Clinical Microbiology: Open Access

Chibueze et al., Clin Microbiol 2017, 6:4
DOI: 10.4172/2327-5073.1000295

Research OMICS International

Biofilm Formation and Antibiotic Susceptibility Profile of Clinical Isolates of *Staphylococcus aureus* Isolated from Clinical Samples in Zaria, Nigeria

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Received date: 22 March 2017; **Accepted date:** August 23, 2017; **Published date:** August 25, 2017

Abstract

Background: *Staphylococcus aureus* is one of the common pathogens associated with nosocomial as well as community acquired infections due to its ability to form biofilms.

Research aim: The aim of this study was to observe the biofilm forming abilities and to study the antibiotic susceptibility profile of clinical isolates of *S. aureus*.

Methodology: A total of 56 isolates from clinical samples were identified using standard techniques and the isolates were further tested for biofilm formation, using microtiter plate assay and antibiotic susceptibility testing was carried out using Kirby-Bauer method.

Results: The results showed that out of the 56 *S. aureus* isolated, biofilm formation was observed in 27 (48.2%). Strong biofilm formation was observed in 5.4% (3) of the isolates, moderate biofilm formation in 8.9% (5) of the isolates, weak biofilm formation in 33.9% (19) of the isolates, while 51.8% (29) of the isolates were non-biofilm formers. Prevalence of **S. aureus** infection was higher in females (67.9%) compared to males (32.1%). The isolates were highly susceptible to Gentamicin (100%), Tigecycline (98.21%), Sulphamethoxazole-Trimethoprim (89.29%), Ciprofloxacin (89.29%) and Linezolide (75%), while the isolates showed low susceptibility to Erythromycin (28.55%), Clindamycin (35.71%) and Vancomycin (41.07%). None of the isolates showed inducible Clindamycin resistance phenotypically. 9 (16.67%) of the isolates showed constitutive phenotype, 3 (5.36%) showed Methicillin-Sensitive (MS) phenotype while 44 (78.57%) showed none of the above phenotypes.

Conclusion: Clinical isolates of *S. aureus* have the potential of producing biofilm and this could influence the rate of resistance.

Keywords: *S. aureus*; Biofilm; Antibiotics susceptibility test; Inducible clindamycin resistance

Introduction

Infections caused by *Staphylococcus aureus* are becoming more difficult to treat because of increasing resistance to antibiotics [1]. One of the resistance/adaptation strategies of *S. aureus* like other bacteria is the formation of an organized communities of aggregated cells, embedded in a hydrated matrix of extracellular polymeric substances (EPS) called biofilms. Biofilm allows bacteria to survive in hostile environment, even in the presence of antibiotics and to colonise new niches by various dispersal mechanisms [2]. For example, populations of *S. aureus* within biofilms exhibit differences in the expression of surface molecules, antibiotic resistance, nutrient utilization and virulence factors [3]. They also coordinate behavior by cell-cell communication using secreted chemical signals [4]. Cell signaling allows them to sense and phenotypically respond to their environment [3]. *S. aureus* biofilm exhibit extraordinary resistance by limiting efficiency of antibiotic therapy and surgical intervention is often required to remove infected tissue or implanted devices. There is little information on the prevalence of biofilm producing *S. aureus* from clinical isolates in Zaria, Nigeria.

Research Objectives

This study was designed to evaluate the antimicrobial susceptibility profile of *S. aureus* isolated from clinical samples and their ability to produce biofilm in University Health Services, (Sick-Bay), Ahmadu Bello University Zaria, Nigeria.

Methodology

Sample collection

Clinical samples (urine, sputum, high vaginal swabs, intracervical swabs, throat swabs, thyroid swabs, ear swabs and urethral samples) submitted for bacteriological analysis at the Microbiology Laboratory, University Health Services (Sick-Bay), Ahmadu Bello University Zaria, between the periods of 6 months (February-August, 2012) were evaluated for the presences of *S. aureus* using the method prescribed by [5].

Biofilm formation assay

Isolates were grown overnight at 37°C in Brain Heart Infusion (BHI) broth supplemented with 2% glucose and sucrose. The overnight culture was then diluted to 1:100 in BHI sterile medium. One hundred and fifty microliters (150 µL) of diluted cell suspension were then transferred aseptically into a 96 wells microtiter plate and incubated at 37°C for 48 h without shaking. After incubation, the wells were gently washed thrice with 300 µL of distilled water and dried in an inverted position for 1 h using sterile air dry oven. The plates were then stained with 300 µL of freshly prepared 2% Crystal violet solution in water for 45 minutes. Then, the plates were washed thrice again with distilled water. Observations were made and recorded as weak, moderate or strong biofilm producers.

Antibiotic susceptibility testing

The antibiotic susceptibility profile of the identified Staph. aureus were carried out using Kirby-Bauer [6] disc diffusion method, CLSI [7] was used for the interpretation of the result while D-test method was used to evaluate for the ability of the isolates to induce Clindamycin resistance [2].

Results and Discussions

Samples	Frequency	Percentage (%)
Urine	31	55.4
HVS	6	10.7
Ear swab	4	7.1
ICS	3	5.4
ECS	3	5.4
Sputum	3	5.4
Throat swab	2	3.5
Wound swab	2	3.5
Urethral	1	1.8
Thyroid	1	1.8
Total	**56**	**100**

Table 1: Distribution of *S. aureus* within the Clinical Isolate.

Biofilm	Frequency	Percentage (%)
Strong producers	3	5.4
Moderate producers	5	8.9
Weak producers	19	33.9
Non-producers	29	51.8
Total	**56**	**100**

Table 2: Prevalence of Biofilm among the Clinical Isolates.

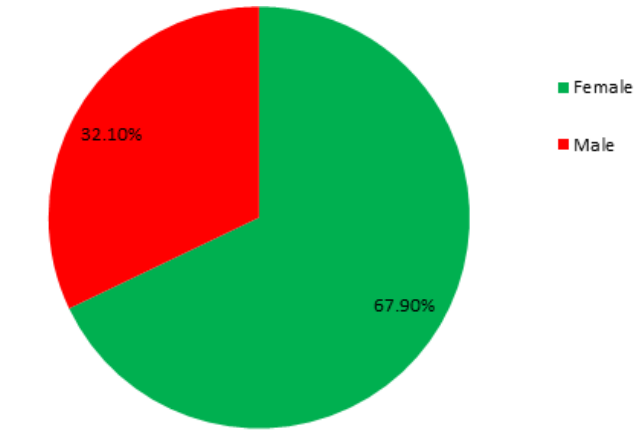

Figure 1: Distribution of Biofilm Production among Genders.

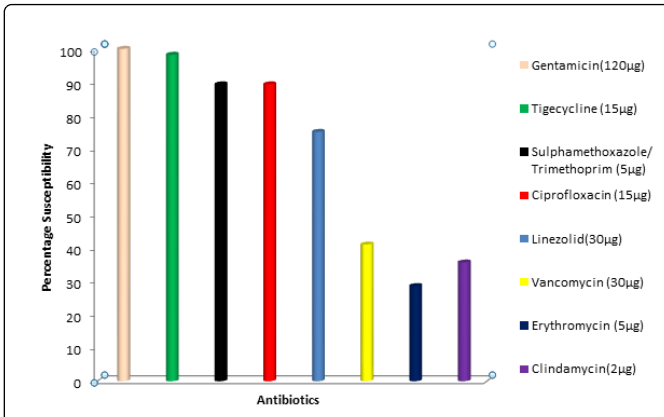

Figure 2: Antibiotics Susceptibility profile of Clinical Isolates of *S. aureus* Isolated from University Health Services, (Sick-Bay), Ahmadu Bello University Zaria, Nigeria.

Phenotype	Frequency	Percentage (%)
MS (Ery^RCli^S)	3	5.4
$iMLS_E$ (Ery^RCli^S i.e. "D"-Shape)	0	0
$cMLS_E$ (Ery^RCli^R)	9	16.1
Non-applicable	44	78.5
Total	56	100

Table 3: Macrolide-Lincosamide Inducible Phenotypic Resistance.

S. aureus develop biofilm for adaptation against external factors in the environment. Biofilm limits the penetration of antibiotics and reduced treatment options especially in endocarditis osteomyelitis and foreign body related diseases (Table 1) [8]. Out of 71 isolates evaluated, the occurrence rate of *S. aureus* was 78.9% (56) with female (67.9%) having the highest infection rate compared to male (32.1%) (Figure 1). This might be attributed to the physiology of women shorter urethra

and its proximity to the anal opening and vaginal introitus [9]. The ability to produce biofilm evaluated in the isolates revealed that 48.2% (27) of the isolates have the potential to form biofilm. 3 (5.4%) were strong biofilm producers, 5(8.9%) were moderate producers, 19 (33.9%) were weak producers while 29 (51.8%) were non biofilm producers (Table 2). Detection of inducible–Lincosamide resistance by disk induction testing also showed that none of the isolates produced inducible Clindamycin resistance phenotypically, 5.4% (3) displayed MS phenotype while 16.1% (9) produced constitutive phenotype resistance (Table 3). This study showed that antimicrobial agents such as Tigecycline, Sulphamethoxazole-Trimethoprim, Ciprofloxacin and Linezolid were the most effective antibiotics agents against *S. aureus* and could be recommended in the treatment of genitourinary tract infection and respiratory tract infection [2], while Gentamicin might not be directly recommended because of its concentration dependent bactericidal activity i.e. not easily absorbed by the body via all routes of administration, the extended post-antibiotic effect, and the possibility of reduced nephrotoxicity and ototoxicity (Figure 2) [10]. High resistance to Vancomycin (58.9%) was observed in this study compared with the study carried out by who observed 14.5% resistance to *S. aureus* [11].

Conclusion and Recommendation

This study showed that 48.2% (27) of the clinical isolates of *S. aureus* isolates in University Health Services, (Sick-Bay), Ahmadu Bello University Zaria, Nigeria have the ability to produce biofilm and women are the mostly infected compared to male. This study also observed that Gentamicin, Tigecycline, Sulphamethoxazole–Trimethoprim, Ciprofloxacin and Linezolid are still effective against *S. aureus* producing biofilm, in University Health Services, (Sick-Bay), Ahmadu Bello University Zaria, Nigeria. However, resistance to Vancomycin is on the increase, as such proper strategies and surveillance should be adopted to prevent increasing resistance to this agent. Also, further study should be carried out using larger population to know the prevalence of biofilm producing *S. aureus* in Zaria, Nigeria.

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