ^{o}C for 24 h. The colonial morphology of the colonies formed was noted and colonies were sub-cultured into nutrient agar plates and incubated at 37 ^{o}C for 24 h and stored for further examination. The isolated bacteria were identified using the Gram staining and biochemical techniques, while fungal isolates were identified using their morphological and colonial characteristics of each colony was identified according to the manual of Pitt *et al*. (1992).

Statistical analysis of data: The data obtained from this research were analysed using statistical package for social scientist (version 21), and Microsoft excel (version 2019). Values were expressed as mean ± standard deviation at 0.05 significance levels (Ogbeibu, 2015).

Results

Table 1 shows the total heterotrophic bacterial count (THBC) on hospital kitchen surfaces. Total heterotrophic bacterial count of sample obtained from Hospital A ranged from 7.00 ± 0.58 x 10^3 to 4.40 ± 0.26 x 10^4 cfu/cm^2, Hospital B ranged from 5.00 ± 0.33 x 10^3 to 4.00 ± 0.00 x 10^4 cfu/cm^2 and Hospital C ranged from 5.10 ± 1.00 x 10^3 to 3.70 ± 0.58 x 10^4 cfu/cm^2. Samples from Hospital A had the highest bacteria count (4.40 ± 0.26 x 10^4cfu/cm^2) while sample from Hospital B had the least bacteria counts of 5.00 ± 0.33 x 10^3cfu/cm^2 in the sink samples respectively.

Table 1: Heterotrophic bacterial count (THBC) on hospital equipment kitchen surfaces (cfu/cm^2)

Hospital	Equipment Sampled			
	Sink	**Gas cooker**	**Freezer handle**	**Table surface**
Hospital A	$4.40 \pm 0.26 \times 10^{4a}$	$7.00 \pm 0.58 \times 10^{3b}$	$4.00 \pm 0.58 \times 10^{4a}$	$2.70 \pm 0.33 \times 10^{4a}$
Hospital B	$5.00 \pm 0.33 \times 10^{3b}$	$6.70 \pm 0.33 \times 10^{3b}$	$4.00 \pm 0.00 \times 10^{4a}$	$2.40 \pm 0.33 \times 10^{4a}$
Hospital C	$6.00 \pm 0.00 \times 10^{3b}$	$3.70 \pm 0.58 \times 10^{4a}$	$2.30 \pm 0.58 \times 10^{4a}$	$5.10 \pm 1.00 \times 10^{3b}$

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript are not significantly different from each other (P>0.05). Mean values with different superscript are significantly different from each other (P<0.05).

The heterotrophic fungal count (THFC) on hospital kitchen surfaces is shown in Table 2. Total heterotrophic fungal count of sample obtained from Hospital A ranged from $5.90 \pm 0.10 \times 10^3$ to $4.00 \pm 0.00 \times 10^4$ sfu/cm^2, Hospital B samples ranged from $5.00 \pm 0.00 \times 10^3$ to $4.30 \pm 0.58 \times 10^4$ sfu/cm^2 while Hospital C samples ranged from $6.00 \pm 0.00 \times 10^3$ to $4.20 \pm 0.33 \times 10^4$ sfu/cm^2. Samples from Hospital B had the highest fungal count ($4.30 \pm 0.58 \times 10^4$ sfu/cm^2) in the sinks, while sample from Hospital B had the least bacteria count $5.00 \pm 0.00 \times 10^3$ sfu/cm^2 on the table surface.

Table 2: Heterotrophic fungal count on hospital kitchen equipment surfaces (sfu/cm^2)

	Equipment Sampled			
Hospital	**Sink**	**Gas cooker**	**Freezer handle**	**Table surface**
Hospital A	$4.00 \pm 0.00 \times 10^{4b}$	$5.90 \pm 0.10 \times 10^{3b}$	$2.70 \times \pm 0.33\ 10^{4a}$	$3.70 \pm 0.33 \times 10^{4a}$
Hospital B	$4.30 \pm 0.58 \times 10^{4a}$	$6.00 \pm 0.00 \times 10^{3b}$	$3.00 \pm 0.00 \times 10^{4a}$	$5.00 \pm 0.00 \times 10^{3b}$
Hospital C	$3.70 \pm 0.33 \times 10^{4a}$	$6.00 \pm 0.00 \times 10^{3b}$	$2.30 \times \pm 0.33\ 10^{4a}$	$4.20 \pm 0.33 \times 10^{4b}$

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript are not significantly different from each other (P>0.05). Mean values with different superscript are significantly different from each other (P<0.05).

The identified and percentage occurrence of bacterial isolates found in hospital kitchen surfaces are shown (Table 3). The bacterial isolates were *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Enterobacter* spp, *Bacillus cereus, Micrococcus luteus*, *Staphylococcus epidermidis* and *Staphylococcus aureus. Staphylococcus aureus* was the most occurring bacterial isolate (28.6%), followed by *B. cereus* (14.3%) while the least occurring bacterial isolate was *Enterobacter* spp (2.5%). Population of bacteria isolates showed that the highest number of bacteria were isolated from kitchen sink surfaces (28, 18 and 21) respectively from the various hospitals (Table 3).

Table 3: Identified and percentage occurrence of bacterial isolates found in hospital kitchen surfaces

Organisms	**Hospital A**				**Hospital B**				**Hospital C**				**Total (%)**
	SK	**GC**	**FH**	**TS**	**SK**	**GC**	**FH**	**TS**	**SK**	**GC**	**FH**	**TS**	
E. coli	6	2	2	0	3	1	1	1	2	0	0	1	19 (16.0)
P. aeruginosa	3	1	0	1	2	0	1	0	2	1	0	1	12 (10.1)
K. pneumoniae	2	1	1	1	2	1	0	0	3	0	0	0	11 (9.2)
Enterobacter spp.	1	0	0	0	1	0	0	0	1	0	0	0	3 (2.5)
B. cereus	5	2	0	1	3	1	1	1	2	1	0	0	17 (14.3)
M. luteus	2	0	0	0	1	0	0	0	3	1	0	0	7 (5.9)
S. epidermidis	4	0	2	0	2	1	1	2	3	1	0	0	16 (13.4)
S. aureus	5	3	3	1	4	2	0	1	5	4	4	2	34 (28.6)
Total	**28**	**9**	**8**	**4**	**18**	**6**	**4**	**5**	**21**	**18**	**4**	**4**	**119 (100)**

Key: SK = Sink, GC = Gas cooker, FH = Freezer handle, TS = Table surface

Table 4 showed the identified and percentage occurrence of the fungal isolates found in hospital kitchen surfaces. The fungal isolates were *Fusarium oxysporum, Aspergillus niger, Mucor mucedo, Saccharomyces* sp. and *Penicillium notatum. Mucor mucedo* was the most occurring fungal isolate (34.0%) followed by *Fusarium oxysporum* (24.5%), the least occurring fungal isolate was *Penicillium notatum* (11.3%) followed by *Aspergillus niger* and *Saccharomyces* sp. (15.1%). Population of fungal isolates showed that the sinks of the hospitals harboured more fungi than the other surfaces.

Table 4: Identified and percentage occurrence of fungal isolates found in hospital kitchen surfaces.

Organisms	Hospital A				Hospital B				Hospital C				Total (%)
	SK	GC	FH	TS	SK	GC	FH	TS	SK	GC	FH	TS	
Fusarium oxysporum	2	0	3	0	1	1	0	2	2	0	0	2	13(24.5)
Aspergillus niger	1	1	0	0	2	1	0	0	1	0	2	0	8(15.1)
Mucor mucedo	4	2	1	1	3	1	0	0	3	2	0	1	18(34.0)
Saccharomyces sp.	0	1	0	1	2	0	0	0	2	1	0	1	8(15.1)
Penicillium notatum	0	0	0	1	1	1	0	0	0	2	0	1	6(11.3)
Total	**7**	**4**	**4**	**3**	**9**	**4**	**0**	**2**	**8**	**5**	**2**	**5**	**53(100)**

Key: SK = Sink, GC = Gas cooker, FH = Freezer handle, TS = Table surface

Discussion

This study indicated that the surfaces of the kitchen equipment sampled were contaminated with bacteria and fungi. The total heterotrophic bacterial counts ranged from $5.00 \pm 0.33 \times 10^3$ to $4.40 \pm 0.26 \times 10^4$ cfu/cm^2. This result correlated with the works of Sinclair and Gerba (2011) that examined kitchen surfaces of rural Cambodian village households and had a geometric mean range of $5 – 16 \times 10^3$ cfu/cm^2. Also, Orogu *et al*. (2017) in their study had total bacterial count (TBC) cfu/ml of $1.8 \times 103 - 6.1 \times 10^3$ for spoons, $2.0 \times 10^3 - 5.4 \times 10^3$ for knives and $4.0 \times 10^3 - 7.7 \times 10^3$ cfu/ml for fork, on examining hostel kitchens in Delta State, Nigeria. More recently, Osaili *et al*. (2020) examined the quality of kitchen sponges in university hostel dormitories and had high counts of mesophilic aerobic bacteria (7.9 $\log_{10}$/cm^3), coliform (7.2 $\log_{10}$/cm^3), enterobacteriaceae (7.3 $\log_{10}$/cm^3) and yeasts and molds (7.0 $\log_{10}$/cm^3). AL-Aejroosh *et al*. (2021) also examined the microbial load of food surfaces, food trays and cooking utensils in domestic kitchens in Saudi Arabia and recorded heavy microbial loads.

Bacteria and fungi identified in this study were: *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter* spp, *Fusarium oxysporum, Aspergillus niger, Mucor mucedo, Saccharomyces* sp. and *Penicillium notatum.* This result is similar to the findings of Janjić *et al*. (2016) who isolated *E. coli, Citrobacter* spp, *Enterobacter* sp., *Klebsiella* sp., *P. aeruginosa, K. pneumoniae* and *B. subtilis* from kitchen surfaces such as (work surface, wooden board, plastic board, floor, and refrigerator. And the works of Moen *et al*. (2023), who isolated the enterobacteriaceae, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Bacillus* from kitchen sponges, cloths, sinks and cutting boards from five European countries. They concluded that kitchen surfaces can be contaminated by microorganisms through raw food items brought into the kitchen, through aerosol and contaminated water.

Prevalence of bacteria isolates revealed that bacteria population in sink surfaces were higher compared to table, freezer handle and gas cooker surfaces. From the result obtained, it is also likely that sinks are among the few surfaces in the kitchens where microorganisms are actively growing because moisture is more available than on other surfaces. This agrees with the studies of Sinclair and Gerba (2011) who reported that sink surfaces harboured more microorganisms because of the moisture content of sink surfaces.

Out of the 110 bacterial isolates, 44 were isolated from Hospital C kitchen surfaces, indicating that individual kitchens are not identical with respect to their design, usage patterns, surface materials, and environmental conditions (e.g. temperature, moisture, and ventilation rates) and any of these factors could influence the composition of bacterial communities found on kitchen surfaces (Gilberto *et al.*, 2016). The total viable counts of microbes in the kitchen surfaces reflect the sanitary or hygienic quality of the hospital kitchen. The high level of contamination of these pathogens could also be as a result of inadequate decontamination of the microbial load from the surfaces (Saka *et al*., 2017).

Staphylococcus aureus is known to be a permanent and ubiquitous colonizer of human skin, though not usually pathogenic but patients with compromised immune systems are often at risk for developing an infection. It is one of the most common causes of both community and hospital acquired infections (Ekhaise *et al*., 2008). Contamination with *Staphylococcus aureus* could occur either by human contact with surfaces or by contacts with contaminated kitchen tools.

Bacillus cereus isolates in this study have been implicated as promoting food borne disease and are health concerns as regards food safety globally (Clarence *et al*., 2009). *Bacillus* species are emetic toxins producers that cause serious illness and fatalities in human consequent to consuming food contaminated with their toxins (Uraih, 2004). Improper hand washing by the kitchen workers before handling of food may lead to contamination of food by *Bacillus* sp. The presence of *Bacillus cereus* is significant because it is associated with gastrointestinal tract infections (Beuchat and Ryu, 2007).

The presence of *E. coli* in this study may be due to closeness of some of these hospital kitchens to wastewater disposal channels and toilet. *Escherichia coli* has also been reported to be harmless and part of normal flora but

can cause serious food poisoning in their hosts and is occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005). Faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. The cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Uraih, 2004).

The presence of *P. aeruginosa* in this study is in agreement with the studies of Gilberto *et al*. (2016) who reported cases of *P. aeruginosa* from home kitchen equipment. Weber *et al*. (2010) also in their work have reported the role played by hospital surfaces in the transmission of emerging healthcare-associated pathogens. Crowded conditions within the kitchen, frequent transfer of kitchen materials from one unit to another may contribute to proliferation of microorganisms in kitchen surfaces. Microbial flora may contaminate surfaces of objects, devices and materials which subsequently contact susceptible body sites (Kramer *et al*., 2006).

The highest heterotrophic fungal count in this study was $4.3 \pm 0.58 \times 10^4$ sfu/cm^2. The high rate of occurrence and distribution of fungi from the hospital kitchen surfaces may be traced to the inadequate handling practices, the ubiquitous nature of these fungi and their ability to withstand and tolerate harsh environmental conditions such as low pH and low moisture content of the environment (Won-Shin *et al*., 2003). The total count produced a means of assuring the conditions obtained when food is prepared in this hospital kitchen environments, it is recognized that an improvement in the cleanliness of equipment reduces the level of contaminants found in kitchen surfaces (Byrne *et al.*, 2009).

The presence of the fungi: *Fusarium oxysporum*, *A. niger*, *M. mucedo*, *Saccharomyces* sp. and *P. notatum* in this study is in agreement with the studies of Adams *et al*. (2013) who reported that fungi such as *Fusarium* species, *C. albicans*, *A. niger*, *P. notatum*, *M. mucedo* and Yeast sp. were present in domestic kitchen surfaces. Darko *et al.* (2017) isolated *A. niger* and *F. oxysporum* from hostel kitchen surfaces. *Aspergillus* sp. is found in the skin flora and most environments according to Zahra *et al*. (2016).

The isolation of *P. notatum* from the kitchen surfaces is in agreement with studies of Addo *et al*. (2007) who reported *Penicillium* spp as one of the predominant microorganisms in school hostel kitchens surfaces in Accra. Dharmage *et al.* (1999) also investigated the prevalence and residential determinants of fungi within homes. They found *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* spp, *Mucor* sp, *Asperosporium* sp. and *Fusarium* spp to be the most predominant isolate.

Conclusion

The results of this study indicated that the hospital kitchen surfaces were contaminated by microorganisms. Proper sanitation of the kitchen facilities surfaces inclusive should be constantly carried out and maintained to reduce the carriage rate of pathogens of hospital kitchen. Also, proper cleaning of kitchen equipment and surfaces before and after food preparation should be strictly observed.

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