Antibiotics and Antifungal Resistance Patterns of Microbial isolates from Dish washing Sponges in the University of Port-Harcourt, Nigeria.

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 *Aspergillusniger* (65.6%)Penicilliumoxalicum (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: Dishwashing Sponges, Disinfectants, *Staphylococcus* sp., Salmonella sp, *Candida albicans*

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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

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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 25^0 °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 VogesProskauer testing, while fungi were characterized using lactophenol cotton blue staining [10] .

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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 Cheesbrough, 2004, 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 (*Aspergillusniger*), 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 tohomogenize, 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

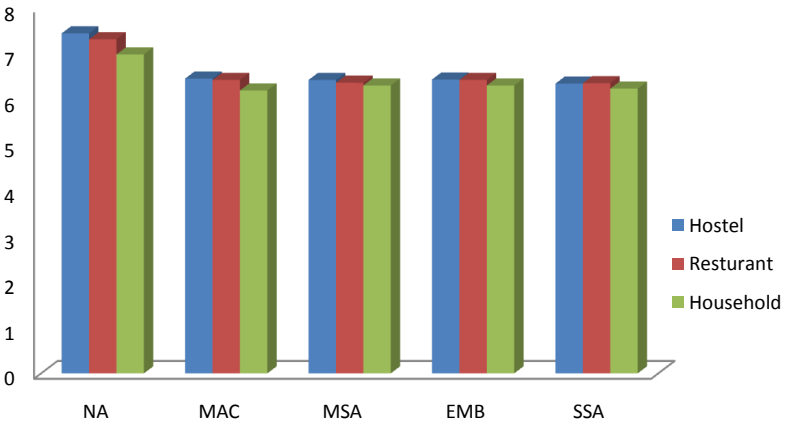

Fig. 1: Mean log cfu/g count of various media

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

Table 1. GRAM STAINING AND BIOCHEMICAL TEST

Medium	Collection site	Samples	Gram reaction	Gram shape	citrate	Oxidase	TISA				Glucose	Lactose	MR	VP	Motility	Suspected Bacteria
							Butt	Slant	Gas	H_2S						
Nutrient Agar	Hostel	A 2	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
		B 1	-	rod	+	-	A	A	+	-	+	+	-	+	-	*Klebsiella spp.*
	Restaurant	C 2	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
		D 1	-	rod	+	-	A	B	+	-	+	-	-	-	-	*Shigella spp.*
	Household	E 1	-	rod	-	-	A	A	+	+	+	+	+	-	+	*E.coli*
MacConkey Agar	Hostel	A 1	-	rod	+	-	A	A	-	-	+	+	-	+	-	*Klebsiella spp.*
		B 2	-	rod	+	-	A	A	+	-	+	+	-	+	-	*Klebsiella spp.*
	Restaurant	C 2	-	rod	+	-	A	A	+	-	+	+	-	+	-	*Klebsiella spp.*
		D 2	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
	Household	E 2	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
Mannitol-Salt Agar	Hostel	A 1	+	cocci	+	-	A	A	-	-	+	+	+	+	-	*Staphylococcus spp.*
		B 2	+	cocci	+	-	A	A	-	-	+	+	+	+	-	*Staphylococcus spp.*
	Restaurant	C 2	+	cocci	+	-	A	A	-	-	+	+	+	+	-	*Staphylococcus spp.*
	Household	E 1	+	cocci	+	-	A	A	-	-	+	+	+	+	-	*Staphylococcus spp.*
Eosin-Methylene Blue Agar	Hostel	A 2	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
	Restaurant	C 2	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
	Household	E 1	-	rod	-	-	A	A	+	-	+	+	+	-	+	*E.coli*
Salmonella-Shigella Agar	Hostel	B 1	-	rod	+	-	A	B	+	-	+	-	-	-	-	*Shigella spp.*
	Restaurant	D 1	-	rod	+	-	A	B	-	+	+	-	-	-	+	*Salmonella spp.*
	Household	E 2	-	rod	-	-	A	B	+	-	+	-	-	-	-	*Shigella spp.*

Table 2. Clonial Characteristics of Fungal Isolates

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Isolates	Colonial Appearance	Microscopy	Probable Organism	Frequency of occurrence	% of occurence
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	*Penicilliumoxalicum*	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 3. SENSITIVITY FOR GRAM-NEGATIVE AND GRAM-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 4. Minimal Inhibitory concentration

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

Table 5. EFFECTS OF DIFFERENT CLEANING AGENTS ON DISH WASHING SPONGES

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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 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 figure 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][13].

Bacterial organisms isolated have percentage occurrence of *E. coli* 40% >*Klebsiella* spp. 20% >*Shigella* spp. 15% >*Salmonella*spp 5%.While the percentage of *Klebsiella* and *Staphylococcus* are the same (20 %) as shown in table 2. The result is in agreement with those of other researchers [5][14], 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 [15] Kusumaningrum*et al* 2010 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 (Kusumaningrum*et* al 2010)

The percentage of occurance of the isolated fungi is 65.6% for *Aspergillusniger, 9.4% for Penicilliumoxalicum*and 25% for *Candida albicans*(Table 3).*Aspergillusniger*and *Candida albicans*are both pathogenic fungi causing Aspergilosis and Candidiasis respectively [16]. Most people breath in *Aspergillus*spore every day withoutgetting sick but immune compromise individual or people with lung disease are affected [17].

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 Amplicin75% for strains that was identified (Table 4).

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 5), 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 *Aspergillusniger* 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. *Aspergillusniger* 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. 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.

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Comment [MB7]: This title should not be separated from the content, move to the next page. There are 7 from 17 references not up to date (more than 10 years)

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