British Microbiology Research Journal
11(6): 1-9, 2016, Article no.BMRJ.19952
ISSN: 2231-0886, NLM ID: 101608140

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Microbiological Examination of Household Kitchen Sponges from Three Communities in Ikwuano L. G. A, Umuahia, Abia State Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author CNO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Authors CNO and CCN managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/19952
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.
Reviewers:
(1) Moustafa El-Shenawy, National Research Center, Cairo, Egypt.
(2) Ilham Zahir, University Sidi Mohamed Ben Abdellah, Morocco.
(3) Lidija Kozacinski, University of Zagreb, Croatia.
(4) Anonymous, Middle Tennessee State University, USA.
Complete Peer review History: http://sciencedomain.org/review-history/12457

Original Research Article

Received 4th July 2015
Accepted 10th November 2015
Published 27th November 2015

ABSTRACT

Sixty kitchen sponges collected from the households in three major communities in Ikwuano L.G.A, Umuariaga, Amawom and Amaoba were examined microbiologically using standard methods. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Aspergillus niger* were recovered from the sponges. The Total Heterotrophic Plate Count (THPC) for Umuariaga community had a mean value of 8.02 x 10^8 cfu/ml while Amaoba had 9.13 x 10^8 cfu/ml as mean value. Amawom had a mean value of 8.47 x 10^8 cfu/ml. The Total Coliform plate count (TCPC) mean values for the three communities are 6.11 x 10^7 cfu/ml, 6.70 x 10^7 cfu/ml and 6.28 x 10^7 cfu/ml respectively. Antibiotics susceptibility pattern of the isolates showed that *Staphylococcus aureus* was most sensitive to Ofloxacin (21.4 mm) but resistant to Gentamycin and Nalidixic acid while *Escherichia coli* was most sensitive to Amoxicillin (22.0 mm). *Pseudomonas aeruginosa* showed the highest sensitivity to Ofloxacin (20.6 mm) and incidentally the highest resistance (to

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five antibiotics). Based on the results, it was evident that kitchen sponges can be contaminated by pathogenic microorganisms which showed varied levels of susceptibility to antibiotics. Regular change of kitchen sponges and the use of washing disinfectants can reduce the level of contamination of the kitchen sponges and the associated infections.

Keywords: Bacteria; coliform; contamination; fungi; infections; kitchen sponges.

1. INTRODUCTION

It is known that during the cleaning process of equipments utensils, sinks in the kitchen, the pre-washing and washing steps are done with the use of sponges to eliminate food residues. As a consequence of these procedures, part of the residues adheres to the kitchen sponges surfaces. These food residues together with the moisture retained in the sponges offer a favourable environment for bacterial growth, and this may lead to the formation of biofilms [1].

The kitchen sponges are made of materials such as fiber and cellulose [2]. This aids in harbouring microorganisms that can cause serious infections thereby posing a constant risk of transferring contaminants. Kitchen sponges have been recognized as important in disseminating pathogens and they cause cross contamination in food leading to foodborne diseases. These sponges can serve as a reservoir of foodborne pathogens. Contaminated sponges can transfer pathogens to surfaces that come in contact with foods, and these microorganisms can remain viable on these surfaces for hours or days after contamination [3].

There has been a significant increase in foodborne illnesses worldwide. The microbial causes of foodborne illnesses include bacteria such as *Escherichia coli, Staphylococcus aureus,* Enterobacteriaceae*, Salmonella* spp*,* Parasites such as *Entamoeba histolytica, Trichinella spiralis* and some viruses with symptoms ranging from mild gastroenteritis to life threatening neurologic, hepatic and renal diseases. Diarrhoeal diseases alone kill a considerable proportion of 2.2 million people globally [4]. These foodborne diseases can result from cross contamination from the household kitchen to other materials that come in contact with food such as kitchen sponges.

Many household kitchen sponges are kept at room temperature, inside containers with water and food residues which can contribute to microorganisms' multiplication. Effective hygienic procedures are important in the prevention of cross contamination, bacterial growth and survival in the kitchen sponges. Disinfection of sponges may prevent the survival and spread of pathogens in the kitchen. As life styles change, individuals spend more time on the kitchen preparing meals and subsequently give less attention to proper food handling and sanitation practices that can reduce foodborne diseases and spoilage of food [5]. Simple fast and effective methods to disinfect kitchen sponges may prevent the spread of pathogenic microorganisms in household kitchens and may lead to better food preservation and fewer cases of foodborne illnesses [6]. To this end, this research work which was carried out in 2015 aimed at microbiological examination of sampled kitchen sponges in three communities of Ikwuano L. G. A. and determining the susceptibility patterns of the isolates.

2. MATERIALS AND METHODS

2.1 Study Area

The kitchen sponges in this research work were collected from three different communities in Ikwuano Local Government Area of Abia state namely: Umuariaga, Amawom and Amaoba. The major population of this area is dominated by peasant farmers and low income workers.

2.2 Sample Collection

Random households were selected in each community and kitchen sponges were collected by going door to door requesting for one's used sponges from the kitchen. The sponges sampled were in use for at least 2 weeks and this information was confirmed by the person who provided the sponge. The sponges were collected with latex gloves and placed in sterile plastic bags with appropriate labels being stored in a cooler containing ice pack at a temperature of 5℃. The sponge was transported to the laboratory 4 hours of collection and was analyzed within 24 hours. A total of 60 sponges were collected. The method of sample collection

described by Lancette and Tatini was used [7] was used.

2.3 Sampling Processing and Analyses

Each kitchen sponge was weighed and 75-100ml of peptone broth was added to the sponges placed inside a sterile 500 ml beaker to guarantee full soaking of the sponge. The sponge was manually kneaded in the peptone broth for five minutes until the broth was completely absorbed by the sponge. The broth was extracted from the sponge by wringing the liquid out aseptically [8]. 1ml of the extracted broth was serially diluted in peptone water and 0.1ml aliquot of appropriate dilution was inoculated onto Nutrient, MacConkey and Sabourand Dextrose agar respectively. The Nutrient Agar plates (Titain Biotech, limited) were used to determine the Total Heterotrophic Plate Count (THPC) while the MacConkey agar plates (Titain Biotech, limited) were used to determine the Total Coliform Plate Count (TCPC). Sabourand Dextrose agar (Titain Biotech, limited) containing 0.1 g/l chloramphenicol to inhibit bacterial contaminants was used for determining the Total fungal count. The inoculated petri dishes for the isolation of bacteria were incubated at 37℃ for 24 hours while the SDA was incubated at 25℃ and incubated for 7 days for the isolation of fungi. The plates were incubated prepared in duplicates while uninoculated plates were kept as control. After incubation, plates containing 30-300 colonies were chosen for enumeration. The results were expressed in cfu/ml [8].

2.4 Identification and Characterization of Isolates

Cultural and microscopic examinations were carried out. Colonies were selected using their morphological characteristics (sizes, pigmentation, elevation, consistency). Gram staining, lactophenol cotton blue staining, biochemical and sugar fermentation tests were performed [9].

2.4.1 Lactophenol cotton blue staining

A drop of 70% alcohol was placed on a microscope slide and the fungal isolate was immersed on the drop of alcohol. Two drops of the lactophenol cotton blue mountant was added before the alcohol dried out and covered with a cover slip while avoiding air bubble and then viewed under the microscope [10].

2.5 Antibiotic Sensitivity Testing

The sensitivity pattern of the pathogenic isolates was determined using the Kirby-Bauer disc diffusion technique [9]. The 24 hour broth culture of the isolates was matched to the turbidity of 0.5 McFarland standards in 0.9% saline and then streaked uniformly on Mueller-Hinton agar plates using swab sticks and allowed to stay for 15minutes. Antibiotic discs were aseptically placed at reasonable equidistance on the inoculated Mueller-Hinton agar plates using sterile forceps and then incubated at 37℃ for 18 hours. The diameter of the zones of inhibition produced by each antibiotic disc was measured using transparent 15 cm rule and recorded in Millimeter.

3. RESULTS AND DISCUSSION

The microbial isolates recovered from the 60 analyzed kitchen sponges were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Aspergillus niger* (Tables 1 and 2). The distribution of the microbial groups shows that *Escherichia coli* had 100% occurrence in all the analysed kitchen sponges followed by *Pseudomonas aeruginosa, Aspergillus niger* and *Staphylococcus aureus* with 43.3%, 17.3% and 13.3% occurrences respectively (Table 3).

The presence of *E. coli* is an indication of faecal contamination. This could present a danger to health, especially for populations at risk including the very young, the elderly and immune-compromised. *E. coli* is responsible for infant diarrhoea and gastroenteritis and its presence in all analyzed kitchen sponges could be due to contaminated water. This would promote cross contamination to the kitchen sponges and the cooking utensils washed with the contaminated water. Mattick et al. [11] isolated 54.1% of *E. coli* in kitchen sponges used in the United States and this is in agreement with our findings here.

Pseudomonas aeruginosa has a percentage occurrence of 43.3%. This bacterium is an opportunistic pathogen responsible for many hospital acquired (nosocomial infections). It thrives in many atmospheric conditions and colonizes natural and artificial environments and thus being able to colonize kitchen environments. Several studies have shown that dish cloths, sponges and towels used daily in the kitchen

contribute to the transmission of pathogenic microorganisms [12]. Relationship between bacterial contamination of countertops and cleaning cloths had been studied [13]. When used for cleaning in a professional caterer's kitchen during working hours, detergent-soaked cloths were heavily contaminated within a few hours. After cleaning of the premises and equipment, their level of contamination and the contamination of the cleaned surfaces were found to be even higher, which indicates the transfer of bacteria from the cloths to the surfaces and vice versa. A study on cleaning cloth contamination confirmed that while cloths could play an important role in the transfer of bacteria to surfaces, there was no significant difference between the microbial load of wet cloths, dry cloths, those used for short and prolonged periods and cloths used for different activities [14].

Kitchen sponges are used through direct contact with hands during washing, therefore, presence of *S. aureus* on the sponges could be due to transfer from human hands were this pathogen lives naturally [15]. However, only 13.3% of sponges had *S. aureus* in them and this could be due to the antimicrobial effect of the washing soaps and detergents used by the homes. *S. aureus* causes staphylococcal food poisoning and may also be present in foods such as meat, poultry and egg products. Gram positive bacteria such as *S. aureus* have a cell wall which is more sensitive to anionic surfactant present in some detergents which can contribute to the inactivation of these microorganisms. Other facts that can explain the low percentage of *S. aureus* in sponges is could be due to high water activity in the sponges and inter-microbial competition in the sponges [11]. Lower results have been found by [16] who isolated 2.7% of *S. aureus* in kitchen sponges used in Netherlands.

Sponges are used frequently to clean surfaces. [17], in a survey of French consumers showed that 89% of respondents said they used a sponge to clean their refrigerators. Due to their large surface/volume ratio, their almost constant humidity and the nutrients for bacterial growth they can contain, sponges are an ideal habitat for bacteria [18]. Chaidez and Gerba [16] studied the microbial quality of sponges used in domestic kitchens in Mexico. They showed that 9.8% of them were contaminated with Salmonella and that 60% of cellulose sponges and 86% of natural sponges were contaminated with *S. aureus*. In contrast, in a North American study published the same year, only 4% of the sponges were contaminated by *S. aureus* and none by Salmonella or Campylobacter [14].

Table 1. Identified Isolates

Colonial morphology	Microscopy	Gram stain	Motility	Catalase	Oxidase	Indole	Coagulase	Lactose	Mannitol	Glucose	Isolate
Smooth large, circular and creamy colonies with outlined edges.	Cocci in clusters	+	-	+	-	-	+	A	A	AG	*Staphylococcus aureus*
Smooth colonies, shiny with grey tint in nutrient agar.	Small scattered rods	-	+	+	+	-	-	-	-	-	*Pseudomonas aeruginosa*
Circular, smooth with entire margin mucoid and translucent colonies on nutrient agar.	Short rods single and separate	-	+	+	-	+	-	AG	A^{o}	AG	*Escherichia coli*

+ = positive, - = negative, AG = Acid and Gas production; A = Acid production only; Ao = Slight gas and acid production

The only fungal species isolated in the analyzed sponges was *Aspergillus niger* with 17.3% occurrence (Tables 2 and 3). This pathogen is present in some type of foods especially grains and cereals where it produces some toxins which are life threatening. Damp environment favours its growth and this could be one of the reasons for its survival in kitchen sponges. *A. niger* causes some types of infections in humans such as aflatoxicosis.

The bacterial load of the kitchen sponges was high and varied between the different locations. From Tables 4, 5 and 6, the sponges had THPC mean value of 8.02×10^8cfu/ml; 9.13×10^8 cfu/ml and 8.47×10^8 cfu/ml for Umuariaga, Amaoba and Amawom respectively. This study shows that sponges can be contaminated with a diverse group of organisms, supporting the results found by other researchers. For example, heterotrophic microorganism contamination was also found by [3] who observed approximately 6 log cfu/ml of hetetrophic microorganism in kitchen sponges used in Netherlands. [19] found 6.9 log cfu/ml of heterotrophic microorganisms in kitchen sponges used in Turkey.

Total coliform plate count (TCPC) from the kitchen sponges ranges from 3.60×10^7 - 9.7×10^7cfu/ml with a mean of 6.11×10^7 cfu/ml; 3.59×10^7 - 1.06×10^8 cfu/ml with a mean of 6.70×10^7 and 3.55×10^7 - 1.01×10^8 cfu/ml with a mean of 6.28×10^7 cfu/ml for the three locations namely Umuariaga, Amaoba and Amawom respectively

Table 2. Morphological identification of fungal isolate

Macroscopic characteristics	Microscopic characteristics	Probable isolate
White colonies later turns black, reverse side is brown.	Septate hyphae, unbranched of variable length double sterigmata covers the vesicle and forms a radiate head	*Aspergillus niger*

Table 3. Percentage occurrence of bacteria and fungus in the kitchen sponges

Isolates	No. of sponges examined	No. of isolates present	% occurrence
Escherichia coli	60	60	100
Staphylococcus aureus	60	8	13.3
Pseudomonas aeruginosa	60	26	43.3
Aspergillus niger	60	9	15.0

Table 4. Total viable counts from kitchen sponges collected from Umuariaga (cfu/ml)

Sample code	THPC	TCPC
1	7.70×10^8	6.15×10^7
2	6.37×10^8	7.65×10^7
3	8.20×10^8	3.60×10^7
4	6.44×10^8	5.45×10^7
5	7.50×10^8	4.75×10^7
6	7.33×10^8	7.89×10^7
7	4.00×10^8	4.40×10^7
8	8.73×10^8	6.30×10^7
9	7.55×10^8	6.60×10^7
10	6.10×10^8	5.35×10^7
11	5.71×10^8	6.75×10^7
12	5.34×10^8	3.60×10^7
13	6.94×10^8	9.73×10^7
14	9.43×10^8	6.65×10^7
15	4.37×10^8	8.63×10^7
16	8.63×10^8	5.92×10^7
17	1.73×10^9	4.77×10^7
18	7.71×10^8	7.19×10^7
19	1.46×10^9	5.77×10^7
20	6.44×10^8	5.05×10^7
Range	$4.00 \times 10^8 – 1.73 \times 10^9$	$3.60 \times 10^7 – 9.73 \times 10^7$
mean	8.02×10^8	6.11×10^7

(Tables 4, 5 and 6). Presence of coliforms in kitchen sponges indicates the presence of faecal materials and may indicate the presence of foodborne pathogens. Coliform contamination in sponges can be attributed to different factors such as inappropriate hygienic and sanitary practices during food preparation. When the mean coliform counts were compared from the various locations, the highest number of contamination by coliforms was obtained from sponges collected from Amaoba. This could be attributed to the use of faecally contaminated water in washing of kitchen utensils.

Table 5. Total viable counts from kitchen sponges collected from Amaoba (cfu/ml)

Sample code	THPC	TCPC
1	5.37×10^8	6.05×10^7
2	1.77×10^9	9.75×10^7
3	7.25×10^8	7.20×10^7
4	8.68×10^8	8.20×10^7
5	1.29×10^9	1.06×10^8
6	7.85×10^8	4.65×10^7
7	8.43×10^8	4.20×10^7
8	1.28×10^9	4.35×10^7
9	9.65×10^8	8.70×10^7
10	7.25×10^8	7.66×10^7
11	7.80×10^8	9.63×10^7
12	1.86×10^9	5.65×10^7
13	5.85×10^8	4.30×10^7
14	1.03×10^9	9.80×10^7
15	3.65×10^8	5.65×10^7
16	5.10×10^8	3.59×10^7
17	9.71×10^8	6.55×10^7
18	8.40×10^8	6.75×10^7
19	5.62×10^8	4.63×10^7
20	9.60×10^8	6.10×10^7
Range	$3.65 \times 10^8 – 1.77 \times 10^9$	$3.59 \times 10^7 – 1.06 \times 10^8$
mean	9.13×10^8	6.70×10^7

Table 6. Total viable counts from kitchen sponges collected from Amawom (cfu/ml)

Sample code	THPC	TCPC
1	6.55×10^8	9.50×10^7
2	9.95×10^8	7.25×10^7
3	9.76×10^8	3.55×10^7
4	7.80×10^8	6.15×10^7
5	1.62×10^9	4.05×10^7
6	6.85×10^8	5.65×10^7
7	4.65×10^8	1.01×10^8
8	9.05×10^8	7.65×10^7
9	6.13×10^8	9.76×10^7
10	4.95×10^8	8.65×10^7
11	6.79×10^8	4.37×10^7
12	6.13×10^8	3.63×10^7
13	9.10×10^8	5.60×10^7
14	6.89×10^8	8.63×10^7
15	8.25×10^8	6.15×10^7
16	7.58×10^8	6.70×10^7
17	1.93×10^8	5.35×10^7
18	9.55×10^8	4.50×10^7
19	7.05×10^8	4.80×10^7
20	6.95×10^8	3.65×10^7
Range	1.93×10^8 - 1.62×10^9	$3.55 \times 10^7 – 1.01 \times 10^8$
mean	8.47×10^8	6.28×10^7

The higher number of kitchen sponges contaminated by various microorganism in the present research which was conducted in rural communities dominated mainly by population of middle to low income residents when compared to the results found by other researchers who conducted their research mainly in developed areas dominated mainly by high income workers may be attributed to factors such as the use of contaminated water in washing [20].

Sanitizers should be also used so long as they are used in accordance with the manufacturer's use direction included in the labeling. The aim is to sanitize the sponges after they have been contaminated through use.

The method of washing in the kitchens of developed areas makes use of running tap waters which has greater flow rate and thus displaces entrapped microorganisms. The rural communities make use of bowl water which has low flow rate and thus microorganisms are not easily removed leading to the formation of biofilms. Most of the sponges in the rural communities are stored in a moisture laden environment which promotes the fast colonization and multiplication of microorganisms as opposed to developed areas where sponges are kept in air dry condition. Delay in the washing of cooking utensils leads to the rapid multiplication of microorganism which could be cross-contaminated to the kitchen sponges during washing. Most sponges used in the rural communities are made of fiber and there are also natural sponges and this also leads to rapid colonization. There are usually enough nutrients remaining in dish cloths and sponges to support the growth of most bacteria. If the sponges and dishcloths are dried after use, the bacterial growth is halted. However, the best practice is to use a clean sponge or dish cloth each day and to use a brush for washing dishes and kitchen utensils. The brush can be rinsed out easily and dried rapidly. Among other answers to this problem is the FDA Food Code recommendation that cleaning cloths should be kept in the sanitizer bucket. But this practice does not prevent the microbial population from increasing because after sometime, the organic material obtained from cleaning neutralizes the sanitizer and the microorganisms begin to multiply.

Antibiotic sensitivity testing (Table 7) of the isolates showed that all the bacterial isolates showed intermediate susceptibility to Ofloxacin. However, *S. aureus* was resistant to Amoxicillin (14.0 mm), Gentamycin (7 mm) and

Table 7. Antibiotic sensitivity pattern of bacterial isolates (mm)

Bacterial Isolates (25 µg)	Amoxicillin (25 µg)	Augmentin (10 µg)	Gentamicin (10 µg)	Nalidixic acid (30µg)	Nitrofurantoin (300µg)	Ofloxacin (30 µg)	Tetracycline (30 µg)	Cotrimoxazole (25 µg)
Staphylococcus aureus	14.0(S)	20.0(S)	7.0(R)	10.0(R)	21.0(S)	21.4(I)	19.0(S)	20.0(S)
Escherichia coli	22.0(S)	23.5(S)	12.0(R)	22.0(S)	8.0(R)	20.09(I)	22.3(S)	21.0(S)
Pseudomonas aeruginosa	8.5(R)	8.0(R)	18.5(S)	22.5(S)	10.5(R)	20.6(I)	9.5(R)	14.0(S)

Key: R = Resistant; S = Sensitivity; I = Intermediate

Interpretative reference range code

	Sensitive	Intermediate	Resistant
Amoxicillin (25 µg)	≥ 18	14-17	≤ 13
Augmentin (10 µg)	≥ 15	13-14	≤ 13
Gentamicin (10 µg)	≥ 15	13-14	≤ 12
Nalidixic acid (30 µg)	≥ 19	14-18	≤ 13
Nitrofurantoin (300 µg)	≥ 17	15-16	≤ 14
Ofloxacin (30 µg)	≥ 22	14-21	≤ 13
Tetracycline (30 µg)	≥ 19	15-18	≤ 14
Cotrimoxazole (25 µg)	≥ 19	11-15	≤ 10

Nalidixic acid (10.0 mm) respectively. *Escherichia coli* was sensitive to Augmentin (23.5 mm) but resistant to Nitrofurantoin and Gentamycin. *P. aeruginosa* showed the highest resistance among the bacterial isolates being resistant to Amoxicillin (8.5 mm), Augmentin (8.0 mm), Nitrofurantion (10.5 mm), Tetracycline (9.5 mm) and cotrimoxazole (14 mm). The antibiotics that inhibited the bacterial isolates can be used in the treatment of some foodborne diseases associated with the isolates. However, development of resistance to these antibiotics could pose a problem to antimicrobial therapy.

4. CONCLUSION

The kitchen sponges analyzed in this work were contaminated and could cause health challenges to the users. The risk would be lowered when sponges are kept dry, partly because bacterial growth and survival would be reduced. Simple, fast and effective methods to disinfect kitchen sponges may prevent the spread of pathogenic microorganisms in household kitchens and may lead to better food preservation and fewer cases of foodborne illnesses.

Since results have shown that microbial inactivation in kitchen sponges depends on a number of factors and is largely changeable, it is therefore recommended that self-disinfecting sponges should be considered for use, re-usable sponges should be dried after use and detergent washing should be employed. Additional analysis should be done to improve the hygienic status of kitchen sponges. The use of disinfectants should be encouraged too.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12457