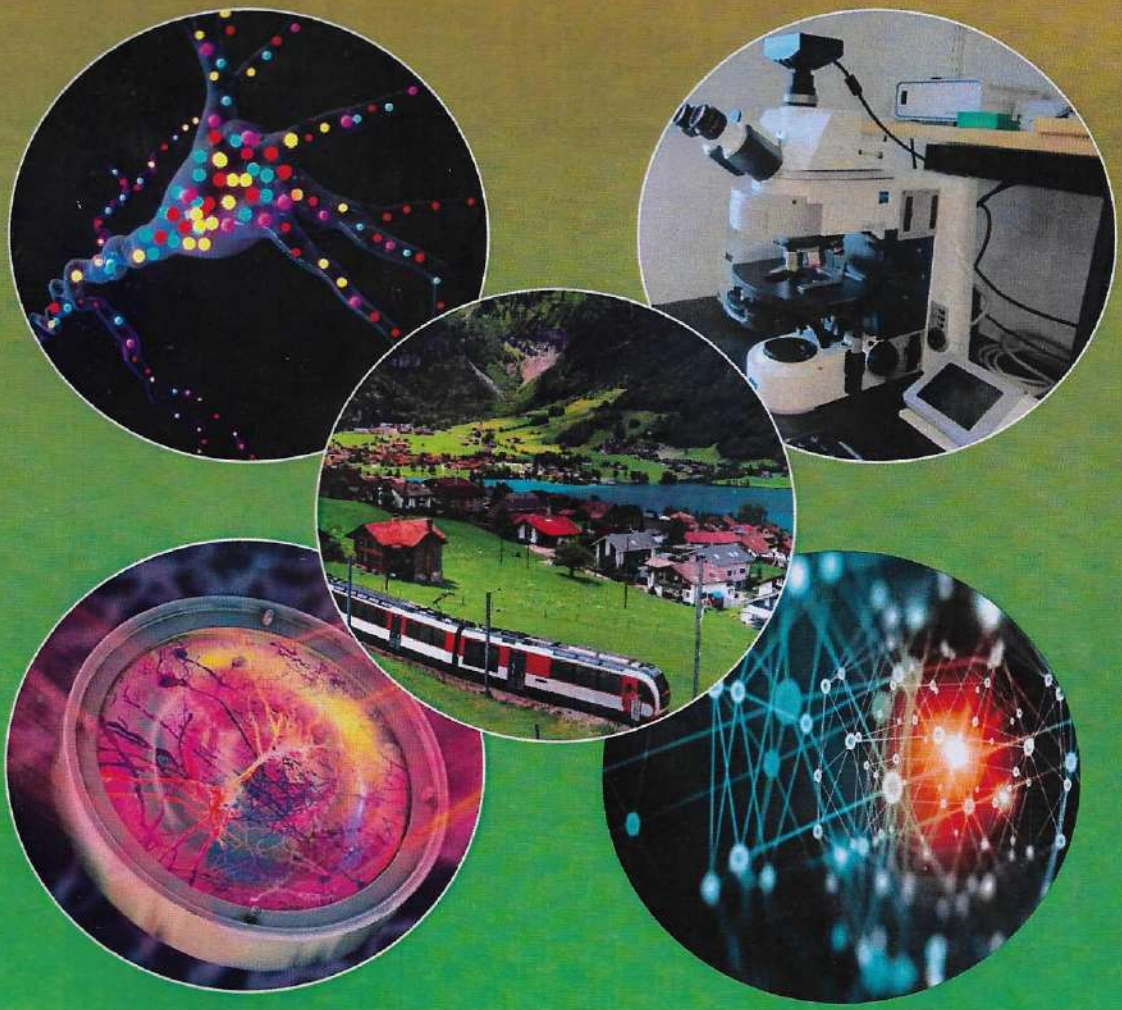
Third Edition: Vol. 1, No. 3, 2023

THE NIGERIAN JOURNAL OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES



A PUBLICATION OF THE FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES (FNSES)
GODFREY OKOYE UNIVERSITY
UGWUOMU NIKE, ENUGU STATE, NIGERIA

The Nigerian Journal of Natural Sciences and Environmental Studies
Godfrey Okoye University, Ugwuomu Nike, Enugu State, Nigeria

BIOFILM FORMING ABILITY AND THE PRESENCE OF *IcaD* GENE IN BACTERIA ISOLATED FROM BATHING TOWELS OF STUDENTS OF A PRIVATE TERTIARY INSTITUTION

By

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Abstract:

Background:

A towel is an absorbent fabric or paper used for drying or cleaning a body or wiping surface. The skin itself provides a large area for microbial colonization and hence the skin ecosystem is not uniform.

Aim:

The aim of this study was to determine the presence and nature of biofilm forming bacteria and the presence of icaD genes found on bathing towels.

Methods:

A total of 20 bathroom towels samples were used following the standard microbiological techniques. Colony morphology, Gram's staining and biochemical tests were used for isolation and identification of bacteria. Antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method and the Congo Red Agar was used for the screening of biofilm production. Finally, the detection of icaD gene was determined by PCR.

Results:

Nineteen(19) out 20 isolates were identified tentatively as Staphylococcus spp. and the other was identified as *E. coli* All the isolates produced biofilm. In particular, isolates 3, 5, 12, and 13 produced the most biofilm (strong). On the other isolates 15, 16, 18, 19 were moderate biofilm formers. Isolates 1,2,4,6,7,8,9,10,11,14, 17,20 were weak producers of biofilm.

Conclusions:

The findings from this study indicated that there is a high level of bacterial contamination on bathroom towels. This is of tremendous clinical significance, because of its potential to cause epidemics in school hostels. Moreover, the antibiotic susceptibility of isolates showed resistance to at least three antibiotics. Furthermore, it indicated a similar scenario in other places.

Keyword: Towels, Bacteria, Biofilm, icaD, Staphylococcus spp.

Article History

1. BACKGROUND

A towel is an absorbent fabric or paper used for drying or cleaning a body or wiping surface. It absorbs moisture through direct contact often either by using a rubbing or blotting motion.

Microbes strive in warm and moist environment that is full of oxygen under optimum temperature of (25°c-37'c) and PH of (5-9) (Liam and Hudson 2004).

The skin itself provides a large area for microbial colonization and hence the skin ecosystem is not uniform. The most common microorganisms found on the skin as its normal flora include *staphylococcus epidermis* (which is found mostly in the regions of the upper body), *staphylococcus hominis* (found on the arms and legs) and *micrococci* yellow pigmented species, some others include gram positive Bacilli belonging to a group of bacteria known as *Coryneform* which include *Corynebacterium and Propionibacterium.* Most organisms have the ability to spread on towels and are then infectious. Some include; *Escherichia coll* (Arch chemical 2012), *Salmonella typhimiiruim, Staphylococcus aureus. Bacillus aureus* (Spicer 1959), *Campylobacter* (Liam and Hudson 2004).

A biofilm may be defined as a microbe-derived sessile community featured under organisms that are attached to a substratum, interface or each other embedded in matrix of extracellular polymeric substance and exhibit an altered phenotype with respect to growth, gene expression and protein production.

The biofilm infection life cycle generally follows the same steps of attachment (which has to do with the interaction between bacteria and the implant) accumulation(which involes interactions between bacterial cells) maturation (formation of viable 3D structure) and dispersion/detachment (release from the biofilm).

2. METHODS

2.1 Collection of samples and Isolation of Bacteria

A total of 20 bathroom towels were swabbed to extract bacteria from the towels with the use of sterile cotton swabs. Once the sample was obtained, the swab was placed in a sterile 15ml centrifuge tube and placed in a 4°C refrigerator until further analysis. After 24 hours, each sample was streaked onto Nutrient agar, MacConkey agar, Mannitol salt agar and Membrane faecal coliform agar plates. Four-quadrant streak plate technique wasperformed. All the plates were incubated for 24 hours at 37°C. After the overnight incubation, the plates were observed for colony characteristics. Isolated colonies were then sub-cultured onto fresh nutrient agar. Single isolated colonies from nutrient agar plates were subjected to Gram staining, and Standard Biochemical tests to identify the organism.

2.2 Phenotypic Characterization of Biofilm Producers

Bacteria Isolates were incubated on a Congo red medium for 24-48hours at 37°e. Indication of black colonies represented positive results. The weak producers indicated tremains pink and then an intermediate result is indicated with the presence of occasional darkening at the center of the colonies present with absence of dry colonial crystalline morphology (Cappuccino & Sherman, 2005). Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide by producing the enzyme catalase. An immediate bubble formation indicated a positive result and no bubble formation indicated catalase negative result (Cappuccino &Sherman, 2005).

2.3 Antibiotic Susceptibility Testing

In this research work the antibiotic susceptibility testing of the organisms were performed by Kirby-3auer disc diffusion method.

2.4 Dna Extraction Using Zr Fungal/Bacterial Dna Miniprep (Manufactured By Zymo Research Cat Number: D6005)

uld 2mLs of bacterial cells broth to to a ZR lashingTM Lysis Tube. Add 750ul Lysis Solution) the tube. Secure in a bead fitted

with 2 ml tube-older assembly and process at maximum speed for >5 minutes. Centrifuge the ZR BashingBeadTM ysis Tube in a microcentirifuge at > 10,000 x g for minute. Transfer up to 400 ul supernatant to a ymo-SpinTM IV Spin Filter (orange top) in a oliection Tube and centrifuge at 7.000 x g for 1 inute. Add 1,200 ul of Fungal/Bacterial DNA inding Buffer to the filterate in the Collection jbe from Step 4. Transfer 800 ul of the mixture)in Step 5 to a Zymo-SpinTM HC Column in a Election Tube and centrifuge at 10,000 x g for 1 inute. Discard the flow through from the Election Tube and repeat Step 6. Add 200 ul NIA Pre-Wash Buffer to the Zymo-Spin TM IIC

Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute. Add 500 ul Fungal/Bacterial DNA Wash Buffer to the Zymo-SpinTM IIC Column and centrifuge at 10,000 x g for 1 minute. Transfer the Zymo-SpinTM IIC Column to a clean 1.5 ml microcentrifuge tube and add lOOul (35 ul minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

2.5 Electrophoresis for DNA and PCR

Measure 1 g of agarose (for DNA); 2g of agarose for PCR. Mix agarose powder with 100 mL IxTAE in a microwavable flask. Microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins. Add 10µL EZ vision DNA stain. EZ vision binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light. Pour the agarose into a gel tray with the well comb in place. Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

2.6 Loading Samples and Running an Agarose Gel

Add loading buffer to each of your DNA samples or PCR products. Once solidified, place the agarose gel into the gel box (eleetrophoresis unit). Fill gel box with IxTAE (or TBE) until the gel is covered. Carefully load a molecular weight ladder into the first lane of the gel. Carefully load your samples into the additional wells of the gel. Run the gel at 80-150 V for about 1-1.5 hours. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

Visualize DNA fragments or PCR product under UV transilluminator.

2.7 PCR Mix Components

The PCR mix is made up of *12.5µL* of Taq 2X Master Mix from New England Biolabs (M0270); 1 µL each of

1 0µM forward and reverse primer; *2µL* of DNA template and then made up with 8,5µL Nuclease free water. Initial denaturation at 94'C for 5mins, followed by 36 cycles of denaturation at 94°C for 30sec, annealing at 55°C for 30secs and elongation at 72°C for 45sec. Followed by a final elongation step at 72°C for 7 minutes and hold temperature at 10 °C forever.

3. RESULTS

Out of the 20 presumptive isolates, nineteen (100%) were identified tentatively as *Staphylococcits* spp. The isolates were all resistant to cefiazidime (100%), cefuroxime (100%), gentamycin (20%), ceftriaxone (100%), erythromycin (90%), cloxacillin (100%), ofloxacin (10%) and augmentm (100%). A moderately high susceptible activity was observed against gentamicin (30%) and augmentin (35%). Moderate sensitivity activity was observed in gentamicin (50%), erythromycin (10%), while a high level of susceptibility was observed in ofloxacin (55%).

All the isolates (1-20) produced biofilm. In particular, isolates 3, 5, 12, and 13 produced the most biofilm (strong). On the other isolates

15, 16, 18, 19 were moderate biofilm formers. Isolates 1,2,4,6,7,8,9,10,11,14, 17, 20 were weak producers of biofilm. There is a significant difference between the three groups ($p < 0.05$) as shown in appendix 11, The gDNA extracted from the isolates is presented in figure 1 while Figure 2 showed *icaD* genes in isolates. The figure suggests absence of these genes in 3 and 4.

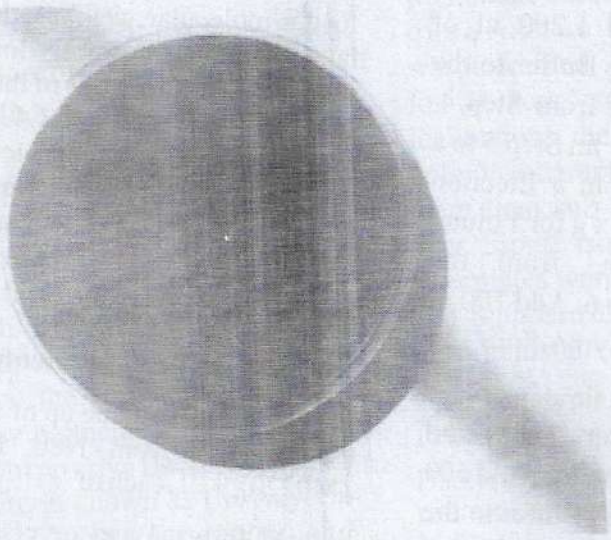

Fig 1: Biofilm production by S. *aureus;* Strong producer and on Congo Red Agar

Table 1: Biofilm producer isolates on Congo red agar (CRA)

Isolate	Biofilm formation		
	Strong biofilm	Moderate biofilm	Weak biofilm
1	−	−	+
2	−	−	+
3	+	−	−
4	−	−	+
5	+	−	−
6	−	−	+
7	−	−	+
8	−	−	+
9	−	−	+
10	−	−	+
11	−	−	+
12	+	−	−
13	+	−	
14	−	−	+
15	−	+	−
16	−	+	−
17	−	−	+
18	−	+	−
19	−	+	−
20	−	−	+

KEY;

+ = Strong biofilm producers

- = Non biofilm produced

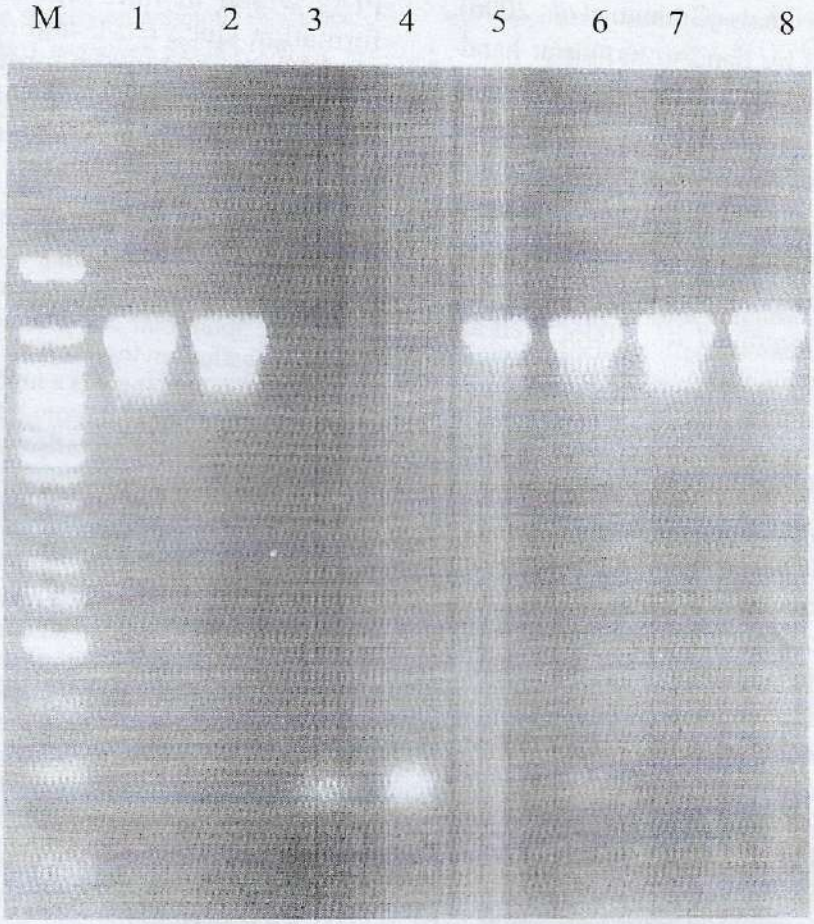

Figure 2: *icaD* genes in isolates, absent in 3 and 4

4. DISCUSSION

The result obtained from this study was out of 20 samples after conducting the biochemical tests, 19 of the isolates were confirmed presumptively as *Staphylococcus'* spp. and 1 was identified as *E. coli.* Therefore, in this study, among the isolates, the most predominant bacteria were *Staphylococcus* spp. This is anticipated as it is a major component of the normal flora of the human skin which the bathroom towel is used for. The findings of other researchers (Nworie *et al.,* 2012; Ducel *et al,* 2002; Brooks *et al.,* 2007), is in accordance with this finding.

The result of this study is also consistent with Jalalpoor et ah, (2009) who reported that *Staphylococcus species* (54.7%) was the most frequent bacteria isolated in bathroom towels and particularly bathroom environment. In contrast, the result of this study did not agree with the work of Orji *et al,* (2005) which showed that *Staphylococcus aureus* was the least isolated bacteria.

Gram-positive bacteria are found more in the bathroom towels than Gram negative one. This can become dangerous as Gram positive bacteria are causing more infections than ever before especially in surgical patients, who are increasingly aged, ill and debilitated (Barie, 1998).

Isolation of more Gram positive bacteria than Gram negative can be explained, as they are members of the body flora of both asymptomatic carriers and sick persons. These organisms can be spread by the hand, expelled from the respiratory tract or transmitted by animate or inanimate objects (Chikere *el a!.,* 2008). Their main source(s) of colonization on the bathroom towel might likely be nasal

carriage by individuals (Graham *el al.*, 2006), likely facilitated by hand-to-mouth or hand-to-nose contact while using these fabrics (ASM, 2005). Isolation of *Staphylococcus aureus* from almost all the bathroom towels indicates their ubiquitous nature. Additionally, they can be sources of infection to humans as previously noted (Hartmann *el al.*, 2004; Inweregbu *e1 a!.*, 2005; Ikeh and Isamade, 2011).

From the findings in this study, it was observed that most of the isolates obtained were resistant to most commonly used antibiotics. These antibiotics are ceftazidime (100%), cefuroxime (100%), gentamycin (20%), ceftriaxone (100%), erythromycin (90%), cloxacillin (100%),ofloxacin (10%) and augmentin (100%). The resistance to these antibiotics which is in accord with the research earned out by Adewoyin *el al.*,(2013), who reported that antibiotic resistant microorganism contaminates fabric surfaces such as bathroom towels. Moreover, reported that most of the isolates obtained in their study were resistant to commonly used antibiotics such as ceftazidime, gentamycin, augmentin and erythromycin.

The biofilm producing ability of the isolates was also studied using the CRA plate test method (Handke *el al.*, 2004; Oliveira *el al*, 2006). All the isolates (1-20) produced biofilm. In particular, isolates 3, 5, 12, and 13 produced the most biofilm (strong). On the other isolates 15, 16, 18. 19 were moderate biofilm formers. Isolates 1,2,4,6,7,8.9,10,11,14, 17, 20 were weak producers of biofilm. There is a significant difference between the three groups ($p < 0.05$). This is consistent with the findings by Stewart and Costerton, (2001) and Ito *el al.*, (2009) who documented that the structure of biofilm in *Slaphylococcus* spp including the robustness and its components show association with antibiotic resistance. Also, Arciola *el al.*, (2015) reported that intercellular adhesion *(ica)* genes encode PIAs which in turn regulate the biofilm formation since *icaA.* and *icaD* genes are associated with biofilm formation. Biofilm production was shown by isolates on Congo Red Agar and presence *oficaD* gene.

CONCLUSION

Recently, infections from bathroom fabrics particularly towels are rising at an alarming rate. The causes of these infections can be connected to increased microbial load of bathroom fabric including bathroom towels. The findings from this study indicate that there is a high level of bacterial contamination on bathroom towels. This is of tremendous clinical significance, because of its potential to cause epidemics in school hostels. Moreover, the antibiotic susceptibility of isolates showed resistance to at least three antibiotics. Furthermore, it indicated a similar scenario in other places. The rise of antibiotic resistance in microbes, especially pathogenic organisms can lead to lethal outcomes. Therefore, it should be tackled with high importance. However, this problem is not limited to this area of study alone. Thus, this will require combined effort of governmental, private organizations and individuals to educate the population on personal and environmental hygiene.

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