International Journal of Advanced Technology and Social Sciences (IJATSS)
Vol.1, No.3, 2023: 197-206

Biofilm Formation Capacity of Bacterial Isolates from Kitchen Sponges

[1]Amadi-Ikpa, C.N.
[2]Okwelle, A.A.
Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt
Corresponding Author: Amadi-Ikpa, C.N. chidi.amadiikpa@iaue.edu.ng

A R T I C L E I N F O

Keywords: Moringa Oleifera Plant, Bacteria, River, Seed Powder, Rumuolumeni Community

Received : 3 October
Revised : 20 October
Accepted: 22 November

A B S T R A C T

This study was aimed at determining biofilm forming bacteria isolates in kitchen sponges of some homes. The investigation involved collection of kitchen sponges from homes before and after the sponges have been used, and thereafter standard microbiological procedure was adopted. Analysis employed the spread plate count and the streak plate techniques. Counts of the bacterial isolates after a 24 hours incubation at 35 degrees centigrade were observed on freshly prepared nutrient agar, Salmonella/Shigella agar, Mannitol salt agar and MacConkey agar media that have been plated with the sample. Also isolates recovered were identified and streaked on Congo red media. Results obtained showed heavy colony counts on nutrient agar plates, Salmonella/Shigella agar plates, Manitol salt agar plates with counts above 103CFU/g. Biochemical identification of the isolates recovered four (4) bacteria species out of a total of 19 isolates from the study namely ; Staphylococcus aureus, Proteus spp., Shigella spp. and Salmonella spp. Proteus spp. were observed with heavy biofilm formation while Shigella spp. was least, Staphylococcus aureus also expressed biofilm property. Result thus indicated that sponge contact on dishes possess some risk to dish users, due to persistence of biofilm expressed by bacteria isolates even after sponge disinfection with Sodium Hypochlorite. This further suggests that kitchen sponges may play a critical role in the health of household occupants and therefore be discarded after every use

INTRODUCTION

The use of kitchen sponges, a material with the aid of soap or detergent to wash and clean-off surfaces of dishes is reported as one of many domestic hygienic practice in the home Enriquez et al. (1997). Kitchen sponges have been reported to harbor microbes specifically, bacteria (Philip et al. (2010). The use of kitchen sponge for cleaning plates/dishes and pots have become a routine domestic practice in the home (Ojima et al., 2002). According to Cardinal et al. (2017) dishes before cleaning may contain pathogenic microorganisms due to food spoilage, and during washing, the microorganisms (bacteria) gets attached to the sponge, remain there and sometimes cross contaminate other surfaces. Basically, several studies agreed that vast number of bacteria accumulate and accommodate kitchen sponge (Osali et al., 2020). Enriquez et al. (2017) reported that 15.4% of sponge samples taken from some households were contaminated with Escherichia coli, klebsiella spp, Proteus spp, Salmonella and Staphylococcus aureus (Cardinal et al., 2017). Similarly, in a hygiene study carried out by Ojima et al. (2002) on the distribution of microorganism in Japanese household, they reported that kitchen sponges had the highest load of coliforms far above counts obtained from drain traps in the home. So also, Rossi et al. (2012) noted that kitchen sponges were observed to harbor high counts of aerobic mesophilic bacteria, coliforms, enterobacteriaceas, yeast and mold microbes due to poor sanitization practices. Rossi et al. (2012) showed that 21% to 43% of bacteria present in sponges can be transferred to new surfaces. However, the number of microbes transferred to surfaces was not dependent on the surface type but on the initial contamination of the sponge, in order words sponges with great number of bacteria transfer more bacteria to surfaces (Taylor & Collins 1948).

LITERATURE REVIEW

The harbor of these bacteria in sponges creates a hazardous features on users (Taylor & Collins 1948). According to Taylor & Collins (1948), bacteria associated with biofilm formation are threats to human health specifically, when the bacteria are associate with consumable. Bacteria born-biofilms are protected from antibiotics, disinfectants and other environmental hazards. According to Taylor and Collins (1948) these inhibitors have little or no effect on bacteria biofilm as the formation allows the bacteria to exist or persist for a longer time. The kitchen sponges in the home provides surfaces area for the attachment. Notable nature of biofilm as stated by Amadi-Ikpa et al. (2020) reported that biofilm formation by bacteria is associated with increased bacteria population, thus, a low/ minimal heterotrophic bacteria population will not initiate sufficient biofilm output whereas a heavy heterotrophic bacteria population initiates high biofilm development. Most kitchen sponge users adopt poor practices regarding food safety, hygiene and handling of kitchen utensils Wolde & Bacha, (2016). Thus, the study will identify bacteria biofilm isolates that are important to users, and hence determine biofilm formation capacity of bacterial isolates from kitchen sponges.

METHODOLOGY

Description of Kitchen Sponges

Kitchen sponges are of different types, range from the absorbent types to the non-absorbent type. As an absorbent it can take the form of polysester, dishcloths, cellulose sponges and many more forms. As the forms differs, so also the color, texture and sizes.

Collection of Kitchen Sponges

Kitchen sponges were collected aseptically from volunteered households in Ubordu town of Omuanwa community of Rivers State Nigeria. Sixty (60) sponges comprising of 30 each for pre-used and post-used sponges were collected from some homes in a sterile bag and then taken to the Biology Laboratory of Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Nigeria.

Preparation of the Kitchen Sponge Samples

The preparation of kitchen sponge samples adopted the method of kotula et al. (1997), ten (10g) gramms of the kitchen sponges (both pre-used and post-used) were chopped aseptically, and introduced into a sterile prepared normal saline, which acted as a buffer and a stock culture. A serial dilution of the buffered or stock culture was carried out and the dilutions of 10- 2 and 10-3 were adopted for the study.

Preparations of the Media

The agar media, Nutrient agar medium, Mannitol salt agar media, Salmonella/Shigella agar medium as used by Amadi-Ikpa et al. (2020) were adopted and prepared according to manufactures direction. Similarly, biofilm screening of the recovered isolate required the compounding of a Congo red agar which is composed of agar/agar, sucrose, brain heart infusion agar and a red stain .

Determination of the Bacterial Load in the Sponges (Pre-Used and Post-Used)

The determination of the bacteria load on the kitchen sponge adopted the method modified by Amadi-Ikpa et al. (2020), were 10- 2 and 10-3 dilutions from the stock culture were plated, using a one (1) ml sterile pippet to transfer 0.1 volume of inoculum onto the various media of Nutrient agar media, Mannitol salt agar media, Salmonella/Shigella agar medium.

Morphological Characterization of the Bacterial Isolates

Morphological characterization of the isolates considered the colour, shape, elevation, opaque etc of the isolates on the media plates after inoculation and incubation. (Ryu & Benchatt, 2005).

Coagulase Test

Coagulase test was done to identify Staphylococcus aureus. Staphylococcus aureus expresses coagulase enzyme features. In carrying out the coagulase test, the isolates were introduced on a clean microscopic slide, thereafter with the adding of a drop of normal saline and rabbit plasma. All substance was mixed gently and after 10 seconds clumping of the isolate indicated positive reaction while the absence of clump indicated a negative result.

Catalase Test

Catalase test was done to note the presence of catalase enzyme on the isolate. This test was achieved by introducing 3% hydrogen peroxide on the isolate that was placed on a slide aseptically. Catalase presence by the isolate was observed with the presence of bubbles after few seconds of the hydrogen peroxide application while a negative catalase showed the failure of a bubble observation.

Citrate Test

The citrate test was done to detect the ability of the isolate to breakdown citrate. The procedure involved introducing the isolate into a test tube containing a freshly prepared sterile citrate agar media. The breakdown of citrate agar media by the isolate marked a color change from green to blue after 24 hours at 37 degrees centigrade incubation temperature. The absence of a color change signified a negative result.

Sugar Fermentation Test

The sugars, glucose, lactose, sucrose and maltose were used for the biochemical identification of the isolates. The sugars were compounded to a broth consisting of 1% each of peptone water, alcohol, the sugar and a phenol red indicator stain. In each of the compounded sugar in a broth, the isolates were introduced into them. The broth cultures were allowed to incubate for 24-48 hours at 37 degrees centigrade. A change in color from red to yellow indicated a positive sugar test while a negative sugar text is indicated by no change.

Methyl Red / Voges Proskauer Test

The test was done to determine the ability of the isolate to breakdown glucose phosphate broth. The glucose phosphate broth was prepared as directed by the manufacturer and the isolates inoculated into the medium in a test tube. The test tube containing the broth was then incubated for 24 hours at a temperature of 37 degrees centigrade. This was then followed by sharing the broth culture medium into two parts/potions. To one of the part/potions, few drops of methyl red solution was added and after 30 to 60 seconds, a change in color of the medium from red to yellow indicated positive reaction while an absence of color change indicated negative reaction. To the other potion of the glucose phosphate broth, few drops of Barrit's reagent was added and after 30 seconds, a formation of red color indicated a positive reaction while yellow indicated negative reaction.

Screening for Bacteria Biofilm Formation

The bacteria recovered and identified were streaked on the already prepared Congo red agar as documented by Amadi-Ikpa et al. (2020). The medium was incubated at 37 degrees centigrade for 24 hours. Inference after 24 hours with a black colonies indicate biofilm formation while pink colonies do not show biofilm formation.

RESULTS

Enumeration of Bacteria Isolates Before and After Use of Sponges

Table 1 showed the counts of Bacteria in kitchen sponges before and after use. The counts of bacteria in house hold sponges showed a mean and standard deviation of 2.5 × 104 ± 0. 4, 9.8 × 103 ± 1.6 and 2.21 × 104 ± 1.9 CFU/ml for Salmonella/Shigella, Staphylococcal and Heterotrophic bacteria respectively. Counts of bacteria were however different in sponges after use. The counts are 2.07 × 104 ± 0.9, 5 × 103 ± 1.0 and 2.04 × 104 ± 1.5 CFU/ml for Salmonella/Shigella, Staphylococcal and Heterotrophic bacteria respectively.

Table 1. Counts of Bacteria Isolates on Sponges Before and After Use

Bacteria/Isolates	Counts Before Use (CFU/ml)	Counts After Use (CFU/ml)	T- test
Coliforms	0.0 ± 0.0	0.0 ± 0.0	$P > 0.05$
Salmonella /Shigella	$2.5 \times 10^4 \pm 0.4$	$2.07 \times 10^4 \pm 0.9$	$P < 0.05$
Staphylococcal	$9.8 \times 10^3 \pm 1.6$	$5 \times 10^3 \pm 1.0$	$P < 0.05$
Heterotrophic	$2.21 \times 10^4 \pm 1.9$	$2.04 \times 10^4 \pm 1.5$	$P < 0.05$

Morphological Features of the Isolates on Plates

Table 2 showed the morphological features of the isolates on plates. Coloration of the colonies on culture plates revealed white and grey colors with small sized colonies that had low elevation. The edges of the colonies were curved and they appeared opaque under the microscope. Gram reaction presumed a bacilli shaped Gram positive rod.

Table. 2 Morphological Features of the Isolates on Plates

Isolates	Color	Size	Elevat.	Edge	Opacity	Shape
Salmonella /Shigella	colourless	Small	Low	Curve	Opaque	Circular
Staphylococcal	yellow	Large	Low	Curve	Opaque	Circular
Heterotrophic	colourless	Small	Low	Curve	Opaque	Bacilli

Biochemical Reaction of the Isolates

Table 3 showed the biochemical reaction of the isolates recovered, the Gram negative bacteria were distinguished with a positive lactose and methyl red test, which indicated Salmonella spp..and Shigella spp. while the other test were predominantly negative except for citrate that was positive for Salmonella spp. The other isolates showed positive reactions for citrate and methyl red but distinguished with a coagulase positive test which indicated Staphylococcus aureus. Further test showed positive reactions for indole and urease, thus indicated Proteus spp.

Table 3. Biochemical Features of the Bacteria Isolates

M. Red	V. Proskauer	Ind	Urease	Coa	Suc	Lac	Cit	Gram Reac.	Bacteria
+	-	-	-	-	-	+	-	-	*Shigella* spp.
+	-	-	-	-	-	+	+	-	*Salmonella* spp.
+	+	-	-	+	-	+	+	+	*Staphylococcus aureus*
+	-	+	+	-	+	-	+	-	*Proteus* spp.

Note; M. Red= Methyl Red, V. Proskaurer = Voges Proskaurer, Ind=Indole, Cit= Citrate, Coa= Coagulase, Suc = Sucrose, Lac = Lactose, Cat = Catalase, Gram Reac = Gram Reaction, (+) = Positive, (-) =Negative

Frequency Distribution and Percentage Occurrence of the Identified Isolates

Table 4 showed the frequency and percentage occurrence of the isolates. Out of 19 isolates of Shigella spp. Salmonella spp. Staphylococcus aureus and Proteus spp. isolated, fifteen of the isolates were recovered from pre-used sponges while nine was recovered from post-used sponges. Shigella spp. was noted with 13% occurrence for pre used sponges while post used sponges had no occurrence. Salmonella spp., Staphylococcus aureus and Proteus spp. had 27, 20 and 40 % occurrence in pre used sponges respectively. Whereas 22, 44 and 33% occurrence were noted for Salmonella spp, Staphylococcus and Proteus spp. respectively.

Table 4. Frequency Distribution and Percentage Occurrence of the Identified Isolates

Bacteria	Frequency of Occurrence (Before Use) (n=30)	Percentage (%) of Occurrence (Before Use)	Frequency of Occurrence (After Use) (n=30)	Percentage (%) of Occurrence (After Use)
Shigella spp.	2	13	-	0
Salmonella spp.	4	27	2	22
Staphylococcus aureus	3	20	4	44
Proteus spp.	6	40	3	33

Quantification of Bacteria Biofilm

Table 5 showed the quantification of the bacteria in percentages. All bacteria isolated under investigation, were able to form biofilm. A high percentage biofilm was evident with Proteus spp with an occurrence of 38%, followed by Staphylococcus aureus with a 29.5 %. Salmonella spp. and Shigella spp. recorded a biofilm percentage occurrence of 25 and 8.3 respectively.

Table 5. Quantification of Bacteria Biofilm

Bacteria	No. of Bacteria Isolates	No. of Biofilm Positive Isolates	% of Biofilm Forming Capacity
Shigella spp.	2	2	8.3
Salmonella spp.	6	6	25
Staphylococcus aureus	7	7	29.2
Proteus spp.	9	9	38

DISCUSSION

The counts of heterotrophic bacteria, Staphylococcal and Salmonella/Shigella obtained before and after use of the kitchen sponges are quite different from studies carried out by Moreto et al.(2020). Although, Moreto et al. (2020) considered in their methods millimeter of the sponges whereas this present study considered the grams of the sponge samples. Coliform counts were absent in the study however, kitchen sponges used to clean food contact surfaces have been reported to have high counts of mesophilic aerobic bacteria (coliform) and Enterobacteriacea between 7.43 to 12.44log cfu/mm3. The absence of coliform in these study implies the sponges had no microorganism of fecal origin. This showed good hygienic practices in terms of fecal matters. Even at these counts recorded for Staphylococcal and bacteria, the use of disinfectant like hypochlorite did not inhibit the bacteria population in the sponge.

The study noted Proteus spp. and Staphylococcus aureus as the most dominant bacteria where as in studies carried out by Wolde (2016) Pseudomonas was the most dominant. Wolde (2016) noted, Gram positive bacteria were higher with a 61 % occurrence (Chryseobacterium, Enhydrobacter, Enterobacteriaceae and Pseudomonas). In the course of all recovered bacteria forming biofilm within the sponges definitely will cross contaminate biotic surfaces, food surfaces, dishes or plates and other kitchen utensils and by extension cause food poisoning (Pong et al.,2023). The sponges provided the attachment or receptor sites for biofilm formation and growth Taylor and Collins, (1948). These attachment sites harbor the bacteria isolates and allow the bacteria form biofilm, increasing the persistence of the bacteria. Again, according to Pong et al. 2023. The implication of the bacteria in the sponges forming biofilm gives account of the ineffectiveness of disinfectants and other detergents used in washing to ward-off bacteria present in the sponge. Therefore, elimination of biofilm bacteria from the sponges remain an issue of concern considering the risk of food born disease infection

where the sponges used in washing plates do not completely clean the plates and thereafter the plates used to serve food. In the forgoing an effective measure to ward-off bacteria biofilm in sponges needs to be proposed.

CONCLUSIONS AND RECOMMENDATIONS

Coliform counts were absent in the study however, kitchen sponges used to clean food contact surfaces have been reported to have high counts of mesophilic aerobic bacteria (coliform) and Enterobacteriacea between 7.The absence of coliform in these study implies the sponges had no microorganism of fecal origin and Staphylococcus aureus as the most dominant bacteria where as in studies carried out by Wolde (2016) Pseudomonas was the most dominant.

Wolde (2016) noted, Gram positive bacteria were higher with a 61 % occurrence (Chryseobacterium, Enhydrobacter, Enterobacteriaceae and Pseudomonas).In the course of all recovered bacteria forming biofilm within the sponges definitely will cross contaminate biotic surfaces, food surfaces, dishes or plates and other kitchen utensils and by extension cause food poisoning (Pong et al.The implication of the bacteria in the sponges forming biofilm gives account of the ineffectiveness of disinfectants and other detergents used in washing to ward-off bacteria present in the sponge.Therefore, elimination of biofilm bacteria from the sponges remain an issue of concern considering the risk of food born disease infection where the sponges used in washing plates do not completely clean the plates and thereafter the plates used to serve food.In the forgoing an effective measure to ward-off bacteria biofilm in sponges needs to be proposed.

FURTHER STUDY

This research still has limitations, so it is necessary to carry out further research related to the topic biofilm formation capacity of bacterial isolates from kitchen sponges in order to improve this research and add insight to readers.

REFERENCES

Amadi-Ikpa, C. N., Akani, N. P., Wemedo, S. A. & William, J.O.(2020). Biofilm Formation and Virulent Properties of Bacterial Isolates in stored Drinking Water of Some Homes. International Journal of Research and Innovation in Applied Sciences 5, 8:2454-619

Akani,N. P., Amadi-Ikpa, C.N. & Wemedo, S. A. (2020). Population of Microbes Associated with Stored Drinking Water in Some Diobu Homes, Port Harcourt. International Journal of Research and Inovation in Applied Sciences 5, (8) 2454-619.

Cardinal,M., Kaiser,D., Lueders, T., Schnell,S. & Egert, M. (2017). Microbiome Analysis and Confocal Microscopy of Used Kitchen Sponges Revealed Massive Colonization by Acinetobacter, Moraxella and Chryseobacterium sp. Scientific Report 7, 5791

Enriquez, C.E., Enriquez-Gordillo, R., Kennedy, D.I. & Gerba, C.P. (1997). Bacteriological Survey of Used Cellulose Sponges and Dish Cloths from Domestic Kitchens. Dairy Food Environment Sanitation 17,20-24.

Kotula.K.L.,Kotula,A.W., Rose, B.F., Pierson,C.J. & Camp.M. (1997). Reductions of Aqueous Chlorine by Organic Material, Journal of Food Protection 10,276-282.

Moreto, T., Birgitte, M., Solveig, L.(2022). Bacterial Levels an Diversity in Kitchen Sponges and Dishwashing Brushes Used by Consumers. Journal of Applied Microbiology 113(3).

Ojima, M., Tochima,Y., Koya,E., Ara,K., Tokuda,H., Kawai,S. &Kasuga, F.(2002). Hygiene Measures Considering Actual Distributions of Micro-organism in Japanese Households, Journal Appl. Microbiol 93:300-9.

Osali,T.M., Obaid,R.S., Alowais,K., Almahmood,R.,Almansoori,M.,Alayadhi, Y., Alowaisi, N. & Waheed,K.(2020). Microbiological Quality of Kitchen Sponges Used in University Student Dormitories, BMC Public Health 20: 1322.

Philip, P.L., Wolcott,R.D.,Fletcher,J. & Schultz, G.S.(2010). Biofilm Made Easy Wounds International 1, (3).

Pong, Q., Tang, X., Dong, W., Sun, N. & Yuan, W. (2023). A Review of Biofilm Formation of Staphylococcus aureus and its Regulation Mechanism, Antibiotics 12(1), 12.

Rossi, E. M, Scapin,D., Grando, W. &Tondo, E. C. (2012). Microbiological Contamination and Disinfection Procedures of Kitchen Sponges Used in Food Services. Food Nutrition Sciences 3, 976-980.

Ryu, J.H. & Benchat, L.R.(2005). Biofilm Formation by Escherichia coli 0157:H7 on stainless steel. Effect of Exopolysaccharide and Curli Production on its Resistance to Chlorine. Applied and Environmental Microbiology 71,247-254.

Taylor, C. B. & Collins, V.G. (1948). Development of Bacteria in Water Stored in Glass Container, Fresh Water Biological Association 3, (1), 32-42.

Wolde, T. (2016). Microbiological Safety of Kitchen Sponges Used in Food Establishment, International Journal of Food Science 1659784.