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Biofilm Forming Potential of *Escherichia coli* from Various Sources

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Abstract

Majority of bacterial communities exist as biofilms and these contribute to the survival of the bacteria. Biofilm development has been associated with protection from adverse environmental conditions and resistance to harmful agents. Generally, however data on biofilm-forming potential of bacteria in Nigeria is sparse. This study was therefore aimed at analyzing variations in biofilm-forming potential of *Escherichia coli* from various sources in Port Harcourt, Nigeria. Previously characterized clinical (30) and non-clinical (30) *E. coli* isolates were assessed for their biofilm-forming potential using the Congo Red agar method and variations in this potential determined as weak, moderate or strong. Majority of isolates (67%) had the potential to form biofilms but only 40% of isolates exhibiting biofilm-forming potential were from clinical sources. Isolates exhibited variable degrees of biofilm-forming potential, with only non-clinical isolates exhibiting strong potential. Majority of both clinical and non-clinical isolates (68.7% and 88% respectively) exhibited moderate biofilm-forming potential. The higher occurrence of *E. coli* exhibiting biofilm-forming potential among non-clinical isolates possibly reflects the essential role biofilms play in the survival of bacteria in nature, but not in infection cases. This study reports on a high level association between the isolates and biofilm production and highlights differences in the abilities of biofilm production between clinical and non-clinical isolates.

Keywords: *Biofilm Potential, Escherichia coli, Congo Red Agar Method, Clinical vs Non-clinical*
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I. INTRODUCTION

Biofilms are protective communities of bacteria surrounded by extracellular polymeric substances (EPS) which are formed on surfaces, multicellular in nature, sessile and have a complex structure (Sauer *et al*., 2007). Majority of bacterial communities exist as biofilms and contribute to the survival of the bacteria. In nature, these biofilms act as a protection from adverse environmental conditions such as desiccation, chlorinated water, oxidative and osmotic stresses, as well as protects from protozoan predators (DEPas *et al.,* 2014). In clinical settings, biofilm associated bacteria have also been noted to play a key role in infections particularly in chronic, nosocomial and medical device associated infections such as catheters.

In particular, *E. coli* existing as biofilms has been described as a major contributor to the disease causing ability of pathogenic strains, particularly as a major cause of recurrent urinary tract infections associated with medical devices such as catheters (Sharma *et al.,* 2016). Biofilm formation has been associated with resistance to host immune responses, persistence and antimicrobial resistance (Adamus-Bialek *et al.,* 2015; Bajpai *et al.,* 2016). Potentially, all of these could play a role in increased levels of morbidity and mortality.

A recent study noted that an extensive focus in biofilm studies has been on laboratory rather than pathogenic strains (Schiebel *et al.,* 2017). The study went on to assess a collection of pathogenic *E. coli* isolates and found a high association of biofilm-forming capabilities with a specific group of *E. coli*, the enteroaggregative *E. coli* (EAEC) using the Congo Red Agar method. Generally, however data on biofilm-forming potential of bacteria in Nigeria is sparse. An assessment of biofilm-forming potential of bacteria in this specific locale will be the first step in extensive biofilm

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studies aimed at elucidating pathogenesis and eventually instituting adequate control measures. This study was therefore aimed at analysing variations in biofilm-forming potential of *E. coli* from various sources in Port Harcourt, Nigeria.

II. Materials And Methods

A. Bacterial Isolates

E. coli isolates used in this study were obtained from the bacterial collection of the Bacteriology group, Medical Microbiology Unit, University of Port Harcourt. These isolates consist of sixty previously characterized clinical (30) and non-clinical (30) *Escherichia coli* (Otokunefor *et al.,* 2019).

B. Detection of Biofilm-forming potential

Biofilm-forming potential of isolates was determined using the Congo Red agar method (Ochi *et al.,* 2020; Mathur *et al.,* 2006). Purified bacterial isolates were subcultured on Congo red agar plates and incubated for 24 hours at 37°C. The biofilm producing ability and the degree were determined as previously described. In brief, isolates producing black colonies are noted as biofilm formers, while non-biofilm formers produce red colonies. Biofilm producers were then further categorized as strong, moderate or weak biofilm formers according to the intensity of the colour of the colonies as previously described (Darwish and Asfour, 2013).

C. Statistical Analysis

Student t-test was used to analysis the data generated at 5% (0.05) significance level using IBM® SPSS® Statistics version 20.0.

III. Results

A. Determination of biofilm-forming potential

An assessment of the biofilm-forming potential of the *E. coli* isolates revealed that the majority of isolates, (67%) had the potential to form biofilms (Figure 1). Non-clinical isolates however made up this majority of biofilm-forming isolates (Figure 2), though the difference was not statistically significant (P = 0.2396, two-tailed T test). Only 40% of isolates exhibiting biofilm-forming potential were from clinical sources. For both sets of isolates, the occurrence of isolates with biofilm-forming potential was higher than those without biofilm-forming potential (Figure 3). Unlike the non-clinical isolates where a 60% difference was noted in occurrence levels, for the clinical isolates the difference was only 6.8%. This variation in biofilm forming ability between clinical and non-clinical bacteria was statistically significant with a P value of 0.030.

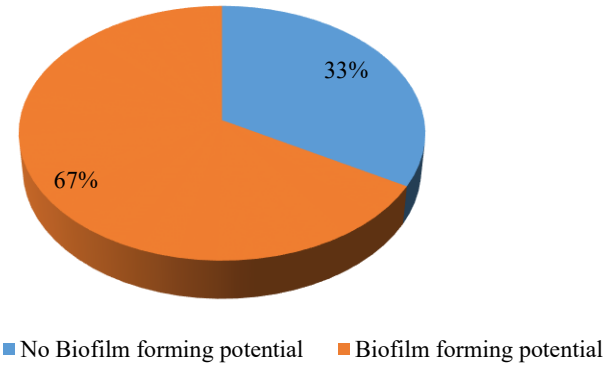


Figure 1. Biofilm-forming potential of *E. coli* isolates

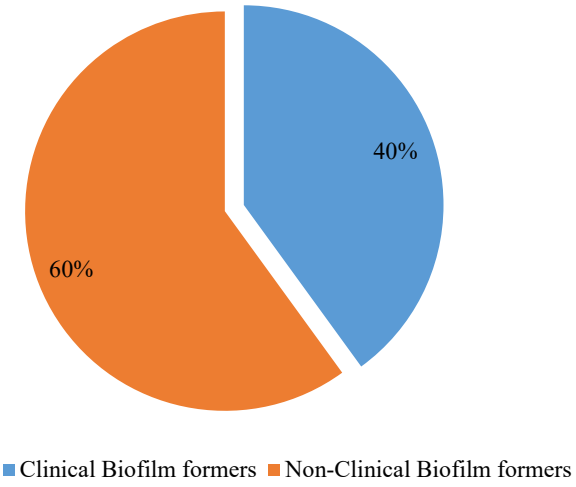


Figure 2. Effect of isolate source on Biofilm-forming potential

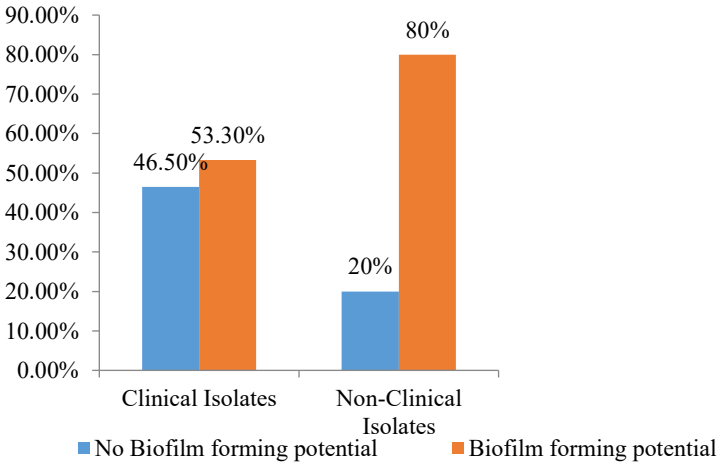


Figure 3. Variation of biofilm-forming potential based on source

Despite the majority of isolates exhibiting biofilm-forming potential, the degree of this potential varied from weak to strong (Figure 4). Only non-clinical isolates exhibited strong biofilm-forming potential (Figure 5), while majority of both clinical and non-clinical isolates exhibited moderate biofilm-forming potential. A higher ratio of bacteria exhibiting a weak biofilm-forming potential was noted among the clinical rather than non-clinical isolates. These variations were however not statistically significant (P = 0.9651).

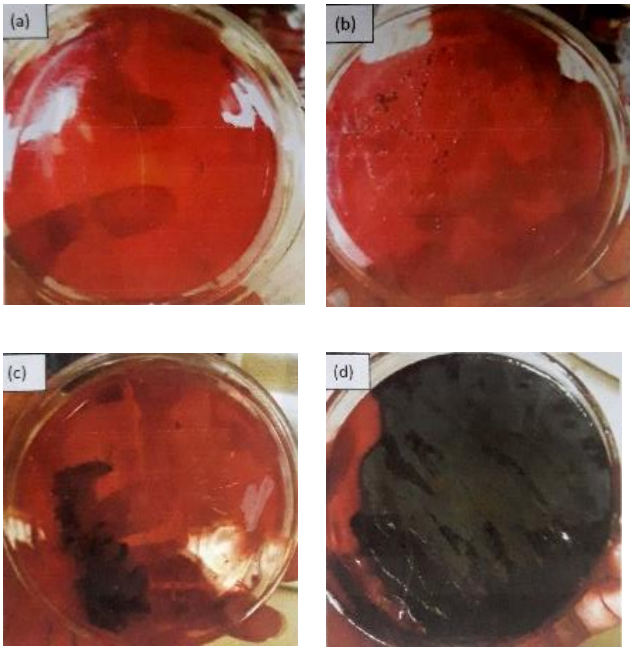


Figure 4. Variation in levels of biofilm-forming potential where (a.) no biofilm-forming potential; (b.) weak biofilm-forming potential; (c.) moderate biofilm-forming potential and (d.) strong biofilm-forming potential.

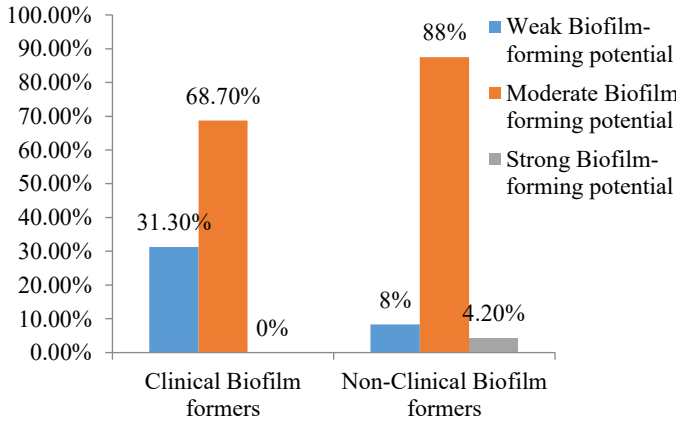


Figure 5. Effect of source on degree of biofilm-forming potential

IV. Discussion

Biofilm formation has long been associated with survival of bacteria both in nature and clinical settings. Studies have previously reported that the majority of bacteria are found in association with biofilms rather than free-living (Donlan and Costerton, 2002). Results of this present study noting a 67% occurrence of bacteria with biofilm-forming abilities agree with this. This study however noted a higher representation of non-clinical isolates among the isolates exhibiting biofilm-forming potential. In nature, biofilms are particularly advantageous as they play an essential role in the ability of the bacteria to survive (DEPas *et al.,* 2014). Furthermore, environmental stresses have been reported to enhance the production of biofilms (Barbosa *et al.,* 2013). Therefore, the 80% occurrence of isolates with biofilm-forming potential among the non-clinical isolates is probably a reflection of adaptive mechanisms which these bacteria have adopted to avoid destruction.

Biofilms in clinical settings give the bacteria an increased chance of resistance. It may however not particular be essential in the initial colonization and invasion of the host which is the first step in pathogenesis. This lack of necessity of biofilms for the survival of clinical isolates is probably why a lower proportion of clinical isolates were found to possess biofilm-forming potential. The value associated with biofilm formation in clinical isolates is similar to the reported values of microbial infections associated with biofilm formation. Costerton *et al.,* 1999 has been reported as describing a more than 50% association with bacterial infections (Hancock *et al.,* 2007), while another review mentions a more than 60% association (Moscoso *et al.,* 2009). Another study assessing biofilm formation in clinical isolates noted varying potential. Invasive isolates had the highest potential (79%), followed by colonizers (73%), with only 28% of commensals exhibiting this potential (Agarwal and Jain, 2013). Iorio and colleagues (Iorio *et al.,* 2011) evaluating biofilm production in isolates from blood culture detected 85% of biofilm producers among their isolates. Banerjee and colleagues studying Enterobacteriaceae from healthy ducks noted biofilm-forming potential of 46.15% in Salmonella, 46.79% in *E. coli* and 70% in *Klebsiella pneumoniae* (Banerjee *et al.,* 2019). The present study simply provides information on biofilm-forming potential of clinical isolates in general without a comparison with the specific role the isolates played in the clinical setting. This might contribute to the much lower figures observed.

Results of this study showing majority of the potential biofilm producing clinical isolates exhibiting moderate or weak biofilm producing abilities were similar to that of a previous study (Tajbakhsh *et al.,* 2016). This study on biofilms in clinical isolates of *E. coli* observed that 81.25% of isolates exhibited either moderate or weak biofilm reaction.

V. CONCLUSION

This work reports on a high level of association between *E. coli* isolates and the ability to form biofilms. Additionally, it highlights differences in the abilities of biofilm production between clinical and non-clinical isolates.

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