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Research Article

Comparative Assessment of Biofilm production capability of *Staphylococcus aureus* Isolated from Clinical and Community Settings in Kano Metropolis, Nigeria

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ABSTRACT

Biofilm are structured communities of microorganisms embedded in a self-produced matrix that enhances survival under extreme and harsh conditions. In *Staphylococcus aureus*, biofilm formation contributes to persistent infections and increased resistance to antibiotics. This study aimed to assess the biofilm production capacity of *S. aureus* isolated from clinical and community settings within Kano Metropolis using Tissue culture method and Tube method. Sixty (60) *S. aureus* isolates were obtained from patients, healthcare workers, apparently healthy individual, food handlers and surgical equipment. Methicillin resistance was determined using EUCAST disk diffusion method (Cefoxitin 30 µg) and Biofilm production was assessed using both TCP and Tube methods. Among the 60 *Staphylococcus aureus* isolates, 24 (40.0 %) were MRSA and 36 (60.0 %) were MSSA. TCP method detected biofilm production in 43 isolates (71.70 %) while TM identified 31 (51.70 %) biofilm producers. MSSA showed higher frequency of biofilm production compared to MRSA using both TCP and Tube methods. Biofilm production was most prevalent among isolates obtained from surgical equipment (100 %) and less among isolates obtained from healthcare workers (57.1 %). TCP methods was more sensitive than Tube Method in detecting biofilm production, there is a slight agreement (k~0.15) between Tissue culture plate method and Tube method suggesting that the methods classified the isolates differently. MSSA isolates had slightly higher biofilm production tendency and surgical equipment appears to be a significant reservoir for biofilm producing *S. aureus* underscoring the importance of infection control practices.

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INTRODUCTION

Staphylococcus aureus is a major opportunistic pathogen responsible for wide range of infections, including skin and soft tissue infections and infections associated with implanted medical devices (Puja *et al*., 2018). *S. aureus* is found to cause biofilms and these biofilm productions provide them mechanism to resist most conventionally used antibiotics use to treat such infections (Taj *et al*., 2011, Abdel-Halim *et al*., 2018). Biofilm associated infections are particularly challenging in healthcare setting and are linked to poor treatment outcomes and prolong hospital stay (Moormeir *et al.,* 2013). Methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) differ in their genetic and phenotypic profiles, including biofilm production capabilities, understanding the biofilm production potential of these isolates is essential for improving infection prevention strategies. Also, Biofilm production in both MRSA and MSSA strains plays a significant role in their ability to survive within hosts and in the surrounding environment. In *Staphylococcus aureus*, biofilm production is primarily driven by the production of polysaccharide intercellular adhesin (PIA) (Piechota *et al*., 2018). Biofilm production among bacterial isolates can be detected using several methods, however this study adopts the use of TCP and Tube methods as TCP is considered the gold standard due to its quantitative nature and TM is considered due to its simplicity and is widely adopted. Comparative data on the performance of these two methods, particularly in resource limited setting like Kano is unavailable. The study aimed to assessed and compare the biofilm production capacity of *S. aureus* isolated from both hospital and community setting and also detect the relationship between methicillin resistance and biofilm production providing insight that may inform infection control practices and antimicrobial stewardship.

MATERIALS AND METHODS

Study area and Design

The study design was cross-sectional and the study was conducted in the Department of Microbiology laboratory Bayero University Kano. The study areas were Bayero University Old campus and Rimi Market (Community) and Aminu Kano teaching hospital and Murtala Muhammad Specialist hospital (Hospital).

Ethical Consideration

Ethical clearance was sought out from Aminu Kano Teaching Hospital (Reference: AKTH/MAC/SUB/12A/P-3/VI/2078) and Kano Hospital Management board for Murtala Muhammad Specialist Hospital Kano (Reference: MOH/OFF/797/T.I/2053) before the commencement of the study and the consent and approval of patients and apparently healthy volunteers and food handlers were obtained before enrollment in the study.

Sample Collection

Sixty swab samples were collected at random using sterile moist swabs and transported on ice to the Department of Microbiology Bayero University Kano for processing. Sample sources included Healthcare workers (n=28), apparently healthy individual (n=13), food handlers (n=9), surgical equipment (n=7) and patient (n=3).

Isolation and Identification of *S. aureus*

Samples were initially inoculated on Tryptone Soya Agar (CM-TSA107 Rapid labs, England) and were incubated at 37 ˚C for 24 hours after which the isolates were sub-cultured on Mannitol salt agar (MH118-500G HIMEDIA, India). All isolates were confirmed morphologically by Gram staining and biochemically by catalase and coagulase test (Chessbrough, 2009).

Antibiotic Susceptibility test

Methicillin resistance of *S. aureus* isolates was determined using Cefoxtin disc (30 µg) following EUCAST 2019 guidelines for disk diffusion method (EUCAST, 2019).

Biofilm Detection

Tissue Culture plate (TCP) Method

Quantitative detection of biofilm production of *S. aureus* isolates was carried out according to Abdel-Halim *et al.* (2018) with minor modification. A loopful of an overnight culture of the bacterial isolates were added to 96-well microtiter plates and tryptone soy broth supplemented with glucose (TSBglu) was added to the wells and incubated for 24 hours, after incubation, the plates content was emptied and stained with crystal violet and absorbance was measured at 620nm. Biofilm production was categorized as non-producers (OD < 0.120), Moderate (OD= 0.120-0.240) or strong (OD> 0.240).

Tube Method (TM)

Qualitative detection of Biofilm was carried out using Tube method according to Saha *et al* (2014). The test was carried out in a glass test tube containing 10 ml of Tryptone Soy Broth (TSB) supplemented with 1 % glucose and a loopful of an overnight culture was inoculated into the test tube and incubated at 37 ˚C for 24 hours. The tubes are emptied and stained with crystal violet. Biofilm was assessed visually by two independent observers.

RESULTS

Identification of MRSA and MSSA

All isolates were confirmed as *S. aureus* through cultural and biochemical test. Cefoxitin 30 µg was used to determine methicillin resistance of which 24 isolates (40.0 %) were methicillin resistant S. *aureus*

(MRSA) and 36 isolates (60.0 %) were methicillin susceptible *S. aureus* (MSSA).

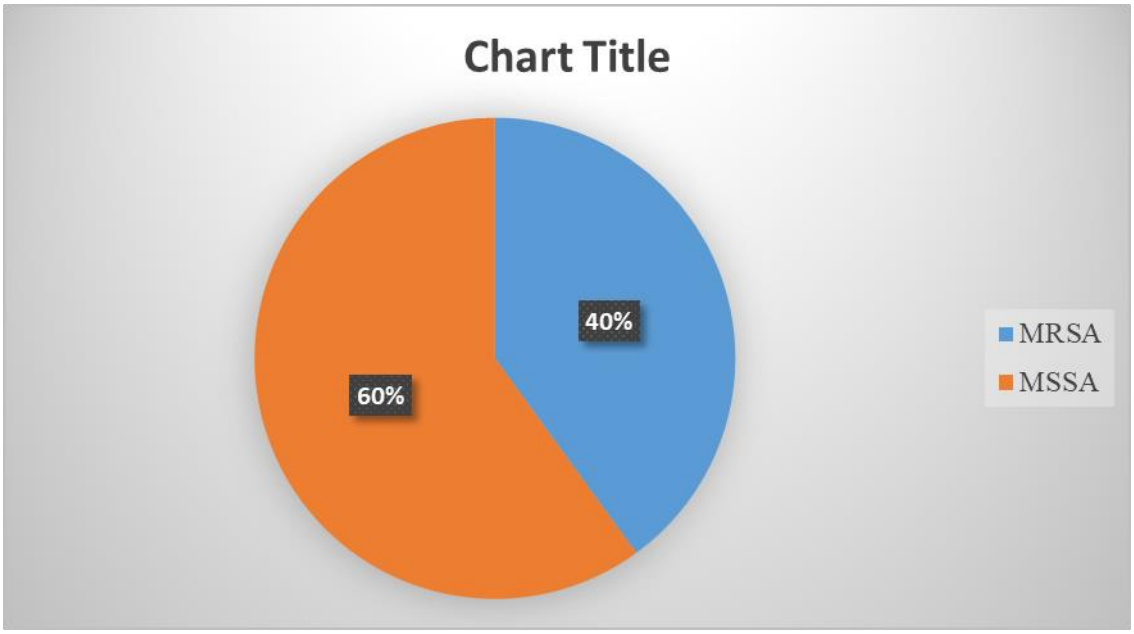

Figure 1: Distribution of MRSA and MSSA from the Study

Biofilm Detection using TCP method

TCP method detected biofilm production in 43 (71.7 %) of isolates. Among these 28 (46.7%) were strong biofilm producers, 15 (25.0 %) were moderate biofilm producers and 17 (28.3 %) were non-biofilm producers. MSSA isolates showed higher rate of biofilm production (27/36; 75 %) compared to MRSA with (16/24; 66.7 %) biofilm producers as seen in table 1. Statistically, chi square showed that there is no significant association between the type of organism (MRSA vs MSSA) and the intensity of biofilm production (non-biofilm, moderate and strong) since p-value = 0.189 at 5% significant level.

Table 1: Detection of Biofilm Production using TCP method

Organism	Non-Producers (%)	Moderate (%)	Strong (%)	Total
MRSA	8 (33.3)	3 (12.5)	13 (54.2)	24
MSSA	9 (25.0)	12 (33.3)	15 (41.7)	36
Total	17 (28.3)	15 (25.0)	28 (46.7)	60

Biofilm detection using Tube Method

Tube Method in this study identified 31 (41.7 %) biofilm producing isolates in which 13 (21.7 %) were strong biofilm producers, 18 (30.0 %) were moderate producers and 29 (48.3 %) were non-biofilm producers. MSSA isolates showed a slightly higher biofilm production rate (19/36; 52.8 %) than MRSA (12/24, 50.0 %). Statistically, chi square showed that there is no significant association between the type of organism (MRSA vs MSSA) and the intensity of biofilm production (non-biofilm, moderate and strong) since p-value = 0.978 at 5% significant level.

Table 2: Detection of Biofilm production using Tube method

Organism	Non-Producers (%)	Moderate (%)	Strong (%)	Total
MRSA	12 (50)	7 (29.2)	5 (20.8)	24
MSSA	17 (47.2)	11 (30.6)	8 (22.2)	36
Total	29 (48.2)	18 (30.0)	13 (21.7)	60

Comparative analysis of Biofilm detection methods

This study highlighted that TCP detects the highest biofilm production with strong biofilm producers having the highest occurrence 28 (46.7 %) compared to TM in which strong biofilm producers had the least occurrence 13 (21.7 %) (Figure 2).

The comparative analysis of both biofilm detection method using Cohen Kappa which measures agreement between methods beyond chance. There is a slight agreement (k~0.15) between Tissue culture plate method and Tube method suggesting that the methods classified the isolates differently

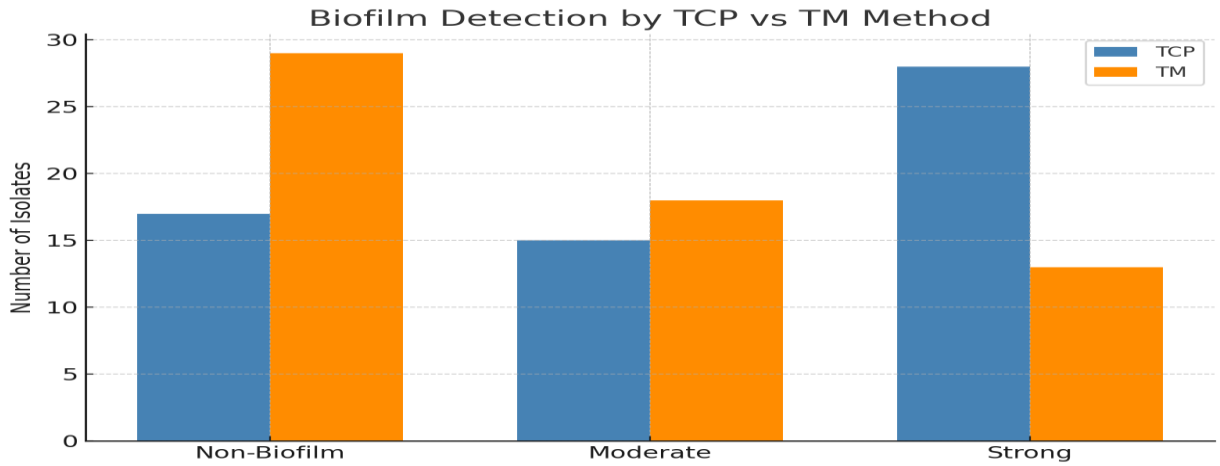

Figure 2: Comparative assessment of Biofilm detection methods**.**

Distribution of Biofilm Production based on Sample Source

The result of the distribution of Biofilm production showed that Surgical equipment had the highest rate of biofilm producing isolates (100 %) while healthcare workers had the least of biofilm producing isolates (57.1 %). Statistically, Chi-square suggested that there is no association between Biofilm production and source of sampling as p-value = 0.138 at 5 % level of significance.

Figure 3: Distribution of Biofilm production based on sample source

DISCUSSION

In the study, 60 clinical and community isolates were confirmed to be *S. aureus* by cultural and biochemical characteristics. Antibiotic susceptibility test detects 24(40.0%) MRSA and 36 (60.0%) MSSA, this is not in agreement with similar study carried out by Abdel-Halim *et al.*, (2018) who reported that 87.4% of the *Staphylococcal* isolates are resistant to cefoxitin i.e., they are methicillin resistant. The difference might be due to the differences in the types of the isolates used in their study as their isolates were all clinical isolates where as both clinical and community isolates were used in this study.

TCP method detects a total of 43(71.7%) positive biofilm producing *S. aureus* in which 28(46.7%) were strong biofilm producers and 17(28.3%) were non-biofilm producers which is higher than that of Fatima *et al.*, (2011) who reported 64.89 % *S. aureus* as biofilm producers. Abdel-Halim *et al.*, (2018), also reported 53.2 % of the biofilm producers among *S. aureus.* However, the result obtained in this study is lower than that of Akinkunmi and Lamikanra, (2012) who reported that 36.0% of *S. aureus* in their study were biofilm producers. Ramakrishna *et al.*, (2014) also reported that 38.0 % of *S. aureus* being biofilm producers. The difference could be as a result of variation in the sample sources and the types of strain isolated. Also lack of molecular analysis to detect biofilm producing gene is a limitation which would have given an insight on the molecular footprint of the bacterial isolates. Tube Method (TM) in this study detected 51.7 % Biofilm production among the *S. aureus* isolates which is lower compared to TCP Method this is also in line with work of Abdel Halim *et al.*, 2018, Mahtur *et al.*, 2006 but in concordant with work of Saha *et al.*, 2014 with higher detection rate in TM than TCP. The variation maybe due to observer reading and also observer judgement between strong and moderate biofilm producers, also the type of test-tube used could also affect reading. Also, TCP method is regarded as the gold standard method for the detection of Biofilm in-vitro as reported by Mathur *et al.*, 2006 as it solves the issue of observer biasness as it has a better screening capability than the TM (Bose *et al.*, 2009).

Biofilm producing based on methicillin resistant showed that MSSA in this study had the higher ability to produce biofilm, and MRSA has the lower occurrence of biofilm producing *S. aureus* in the study. This is in agreement with work of Abdel-Halim *et al.*, (2018) who also detects more biofilm producers among MSSA isolates with 80.9 % than in MRSA isolates 77.8 % biofilm producers. It also agrees with the work of O'Neil *et al.*, (2017) who also reported higher biofilm producers in MSSA with 84.0 % than MRSA with 74.0 % biofilm producers. However, the findings in this study are not in agreement with the findings of Sharvari and Chitra, (2012) who reported 80.8 % MRSA as biofilm producers and 31.6 % MSSA as biofilm producers in their study. Fatima *et al.*, (2011) also reported 87.6 % of the MRSA isolated in their study are biofilm producers. MSSA has a strong association with ICA-dependent biofilm production which was first reported when PIA production was found to be essential for biofilm production by MSSA but not MRSA. MSSA biofilm

are significantly induced in growth media supplemented with NaCl which is known to activate ICA open expression (O'Neill *et a*l., 2007).

Biofilm producing *S. aureus* found to be more frequent among surgical equipment in this study. This might be because biofilm producers attached easily to polymer surfaces and *S. aureus* are frequently found to colonized medical devices and catheters and can be able to produce surface adhesins (Bose *et al*., 2009).

CONCLUSION

In conclusion, MSSA is more prevalent and showed higher biofilm formation than MRSA. TCP method was more effective in detection of biofilm compared to Tube method, surgical equipment harbors more biofilm producing *S. aureus* and this highlights potential risks for nosocomial infections. And the limitation of the research includes small and unequal sample size, no local validation was carried out for the biofilm detection protocols and molecular detection of biofilm producing gene was not carried out in this study.

RECOMMENDATION

1. Infection Control: Sterilize and monitor surgical tools routinely.
2. Screening Methods: TCP is recommended for reliable biofilm detection.
3. Antibiotic Stewardship: Curb misuse of antimicrobials.
4. Awareness: Educate healthcare workers and the public.
5. Further Research: Include molecular analysis (e.g., ica, agr, SCCmec)

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