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Variation in Biofilm-Forming Potential of *Staphylococcus aureus* from Clinical and Non-Clinical Sources

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KO and OEA designed the study. Author JJJ carried out most of the lab work. Author KO wrote the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Biofilm forming ability has been described as a potential marker of pathogenicity, particularly in *Staphylococcus aureus*. These biofilms are notable as an important contributor to virulence abilities, further aiding the producing strain in long term survival and resistance to antimicrobial agents. Regional data exploring biofilm forming ability of *S. aureus* from various sources is limited. This study therefore set out to explore variations in biofilm-forming potential of *S. aureus* from clinical and non-clinical sources.
Place and Duration of Study: Medical Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, Nigeria from August to October 2019.
Methodology: Eighty five *S. aureus* clinical and non-clinical isolates were studied. Biofilm-forming potential was assessed using the Congo Red agar (CRA) method which describes both the presence and degree biofilm-forming potential.
Results: Majority of isolates (65.9%) did not exhibit any biofilm-forming potential using the CRA method. Biofilm-forming potential however appeared source based with 100% of non-clinical *S. aureus* isolates lacking biofilm-forming potential, while 58% of clinical isolates showed biofilm-

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forming potential. A higher proportion (65.5%) of the clinical isolates exhibiting biofilm-forming potential where associated with strong biofilm-forming potential.
Conclusion: This study reports a high association of biofilm-forming potential with *S. aureus* isolated from clinical rather than non-clinical settings. If this characteristic can indeed be used as a general marker of pathogenicity would however require more extensive studies.

Keywords: Biofilm-forming; CRA; Staphylococcus aureus; Nigeria; pathogenicity.

1. INTRODUCTION

Though notable as one of the two main causative agents associated with human bacterial infections [1], *Staphylococcus aureus* is also a known commensal, found in association with various systems of the body without causing harm [2]. In addition to being a leading bacteria isolated in clinical microbiology practice, *S. aureus* has also been widely isolated from non-clinical sources such as food, water, environment and inanimate surfaces. *S. aureus* is notorious for its repertoire of associated virulence genes encoding staphylococcal enterotoxin, exfoliative toxins, hemolysins and toxic shock syndrome toxin [3].

Studies on the determining factors differentiating pathogenic and commensal strains of *S. aureus* are still widely ongoing. Several studies found a higher association of staphylococcal enterotoxin and enterotoxin-like genes with clinical strains of *S. aureus*. Aung and colleagues reported prevalence rates ranging from 5.6% to 92.9% [4]. Li and colleagues studying 11 different virulence genes, noted prevalence rates ranging from 15.4% to 100%. Though for 8 of the 11 genes assayed for, prevalence rates were above 35% [5]. A 2016 study, on 14 virulence genes in clinical isolates similarly noted prevalence rates ranging from 3.2% to 100%. Rates above 35% were found to occur only in 6 of the virulence genes [6]. This was opposed to a lower association of these genes with strains of *S. aureus* from food, animals and the environment [7]. Chao and colleagues noted a significantly higher ($P<0.01$) representation of classic staphylococcal enterotoxin genes in foodborne and human isolates than in animal isolates [8]. A 2019 study on enterotoxin carriage in isolates from food handlers, reported rates ranging from 2.7% to 40.2% [9].

Characterization of *S. aureus* from fish revealed prevalence rates ranging from 3 to 12% [10]. While a recent study reporting on isolates from ready to eat foods noted prevalence rates ranging from 18.8% to 56.3% [11]. Much lower prevalence rates (0% to 21.8%) were noted in a study on *S. aureus* isolated from insects [12]. And other studies have noted a variation in gene expression levels rather than in the gene presence [13].

Biofilm forming ability is one of the different characteristics which have been examined in exploring differences between commensal and pathogenic strains of *S. aureus* [14]. Biofilms are microbial communities occurring within a self-produced extracellular polymeric substance (EPS) matrix composed of cells adhered to each other and a solid surface [15]. These biofilms are notable as an important contributor to virulence abilities, further aiding the producing strain in long term survival and resistance to antimicrobial agents [16-19]. Biofilm forming ability has even further been described as a potential marker of pathogenicity particularly in *S. aureus* [14,20]. Of all the studies corroborating these facts, few have been carried out in Africa with only a handful of studies exploring biofilm forming potential with relation to source of isolates in Nigeria. Regional data is key because despite general trends, sometimes regional variations occur. This study therefore set out to explore variations in biofilm forming potential of *S. aureus* from various sources in a bid to highlight possible links to pathogenicity.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

Test bacterial isolates used in this study comprised a total of 85 *S. aureus* isolates. These isolates were obtained from the bacterial collection of the Bacteriology group, Medical Microbiology Unit, University of Port Harcourt. Isolates were stored in agar stab cultures at -4ºC, and comprised of 35 non-clinical isolates and 50 clinical isolates. The identities of the isolates were confirmed using standard phenotypic biochemical test methods [21,22].

2.2 Analysis of Biofilm Forming Potential

The biofilm forming potential of the clinical and non-clinical *S. aureus* was assessed using a

previously described Congo Red agar (CRA) method [23]. In brief, this simply involved the culture of purified test isolates on Congo red agar plates. Following a 24 hour incubation at 37°C, isolates exhibiting biofilm forming abilities show up as black colonies, while red colonies are indicative of isolates lacking biofilm forming potential. Further, based on intensity of black pigmentation, isolates exhibiting biofilm forming potential could then be classed as having strong, moderate or weak potential [24,25].

3. RESULTS AND DISCUSSION

3.1 Results

An analysis of the biofilm-forming potential of the test isolates revealed that majority of the isolates (53/85, 65.9%) did not exhibit any biofilm-forming potential using the CRA test method (Fig. 1).

A further assessment of biofilm-forming potential however showed that the higher proportion of test isolates lacking biofilm-forming potential was related to source, as biofilm-forming potential was not detected in any of the non-clinical isolates (Fig. 2). For the clinical isolates, the majority of isolates (29/50, 58%) actually exhibited biofilm-forming potential.

Furthermore, a higher proportion (19/29,65.5%) of the clinical isolates exhibiting biofilm-forming potential where associated with strong biofilm-forming potential (Fig. 3).

3.2 Discussion

Reports of *S. aureus* isolation are very widespread, with the organism found in association with various different samples. This organism is however both a leading cause of infection and a human commensal with human anterior nares as the primary reservoir of these organisms [26]. It is often difficult though to tell whether a specific strain of *S. aureus* is a commensal or a pathogen. Biofilm formation has however been associated with the pathogenicity of *S. aureus*. Studies assessing biofilm-forming potential in *S. aureus* using the Congo red agar method have reported varying rates ranging from 1.9% to 94% [27-33].

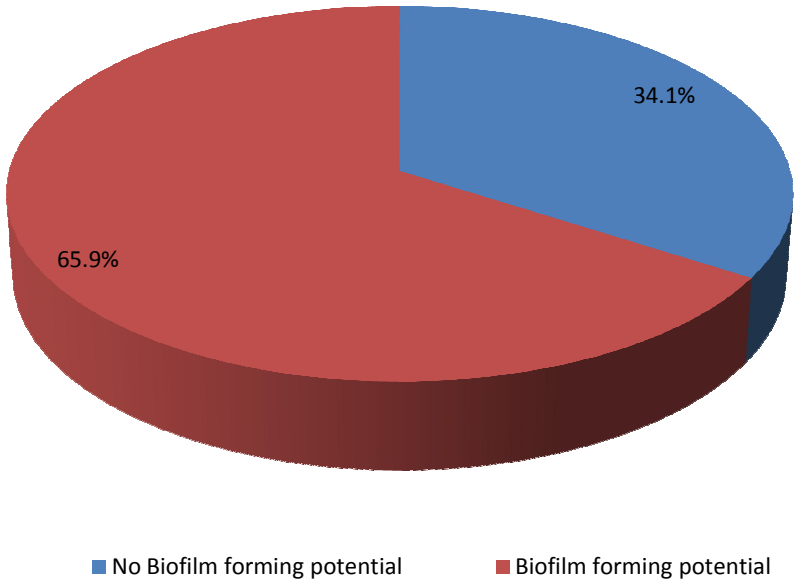

Fig. 1. Assessment of biofilm-forming potential of *S. aureus* isolates

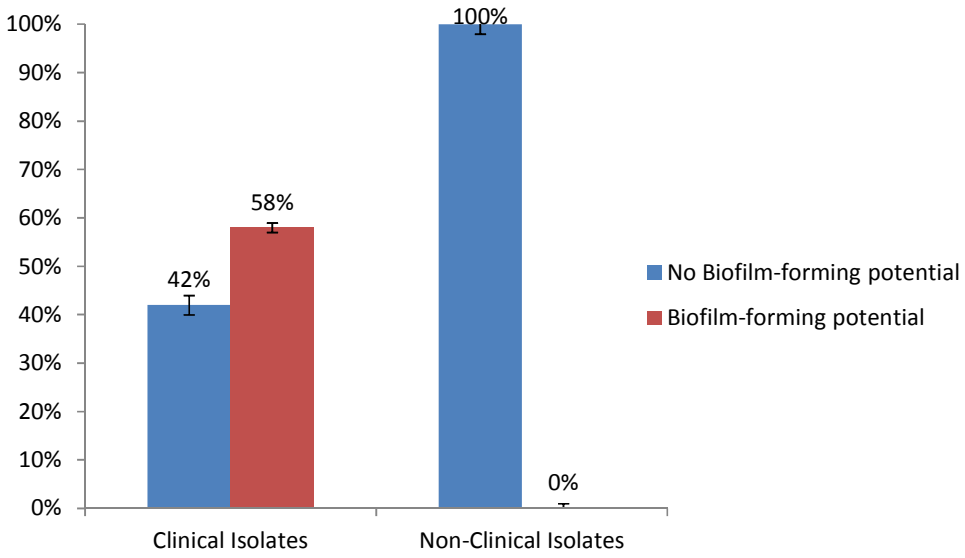

Fig. 2. Source based effect on biofilm-forming potential

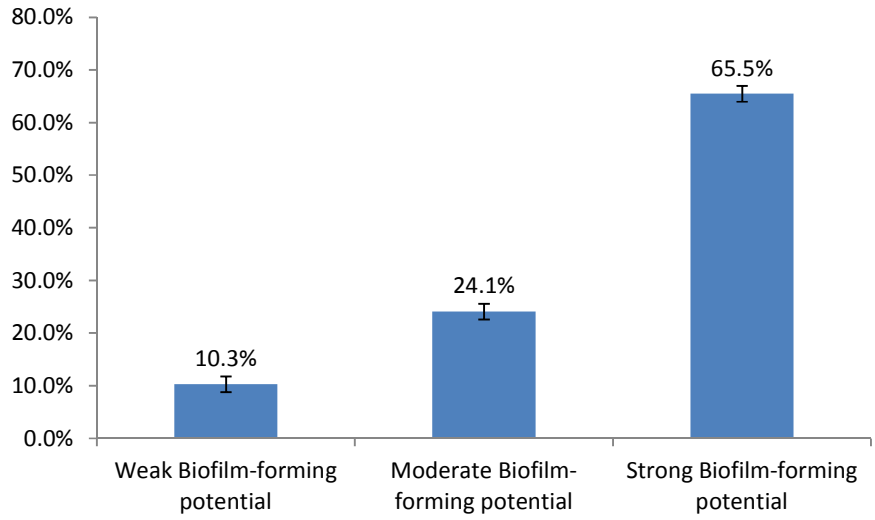

Fig. 3. Variation in degree of biofilm-forming potential

Majority of these studies involved only clinical isolates though some studied isolates from lab coats of medical students and another looked at organisms from surfaces in a dental clinic. The 58% rate of *S. aureus* isolates exhibiting biofilm-forming potential in this present study is more closely related to reports by Torlak and colleagues (46.9%) who studied *S. aureus* from surfaces in a dental clinic [29], and Khan and colleagues (47.71%) who looked at clinical *S. aureus* isolates in general [27]. It is also similar to studies carried out in Nigeria which reported a 52.7% and 64% rate of biofilm-forming potential in clinical *S. aureus* isolates [33,34].

All these studies looked at *S. aureus* isolated from a variety of clinical sources and this could have had an impact on the variation in biofilm-forming potential observed. Ocal and colleagues had previously reported a significant relationship between invasive isolates and biofilm-forming potential [35]. These assertions were

corroborated by a more recent study observing that biofilm formation correlates with infection type [36]. Worryingly however, there have also been reports of a high association of biofilm-forming potential (61%) with *S. aureus* isolated from nasal cavities of healthy volunteers [37].

Studies exploring biofilm-forming potential in organisms from non-clinical sources were surprisingly lacking indicating an unexplored area of research.

4. CONCLUSION

This study reports a high association of biofilm-forming potential with *S. aureus* isolated from clinical rather than non-clinical settings, perhaps pointing at a role for biofilms in pathogenicity. These findings corroborate reports from other parts of the world. Further studies would however be needed to see if these findings are unique or representative of the Nigerian story.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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