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Research Article

Biofilm Formation among *Staphylococcus aureus* Strains from Food Contact Surfaces in Households: A Public Health Concern

***Shu'aibu Isa[1], Abubakar A. Bilkisu[2], Jabir A. Hamza[3], Muhammed M. Isma'ila[2], Kabiru Y. Mustapha[4], Ummu R. A. Rabi'u[1], Abubakar Muhammad[1], Mohammed U. Maikudi[5], Tasiu Sulaiman[6] and Rukayya Ibrahim[2]**

[1]Department of Microbiology, Gombe State University, P.M.B. 127, Gombe, Gombe State, Nigeria
[2]Department of Science Laboratory Technology, Federal Horticulture Dadin Kowa, Gombe State
[3]Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe State, Nigeria
[4]School of Health Sciences, Maryam Abacha American University of Nigeria, Kano, Nigeria
[5]Department of Biotechnology, Modibbo Adama University, P.M.B. 2076, Yola, Adamawa State
[6]Department of Microbiology, Federal University Dutsin-Ma, P.M.B. 5001, Dutsin-Ma, Katsina State
***Corresponding Author's email*:** shuaibuisa2002@gsu.edu.ng +2348065534951

ABSTRACT
Staphylococcus aureus is a foodborne pathogen of serious public health concern due to its high biofilm formation capability. One of the contributing factors to foodborne illnesses is improper cleaning and sanitization of food contact surfaces This study aimed to determine the prevalence of *S. aureus* with biofilm formation potential (BFP) from various food contact surfaces. A total of 100 samples were obtained, 20 from each of stainless steel spoons, plastic plates, plastic eating bowls, plastic cutting boards and glass cups. Samples were obtained by surface swabbing. The cotton swabs were streaked onto nutrient agar plates and incubated overnight. Colonies obtained were subjected to Gram staining, coagulase, catalase and DNAse tests for the identification of *S. aureus*. The identified *S. aureus* strains were subjected to biofilm formation potential assay using crystal violet assay (CV-assay) in polystyrene 96-well microtitre plates. Out of the 100 samples obtained, 26 were found to harbour *S. aureus*. Moreover, out of the 20 isolates, 2 (10%) were from stainless steel spoons, 8 (40%) from plastic plates, 5 (25%) from plastic-eating bowls, 10 (50%) from plastic cutting boards and 1 (5%) from glass cups. Additionally, 11 (42%) of the strains had strong BFP, 11 (42%) had moderate BFP, 3 (12%) and 1 (4%) had no BFP. Presence of *S. aureus* with BFP on food contact surfaces should be checked to avoid foodborne outbreaks due to this organism.

Keywords: *Staphylococcus aureus;* Biofilm formation potential; Food contact surfaces; Crystal violet assay

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most serious pathogens associated with a tremendous threat to the food safety and human health (Zhang *et al.,* 2022). Biofilm is one of the important factors contributing to antimicrobial resistance in the bacteria. *Staphylococcus aureus* usually has strong biofilm formation ability, and it is widely found in animal-based food (Ou *et al.,* 2020). Although *S. aureus* is widespread in nature, foods are still the important matrix harbouring *S. aureus*, which play a vital role in disease causation in humans and animals (McGavin & Heinrichs, 2012; Vrbovská *et al.,* 2020; Zaatout *et al.,* 2020).

Bacteria can form biofilms in natural and clinical environments on both biotic and abiotic surfaces. The

bacterial aggregates embedded in biofilms are formed by their own produced extracellular matrix. *Staphylococcus aureus* is one of the most common pathogens of biofilm infections. The formation of biofilm can protect bacteria from being attacked by the host's immune system and antibiotics; and thus bacteria can be persistent against external challenges. Therefore, clinical treatments for biofilm infections are currently encountering difficulty. To address this critical challenge, a new and effective treatment method needs to be developed. A comprehensive understanding of bacterial biofilm formation and regulation mechanisms may provide meaningful insights against antibiotic resistance due to bacterial biofilms.

Biofilm is an organized bacterial population and refers to the membrane-like extracellular matrix (ECM) formed by the adhesion of bacterial colonies and extracellular polymeric substances (EPS) such as polysaccharides, nucleic acids, and proteins secreted by bacteria during their growth process (Karygianni *et al.,* 2020).

The interaction between EPS and bacterial aggregates endows biofilm with cohesion and visco-elasticity (Di Martino, 2018). As a result, bacteria can attach to both biotic and abiotic surfaces. The formation of pathogenic biofilm plays an important role in causing chronic persistent infection (Guilhen *et al.,* 2017). Researchers generally believe that more than 80% of chronic infections are mediated by bacterial biofilms (Jamal *et al.,* 2018).

The presence of biofilm on food contact surfaces is considered as a health hazard. Microbial biofilms, in fact, may contain a considerable number of both spoilage and pathogenic microorganisms. Exposure of pathogens to surfaces may take place either by direct contact with contaminated materials or indirectly through airborne microflora (Di Ciccio *et al.,* 2015). The purpose of this study was to determine the prevalence and biofilm formation of *S. aureus* in kitchen utensils from various households in Gombe metropolis.

MATERIALS AND METHODS

Sampling

Food contact surfaces from various households in Gombe metropolis were examined in this study. Samples were obtained using cotton swabs moistened with Tryptic soy broth (TSB). A total of 100 samples were collected. All samples were immediately placed and stored in cooler containing frozen ice packs to keep samples cool, and transported to the laboratory within 4 h of collection. In each selected household, surface of stainless steel spoons, plastic plates, plastic eating bowls, plastic cutting boards and glass cups were swabbed with sterile cotton swabs moisten in TSB within areas of 100 cm^2 for plates and 20 cm^2 for cups.

Identification of *S. aureus* Isolates

The swabbed samples were further swabbed evenly onto Nutrient agar (NA) plates and incubated at 37 ^oC for 18 to 24 hours in an inverted position. Upon incubation, characteristic golden yellow or yellowish white, round and smooth as well as slightly raised (convex) colonies of about 2 to 4 mm in diameter presumed as *S. aureus* were subcultured and subjected to Gram staining, catalase, coagulase (both tube and slide methods) and DNAse for confirmation. The confirmed isolates were Gram positive; and positive for catalase, coagulase and DNAse tests.

Determination for Biofilm Formation Potential (BFP)

The procedure for biofilm formation described by Stepanovic et al. (2000) was adopted with slight modification. Isolates were grown in TSB (Oxoid, UK) for 18 hours to observe growth. The culture on TSB was diluted into fresh TSB containing 0.25% NaCl at 1:40 ratio. An aliquot of 200 µL of the prepared suspension was used to inoculate the wells of the polystyrene 96-well microtitre plate (SPL, Life Science, Korea). The inoculated plate was incubated at upright position at 37 ^oC for 24 hours without agitation.

After incubation, the wells were washed 3 times with PBS (pH 7.4±0.1; Thermo Fisher Scientific, Waltham, Massachusetts, USA), air-dried for 1 h at 60^oC. The dried wells were stained with 0.25% crystal violet and incubated at room temperature for 15 min. After washing, the absorbance at OD_{570nm} was determined using spectrophotometer (Bio-Rad, USA). The mean absorbance of each test well was classified according to Stepanovic et al. (2000) either as non-biofilm, weak, moderate or strong biofilm formers relative to the controls (which were the uninoculated wells). Wells with non-biofilm formers had absorbance less than or equal to the control wells ($OD \leq OD_C$); the weak biofilm formers ($OD_C \leq OD \leq 2x\ OD_C$), moderate biofilm formers ($2x\ OD_C \leq OD \leq 4x\ OD_C$) and the strong biofilm formers ($4x\ OD_C < OD$). Each of the isolates used for the biofilms assay was in triplicates. Finally, the mean absorbance was calculated. The analyses were repeated separately in three separate occasions (O'Toole, 2011). The ATCC *S. epidermidis* (ATCC 35983) and *S. epidermidis* (ATCC 12228) were respectively used as positive and negative controls to assess the strains BFP, capacity of local strains in BFP.

RESULTS

Out of the 100 swab samples obtained from kitchen utensils, only 26 were positive for *S. aureus*. However, out of 20 samples collected from each food contact surface, only 2 (10%) from stainless steel spoons were

positive for *S. aureus* (Table 1). Additionally, 8 (40%) samples from plastic plates, 5 (25%) samples from plastic eating bowls, 10 (50%) samples from plastic cutting boards and 1 (5%) from glass cups were found to be positive for *S. aureus*. The negative values for the occurrence of *S. aureus* from the various contact surfaces stand as 18 (90%) from stainless steel spoons, 12 (60%) from plastic plates, 15 (75%) from plastic eating bowls, 10(50%) from plastic cutting boards and 19 (95%) from glass cups.

The distribution of the *S. aureus* isolates from the various food contact surfaces are shown in Table 2. It can be seen that the isolates ST13 and ST26 were found in samples from stainless steel spoons. However, ST1, ST3, ST5, ST7, ST11, ST12, ST15 and ST17 were eight isolates detected in samples obtained from plastic plates. Moreover, ST2, ST4, ST6, ST9 and ST16 were the six isolates from plastic eating bowls. Samples from plastic cutting boards had ST10, ST13, ST18, ST19, ST20, ST22, ST23, ST24, ST25 and ST26 were *S. aureus* isolates from plastic cutting board samples. The glass cup had only one isolate, the ST21.

Table 1: Occurrence of *S. aureus* from various food contact surfaces

Food Contact Surface	Number Examined	No. Positive (%)	No. Negative (%)
Stainless Steel Spoon	20	2 (10)	18 (90)
Plastic Plates	20	8 (40)	12 (60)
Plastic Eating Bowl	20	5 (25)	15 (75)
Plastic Cutting Boards	20	10 (50)	10 (50)
Glass Cups	20	1 (5)	19 (95)
Total	100	26	74

Table 2: Distribution of the *S. aureus* isolates obtained from various food contact surfaces

S/N	Food Contact Surface	*S. aureus* isolated Obtained	No. Obtained
1	Stainless Steel Spoon	ST13 and ST26	2
2	Plastic Plates	ST1, ST3, ST5, ST7, ST11, ST12, ST15 and ST17	8
3	Plastic Eating Bowl	ST2, ST4, ST6, ST9 and ST16	5
4	Plastic Cutting Boards	ST8,ST10,ST14, ST18, ST19, ST20, ST22, ST23, ST24 and ST25	10
5	Glass Cups	ST21	1
Total			26

The values for mean absorbance to quantify the biofilm formation potential of the *S. aureus* strains from the different food contact surfaces are depicted in Table 3. The results were obtained at OD_{570nm}. All the isolates except one (ST14) have various BFPs. Isolates ST1, ST2, ST3, ST5, ST6, ST11, ST16, ST18, ST19 and ST21 were found to be with strong biofilm formation potential. Meanwhile, ST4, ST7, ST9, ST10, ST15, ST17, ST20, ST22, ST23, ST24 and ST25 were found to possess moderate BFP. However, the weak biofilm formation potential was found to be present in the isolates ST12, ST13, and ST26. The control served as a measure for which the BFPs of all the isolates were derived.

The distribution of the isolates based on the intensity of their biofilm formation potential (BFP) is shown in Table 4. All the two isolates obtained from stainless steel spoon (ST13 and ST26) were weak biofilm formers. Meanwhile, four out of the eight isolates from plastic plates; ST1, ST3, ST11 and ST13 had strong BFP, three of them, ST7, ST15 and ST17 had moderate BFP and the last ST12 with weak BFP. There was none without BFP. However, out of the five isolates obtained from plastic eating bowl, ST2, ST6 and ST16 (n=3) were potentially strong biofilm formers, and ST4 and ST9 (n=2) were with moderate BFP. Neither weak biofilm former nor non-biofilm former was found among them. On the other hand, out of the 10 isolates from plastic cutting board, ST8, ST18 and ST19 (n=3) had strong BFP, ST10, ST20,ST22, ST23, ST24 and ST25 (n=6) had moderate BFP and ST14 (n=1) had no BFP. Lastly, the only isolate from glass cup ST21 had strong BFP.

The development of biofilm using crystal violet (CV) assay is depicted in Figure 1. It can be observed that even after washing some isolates were able to adhere to the surface of the wells of the microtitre plates. The wells are with alphabetical and numerical symbols for proper identification of the isolates while running the procedure of determining the BFP.

Table 3:Biofilm formation potential of the*S. aureus* isolates (n=26) at OD570nm

S/N	Isolates	Absorbance at OD_{570nm}	Biofilm Formation Potential
1	ST1	0.247 ± 0.08	Strong
2	ST2	0.203 ± 0.13	Strong
3	ST3	0.212 ± 0.03	Strong
4	ST4	0.135 ± 0.03	Moderate
5	ST5	0.173 ± 0.06	Strong
6	ST6	0.122 ± 0.08	Strong
7	ST7	0.094 ± 0.02	Moderate
8	ST8	0.197 ± 0.11	Strong
9	ST9	0.160 ± 0.10	Moderate
10	ST10	0.148 ± 0.08	Moderate
11	ST11	0.250 ± 0.16	Strong
12	ST12	0.079 ± 0.02	Weak
13	ST13	0.079 ± 0.01	Weak
14	ST14	0.021 ± 0.04	Non-biofilm former
15	ST15	0.104 ± 0.02	Moderate
16	ST16	0.197 ± 0.09	Strong
17	ST17	0.099 ± 0.01	Moderate
18	ST18	0.172 ± 0.08	Strong
19	ST19	0.189 ± 0.12	Strong
20	ST20	0.130 ± 0.03	Moderate
21	ST21	0.280 ± 0.04	Strong
22	ST22	0.132 ± 0.06	Moderate
23	ST23	0.140 ± 0.06	Moderate
24	ST24	0.120 ± 0.03	Moderate
25	ST25	0.162 ± 0.04	Moderate
26	ST26	0.083 ± 0.02	Weak
27	Control	0.042 ± 0.08	OD_C

Non adherent (0) $OD \leq OD_C$
Weakly adherent (+) $OD_C \leq OD \leq 2x\ OD_C$
Moderately adherent (++) $2x\ OD_C \leq OD \leq 4x\ OD_C$
Strongly adherent (+++) $4x\ OD_C < OD$

Table 4: Distribution of the *S. aureus* isolates based on BFP intensity

		Biofilm Formation Potential (BFP)				
S/N	Food Contact Surface	Strong	Moderate	Weak	Non-Biofilm	Total
1	Stainless Steel Spoon	ND	ND	ST13, ST26,	ND	2
2	Plastic Plates	ST1, ST3, ST5, ST11	ST7, ST15, ST17	ST12	ND	8
3	Plastic Eating Bowl	ST2, ST6, ST16	ST4, ST9	ND	ND	5
4	Plastic Cutting Boards	ST8, ST18, ST19	ST10, ST20, ST22, ST23, ST24, ST25	ND	ST14	10
5	Glass Cups	ST21	ND	ND	ND	1
Total		11	11	3	1	**26**

Keys: ND = Not Detected

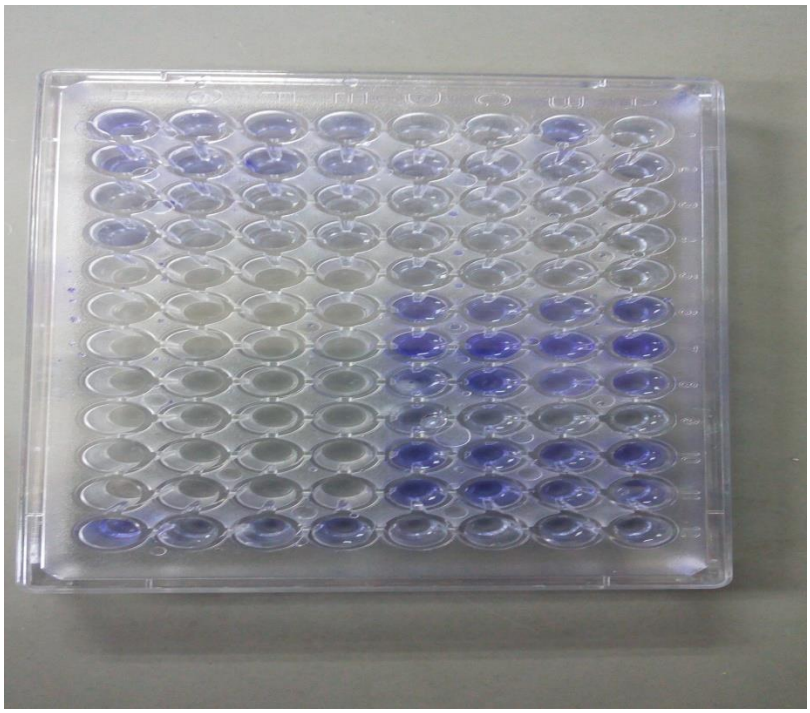

Figure 1: A 96-well polystyrene microtitre plate showing various isolates subjected to BFP determination using CV-assay

DISCUSSION

Microbial attachment and biofilms are of great concern to the food industries and households, and they occur on various surfaces of food contact (Donlan & Costerton, 2002; Simoes, Simoes, & Vieira, 2010; Di Ciccio *et al.,* 2012; 2015; Flemming, Meier, & Schild, 2013). The bacterial capability of surviving stress is enhanced by biofilms. These biofilms become possible reservoir of pathogens including *S. aureus* (Abdallah *et al.,* 2014). A number of studies have reported the ability of bacteria to form biofilms on common food contact surfaces. These surfaces include glass, stainless steel, rubber, polyurethane, polycarbonate, polystyrene, titanium, polypropylene, ceramic and aluminum (Di Ciccio *et al.,* 2015). Surfaces contaminated with pathogenic microorganisms are well established in food processing and catering for the spread of these microbes (Silva-Meira *et al.,* 2012; Giaouris *et al.,* 2014). Factors such as environmental ones that include pH, humidity, and temperature, availability of nutrients and nature of the contact surface contribute immensely in the attachment, growth and biofilm formation of bacteria. Despite application of disinfection procedure, some studies have revealed the presence of biofilms on food contact (Gounadaki *et al.,* 2008; Gutie'rrez *et al.,* 2012; de Jesus *et al.,* 2014).

The occurrence of *S. aureus* on the food contact surfaces of household kitchens is not surprising for various reasons. First, these surfaces are where food types that harbour microbes such raw meat, vegetables, contaminated foods and so on are processed. This makes it easier for cross contamination with microbes. Second, the surface disinfection procedure may not be well applied and appropriately adhered to the manufacturers' instructions by the householders (Di Ciccio *et al.,* 2015). Lastly, the bacterial pathogens may have the potential to develop biofilm due to the presence of matrix of the extracellular polymeric substance or genes encoding for biofilm formation. Hence, this is deleterious to health of householder and the public health at large (Vlkova *et al.,* 2008).

The *S. aureus* isolates revealed high capability of adherence to food contact surfaces due to biofilm formed at 37 ^{o}C. This is similar to the study conducted by Va'zquez-Sa'nchez et al. (2013) on n.26 *S. aureus* isolated from seafood and n.2 *S. aureus* reference strains showed that most of the strains had a higher biofilm production at 37 ^{o}C than at 25 ^{o}C on polystyrene. However, Pagedar et al. (2010) reported a higher cell count of the *S. aureus* biofilm at 25 ^{o}C contrary to that was obtained at 37 ^{o}C on stainless steel. This indicates that temperature has little effect on the development of biofilms due to *S. aureus.*

Unlike in the past as well as in the developed world whose majority of food contact surfaces are made of stainless steel (Di Ciccio *et al.,* 2015), most household kitchens herein are made of plastics. Formation of bacterial biofilms is difficult on stainless steel due to its smooth nature and, until scratches and cracks are made

on them, biofilm formation proves difficult. This is true as in the case of this study in which only two of the *S. aureus* isolates were found in stainless steel spoon observed in spite of its frequent usage during processing and consumption. The glass cup was able to harbour only one isolates. This is due to the fact that the glass is the weakest of all the materials observed in terms of biofilm formation capability. This is similar to the study of Kim et al. (2017) in which polypropylene (PP) and polyethylene (PE) showed greater biofilm formation ability than stainless steel (SS) and glass (G). The reason for having more biofilm formers on plastic materials in this study is that plastic materials are more hydrophobic in nature than SS and G. Pathogens tend to more readily attach and form biofilms on hydrophobic materials than on hydrophilic ones (Di Bonaventura *et al.,* 2008).During washing, the roughness of the food contact surfaces is increased by the friction between a scrubber and the utensils. This allows for the development of scratch or nicks that can create roughness on their surfaces (Rodriguez *et al.,* 2008; Tang *et al.,* 2011; Kim *et al.,* 2017). This roughness can lower the effectiveness of cleaning and sanitizing processes (Chaturongkasumrit *et al.,* 2011). Cutting boards with cuts and scratches are more difficult to clean than new and intact boards (Yang *et al.,* 2009); hence the higher occurrence of biofilm forming *S. aureus* on plastic surfaces (plates, eating bowls and cutting boards). However, the reason for highest occurrence of biofilm forming *S. aureus* among the plastic contact surfaces may not be unconnected with its frequent usage and the possibility of making cracks during cutting. Also microbes-laden foods such as raw meat and fish are cut into pieces on cutting boards. The frequent use of the microbes-laden foods coupled with the higher chance of making cracks in the course of cutting makes cutting board the highest priority for harbouring *S. aureus* with biofilm formation capability in this study.

CONCLUSION

Microbial biofilms enhance the bacterial ability of surviving mechanical stress and can cause problems in household kitchens. In fact, the persistence of biofilm on food contact surfaces may constitute a continuous source of contamination. The results in this study revealed the occurrence of *S. aureus* in food contact surfaces; but surfaces of plastic utensils harboured more isolates than stainless steel and glass utensils. However, there has been no relationship ($P < 0.05$) observed between the intensity of biofilm formation potential and the surface as isolates with strong biofilm formation was found in the surface with the weakest hydrophobicity, which in turn helps in attaining higher attachment to form biofilm. This is in spite of the fact that all the isolates obtained from stainless steel were weak biofilm formers. One isolates of *S. aureus* was found to be non-biofilm former and was isolated from plastic cutting boards. In order to minimize or eradicate the microbiological risks associated with biofilm in household kitchens, more hygiene practices should be introduced and appropriately applied. Moreover, the knowledge of how *S. aureus* forms biofilms on food contact surface is needed. Programmes showcasing the dangers associated with improper cleaning and sanitization as well awareness on the good hygiene practices is important.

Conflict of Interest

There was no conflict of interest among the authors of this research study.

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