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Antibiotics and Antifungal Resistance Patterns of Microbial Isolates from Dish Washing Sponges in the University of Port-Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study examined the presence or contamination of bacteria in dishwashing sponges as well as the impact of various disinfectants on sponges. The total number of pathogenic organisms present in 120 sponges was determined using the nutritional agar (NA), MacConkey agar (MAC), Mannitol-Salt agar (MSA), Eosin-Methylene Blue agar (EMB), and Salmonella-Shigella agar (SSA) techniques. The efficacy of various disinfectants was evaluated using bleach, sanitizer, liquid soap, and boiled water for 30 minutes, while the remaining one served as a control sample. The result showed that household sponges had the lowest bacteria load across the five media with a mean bacteria count of 6.98 log CFU/g, followed by restaurant sponges with a mean count of 7.31 log CFU/g, and the highest bacteria load of 7.43 log CFU/g was obtained from hostel sponges. *E. coli* (40%), *Klebsiella* sp. (20%), *Shigella* sp. (15%), *Staphylococcus* sp. (20%), and Salmonella sp. (5) were the bacteria isolated and identified, whereas *Aspergillus niger* (65.6%) Penicillium oxalicum

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(9.4%), and *Candida albicans* (25%) were the fungi responsible for the contamination. Tarivid 25%, Reflacine 50%, Ciproflox 0%, Augmentin 50%, Gentamycin 0%, Streptomycin 0%, Ceporex 50%, Nalidixic acid 75%, Septrin 25%, and Amplicin 75% are the antibiotic resistant strains that have been identified. Griseofluvin eliminates both fungi at all concentrations that have been tested. Dishwashing sponges can be extremely contaminated, especially those used in the hostels on the University of Port Harcourt's Abuja campus. However, by applying basic and routine disinfection processes, the microbial contamination can be greatly reduced.

Keywords: Candida albicans; dishwashing sponges; disinfectants; Salmonella sp; Staphylococcus sp.

1. INTRODUCTION

Sponges are made of cellulose fibers and are used to clean surfaces [1] Sponge use in our homes includes washing dishes, cutlery, counters, and sinks. Cutting boards, sinks, oven tops, and refrigerators are just a few of the kitchen items and surfaces that kitchen sponges are used to clean. However, during cleaning, food residues could stick to the sponge's surface, and moist places like sink areas might serve as additional microbial reservoirs that contaminate the sponges as they are being used [2]. Kitchen sponges will continue to support microbial development at room temperature if they are later handled carelessly, improperly stored, or improperly disinfected. As a result of their ability to spread infectious agents, microbial agents that cause deterioration, and food-borne pathogens, kitchen sponges are significant sources of cross-contamination [2]. According to a study done in 10 kitchens in the United States of America, 33 and 67% of the sponges tested positive for fecal coliforms and Escherichia coli, respectively [3]. Contaminated sponges can transfer pathogens to surface that come in contact with food and these microorganisms can remain viable on these surface for hours or days after contamination [1].

The item in the home that has been found to be the most contaminated is a dishwashing sponge. This is a result of both its consistent moisture content and frequent interaction with food particles [4]. This has also been a significant challenge because it contributes significantly to cross-contamination. In other words, food pathogens can spread from sponges to people, increasing the risk of food-borne illnesses. Globally, food-borne infections have significantly increased in recent years. According to estimates, there are 38.6 million illnesses in the US each year, 13.8 million of which are transmitted by food [5]. Furthermore, 37.3% of food borne outbreak in EU in 2014, founded their infection sources in homes environment [6,7].

In addition to the negative effects that microbial illnesses have on people's health, well-being, and economies, the spread of antibiotic-resistant bacteria poses a substantial risk to people's lives in both developed and developing countries. Numerous studies of college students have revealed that they have poor hygiene habits and improper techniques for using sponges and other kitchen cleaning items [8]. The objective of this study was to evaluate the bacterial load in dishwashing sponges. Its precise goals include (i) determining the total bacterial pollutants on dishwashing sponges and isolating them; (ii) assessing the effectiveness of different cleaning agents on dishwashing sponges; and (iii) testing for antibiotic resistance.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 120 sponges were gathered for the investigation. They were acquired from the University of Port Harcourt's Abuja Campus. On the Abuja campus, 40 were collected from the student dormitories, 40 from cafeterias, and 40 from homes.

2.2 Culturing of Total Heterotrophic Organisms

To isolate certain organisms, the samples were streaked on selective media. They include nutritional agar for bacterial total counts, MacConkey agar for coliform isolation, eosin-methylene blue agar for E. coli isolation, monnitol salt agar for Gram-positive organism isolation, and Salmonella-shigella agar for the isolation of Salmonella and Shigella sp. After twenty-four (24) hours of incubation on each plate, the organisms were separated, purified, and kept at 4^0 C for characterization [9].

2.3 Culturing of Total Fungi

The total number of fungi was counted using potato dextrose agar that also contained 0.1 g/l

chloramphenicol to suppress bacterial contamination. According to Obi and Ndukwu (2016), the inoculation plates were incubated at 25°C for 7 days.

2.4 Identification and Characterization of Isolates

Bacteria were characterized using Gram staining, citrate testing, oxidase testing, sugar fermentation testing, triple-sugar infusion agar testing (TISA), motility testing, Methy Red testing, and Voges Proskauer testing, while fungi were characterized using lactophenol cotton blue staining [10] .

2.5 Sensitivity Test

The Clinical Laboratory Institute [11] advised utilizing the Kirby-Bauer disc diffusion technique to assess the sensitivity pattern of the pathogenic isolates. An inoculum of the suspected organism (0.5 McFarland standardized inoculum) was added to 0.9% normal saline using a sterile swab stick, and the swab stick was then emptied onto the test tube wall. On Mueller-Hinton plates, a consistent streak was created using the swab stick. The antibiotic sensitivity disk was removed from its preservation container using sterile forceps. The Muller-Hinton agar plate was impregnated with the antibiotic disc. After that, the plate was incubated for 18 hours at 37 °C. Using the Obi and Ndukwu, 2016, interpretation chart based on the inhibitory zone diameter of standard organisms was carried out. In order to determine whether an isolate was resistant, intermediate, or susceptible, the zone size of each antimicrobial agent was analyzed. Using a transparent 15-cm rule, the diameter of the zones of inhibition generated by each antibiotic dish was measured and recorded in millimeters.

2.6 Minimal Inhibition Concentration Test for Isolated Fungi

This experiment made use of the well-in-agar diffusion technique. The manufacturer's recommended method for preparing potato dextrose agar medium was followed. For 48 hours (*Candida albicans*) and 120 hours (*Aspergillus niger*), the fungal isolates were rehydrated on new medium and cultured at room temperature. A 0.5 McFarland solution of *Candida albicans* was diluted with sterile distilled water, seeded on the medium, and given time to acclimatize. A Bunsen flame was used to sterilize the cork borer before it was used to drill wells in the medium. The extracts were added to the wells at various dilutions. Spores from a sporulated colony were taken out and placed in a tube of sterile distilled water. They were then added to the molten medium and gently stirred to homogenize, then pour into sterilized Petri dishes and let the mixture set up. By soaking in ethanol and going through a Bunsen flame, cork borer was disinfected. It was then used to make wells in the medium, into which different dilutions of the extracts were added. For 5-7 days, all infected plates were incubated at room temperature. Afterward, inhibitory zones around the wells on the plates were looked for. For the susceptibility test and lowest inhibitory concentration, three different antifungals—griseofluvin, ketoconazole, and nystatine—were used at various concentrations (100%, 50%, 25%, and 12.5%).

2.7 Evaluation of Various Disinfectants

Five equal pieces of a two-week-old sponge were aseptically divided apart. One component was cleaned for 10 minutes with 200 ppm sodium hypochlorite, followed by a potable water rinse [12]. The second spent 30 minutes in hot water. The other two were added, followed by a potable water rinse, to 200 ppm sanitizer and liquid soap, respectively, the firth serve as a form of positive control, the first section. For each setup, an aliquot of 0.1 ml was plated on nutritional agar and incubated there for 24 hours at 37°C. CFU/g units were measured and counted for viable colonies.

3. RESULTS AND DISCUSION

The sponge samples from hostel had microbial load ranging from 6.34 to 7.44 log cfu/g, nutrient agar has 23%, MacConkey agar 20%, Mannitol salt agar 19%, Eosin-Methylene Blue agar 19%, Salmonella-Shigella agar 19%. Restaurants have 6.35 to 7.31 log cfu/g: nutrient agar has 22%, MacConkey agar 20%, Mannitol salt agar 19%, Eosin-Methylene blue agar 20%, Salmonella-Shigella agar 19%. While household samples have 6.19 to 6.98 log cfu/g nutrient agar has 22%, MacConkey agar 19%, Mannitol salt agar 20%, Eosin-Methylene Blue agar 20%, Salmonella-Shigella agar 20%. as shown Fig. 1. There is significant different between mean of isolated organisms from various media at P > 0.05. The result obtained is similar to those obtained by other researchers [9,11].

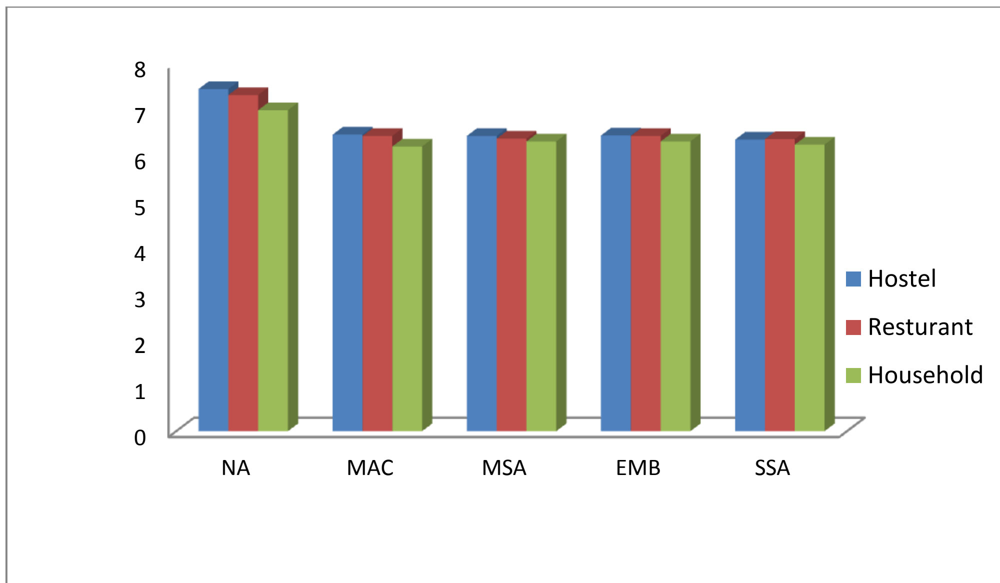

Fig. 1. Mean log cfu/g count of various media

Key: NA- Nutrient Agar; MAC- MacConkey Agar; MSA- Mannitol Salt Agar; EMB- Eosin-Methylene Blue Agar; SSA- Salmonella-Shigella Agar

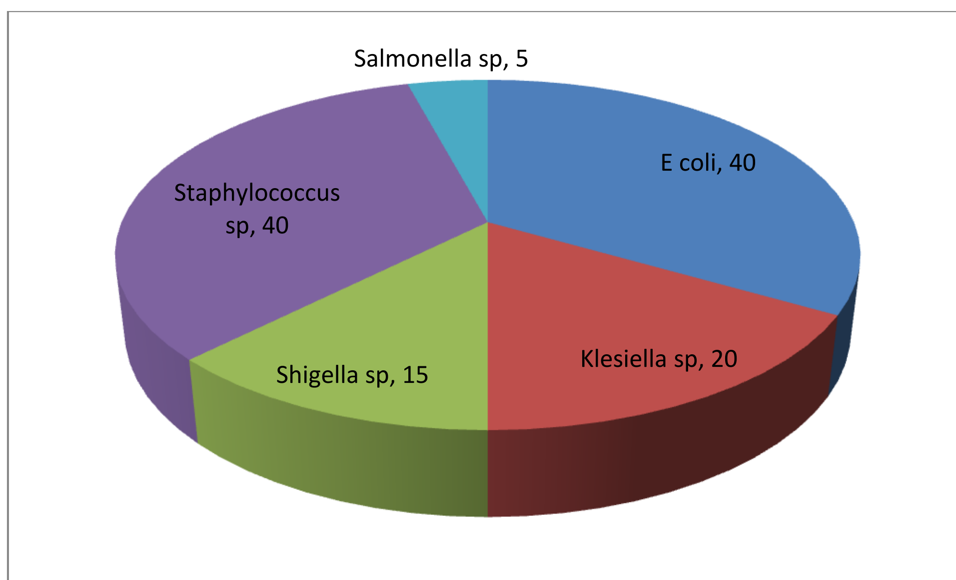

Fig. 2. Percentage (%) occurrence of bacterial isolates

Bacterial organisms isolated have percentage occurrence of *E. coli* > *Klebsiella* spp. > *Shigella* spp. > *Salmonella* spp . While the percentage of *Klebsiella* and *Staphylococcus* are the same as shown in Fig. 2. The result is in agreement with those of other researchers [5,12], showing higher microbial load of Coliforms and lower load of *Shigella* and *Salmonella* attributing to its' cell wall which is easily disintegrated by disinfectants and been a poor competitor to other pathogens although Shigella and Salmonella are of health concern being the main causative agent of foodborne diseases. The result is also similar to that obtained by [13] Kusumaningrum *et al* 2013 where *Listeria*, *Campylobacter* sp., *Bacillus* sp. *Staphylococcus aureus* and *Escherichia coli* were isolated from kitchen sponges. The presence of Escherichia coli indicate faecal contamination of sponge this could been via water source and/or hands of individuals; this microorganisms could be transferred to food, if not properly decontaminated, can lead to food borne disease and illnesses [13].

Table 1. Clonial characteristics of fungal isolates

Isolates	Colonial Appearance	Microscopy	Probable Organism	Frequency of occurrence	% of occurrence
S1	Dark-brown colony with radiating hyphae, Reverse; dark brown	Macroconidia;mature with septa, immature appears septate	*Aspergillus niger*	21	65.6
S2	Blue-greenish spherical shaped, rough raised center with white margin	Long hyphae with brush-like round conidiophores	*Penicillium oxalicum*	3	9.4
S3	White to cream-coloured smooth, glabrous, yeast-like with no secial reverse	Spherical to subspherical budding blastocondia, immature appears asptate	*Candida albicans*	8	25

Table 2. Sensitivity for gram's-negative and gram's-positive microbes

Microorganisms	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN	R%
Escherichia coli	30(S)	30(S)	16(I)	16(I)	28(S)	30(S)	16(I)	16(I)	26(S)	14 (I)	0
Klebsiella spp	28(S)	0(R)	30(S)	12(R)	28(S)	26(S)	0(R)	0(R)	30(S)	0 (R)	50
Salmonella spp	30(S)	30(S)	16(I)	14(I)	28(S)	14(I)	14(I)	12(R)	14(I)	12(R)	20
Shigella spp	0(R)	0(R)	15(I)	0(R)	14(I)	14(I)	0(R)	0(R)	0(R)	0 (R)	70
RESISTANCE (%)	25	50	0	50	0	0	50	75	25	75	
Gram Positive Organism	CPX	CN	S	N	AP	R	ERY	AMP	LEV	CPL	
Staphylococcus spp	28	30	30	14	28	30	30	30	30	16	0

Key: OFX = Tarivid, PEF = Reflacine, CPX = Ciproflox, AU = Augmentin, CN = Gentamycin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic acid, SXT = Septrin, PN = Amplicin N = Norfloxacin, Am = Amoxil, R= Rifampicin, Ery = Erythromycin, Amp = Ampilox, LEV = Levofloxacin, CHL = Chloramphenicol, R = Resistance (diameter ≤ 13), I = Intermediate (diameter ≥ 13 ≤ 20), S = Sensitive or Susceptible (diameter ≥ 20)

Table 3. Minimal inhibitory concentration

Candida albicans	Antifungal	100 %	50%	25 %	12.5 %
	Griseofluvin	-	-	-	-
	Ketoconazole	**24**	**20**	**16**	**11**
	Nystatine	**22**	**22**	**22**	**19**
Aspergillus niger	Griseofluvin	-	-	-	-
	Ketoconazole	**26**	**25**	**19**	**15**
	Nystatine	**27**	**24**	**21**	**18**

Table 4. Effects of different cleaning agents on dish-washing sponge

Disinfecting agent	Dilution factor	Viable count	CFU/g	Log CFU/g
Bleach	10^1	15	1.5×10^3	3.18
Boiled water	10^1	TFTC	TFTC	TFTC
Sanitizer	10^1	123	1.23×10^4	4.09
Liquid Soap	10^1	256	2.56×10^4	4.41
Control	10^1	TNTC	TNTC	TNTC

The percentage of occurrence of the isolated fungi as *Aspergillus niger* > *Candida albicans* > *Penicillium oxalicum* (Table 3). *Penicillium oxalicum* although they are not frequently mentioned in relation to penicilliosis, these fungal species have the potential to cause infections in human that could be fatal. *Penicillium chrysogenum* and *P. expansum* have been reported to be causative agents of necrotizing esophagitis, endophthalmitis, keratitis and asthma [14]. *Aspergillus niger* and *Candida albicans* are both pathogenic fungi causing Aspergilosis and Candidiasis respectively [15]. Most people breath in *Aspergillus* spore every day without getting sick but immune compromise individual or people with lung disease are affected [16]. *Candida* is known for causing infections especially in the gastrointestinal tract, most systemic infections are caused by *C. albicans*, hence may present a potential risk to public health in the event of their present in food which may take place through cross-contamination from sponges [17]. *Aspergillus* sp are the paramount ubiquitous fungi that contaminate various food substates and produce biochemicals known as mycotoxins and mycotoxins is known to exhibit wide range of toxicity to the humans even at nanomolar (nM) concentration. Bioaerosols consisting of spores and hyphale fragments of *Aspergillus* sp are active elicitor of bronchial irritation and allergy and challenging public health [18] hence the presence of *Aspergillus* species in the kitchen sponge is an indication of potential exposure to mycotoxins produced which of health concern especially as they could easily contaminate food prepared in the kitchen owing to their endospores [14]. Species that should cause the most concern are human opportunistic fungi, able to cause diseases in immunocompromised people, particularly species secreting extracellular polysaccharides (EPS). These species can form mixed bacterial-fungal biofilms and can thus adhere more easily to human hands (e.g. species from genera *Candida, Cryptococcus, Malassezia* and *Rhodotorula*). In our study *Candida* was isolated. Fungi spread by air or aerosols, such as representatives of the *genera Aspergillus, Cladosporium, Fusarium and Penicillium*, can pose a risk for people suffering from allergies or asthma [19]. *Aspergillus niger* was also isolated from dish-washing sponge samples from the research work.

Gram's negative isolated organisms have the following antibiotic sensitivity pattern: *Escherichia coli* been sensitive to all tested antibiotic (0 % R). *Klebsiella* spp. was resistance to 50% of the drug, *Salmonella* spp. shows 20% resistance while *Shigella* has a resistant pattern of 70%. Staphylococcus spp. Gram's positive organism has zero (0 %) resistance to all tested antibiotics. The antibiotic tested have resistance pattern of Tarivid 25%, Reflacine 50%, Ciproflox 0%, Augmentin 50%, Gentamycin 0%, Streptomycin 0%, Ceporex 50%, Nalidixic acid 75%, Septrin 25%, and Amplicin 75% for strains that was identified (Table 3). The antibiotics that the organisms are sensitive to could be administered in cases of foodborne diseases cause by the organisms.

The effectiveness of various disinfectant methods tested after two weeks shows that treatment with boiled water is the most effective having colony count that are too few to count with a percentage reduction of 80%. The other disinfectant agent tested recorded effectiveness of bleach > sanitizer > liquid soap (Table 4), this result is similar to that by other researcher [5,13]. Rossi et al., 2013 that worked on microbiological contamination and disinfection procedures of kichen sponges used in food service in Brazil recorded reduction of bacterial counts (99.9999%) for boiling method while (99.9%) for disinfection by 200 ppm sodium hypochlorine. Nicole., 2006 on testing which physical methods are most effective in decontaminating kitchen sponges recorded dishwasher been the most effective reducing bacterial counts by 57.3% then boiling 47.2% and washing machine 43.2%.

The Minimal inhibitory concentration shows *Candida albicans* and *Aspergillus niger* are both inhibited by Griseofluvin at all concentration tested. *Candida albicans* shows sensitive to ketoconazole at 100%, 50%, and 25% but resistance at 12.5% while sensitive to nystatine at all tested concentration. *Aspergillus niger* recorded sensitive to ketoconazole and nystatine at all tested concentration.

4. CONCLUSION

The level of hygiene practice of households, hostels, and cafeteria in the university was revealed, very poor hygiene practice as well as unhealthy lifestyles was evident in the bacterial load from the various sites. Kitchen sponges could be said to be a source of fungi contamination which are of public health concern and their presence could be traced to their humid condition and nutrient composition of the food particles in which it is used to clean. One way of

keeping bacteria and viruses from spreading through dish washing sponges is by keeping them clean and dry regularly as well as maintaining a healthy lifestyle. Dishwaching sponges were colonized by potentially pathogenic bacteria, which encoded for various antibiotic resistances. Students need to be reminded of good hygienic practices in order to reduce the risk of contaminating ready to eat food and dishwashing sponges should be disinfected by boiling and change regularly.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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