Clinical Microbiology: Open Access

Grace et al., Clin Microbiol 2018, 7:6
DOI: 10.4172/2327-5073.1000321

Research Article Open Access

Methicillin Resistance and Biofilm Formation of *Staphylococcus epidermidis* in Blood Culture Isolates from Children under Five: A Multicenter Study in Nigeria

John-Ugwuanya A Grace[1*], Busayo O Olayinka[1], Josiah A Onaolapo[1], Fatimah Hassan-Hanga[2], Huda Munir[3], Paul D Fey[4] and Stephen K Obaro[5]

[1]*Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Kaduna, Nigeria*

[2]*Paediatrics Department, Aminu Kano Teaching Hospital, Kano, Nigeria*

[3]*Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano*

[4]*Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska*

[5]*Division of Pediatric Infectious Disease University of Nebraska Medical Center, Omaha, Nebraska*

[*]**Corresponding author:** John-Ugwuanya A Grace, Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Kaduna, Nigeria, Tel: +2347061145614; E-mail: graceamy2k@yahoo.com

Received Date: Dec 05, 2018; **Accepted Date:** Dec 19, 2018; **Published Date:** Dec 30, 2018

Abstract

The clinical impact of *Staphylococcus epidermidis* in bacteremia remains controversial. The aim of this study is to determine the methicillin resistance and biofilm formation of *S. epidermidis* isolates. A total of 102 *S. epidermidis* blood culture isolates from children under five attending seven selected hospitals in north-central and north-west Nigeria within 2009 to 2016 were analyzed for methicillin resistance using cefoxitin disk agar diffusion test. Phenotypic biofilm formation and molecular detection of the intercellular adhesion locus (*icaA*) gene was performed by the quantitative Microtitre Plate (MTP) method and conventional Polymerase Chain Reaction (PCR) respectively. Seventy-four (72.5%) Methicillin-Resistant *Staphylococcus epidermidis* (MRSE) was observed while biofilm formation was detected in 20 (19.6%) *S. epidermidis* isolates. The *icaA* gene positive and negative *S. epidermidis* were 22.5% (23/102) and 77.5% (79/102) respectively. In correlating the biofilm formation using MTP method and *icaA* gene detection, 19.6% were biofilm producers and *icaA* positive while 2.9% carried the *icaA* gene but did not produce biofilm on the tissue culture plate. Out of the *icaA* positive *S. epidermidis*, 91.3% were MRSE while 69.6% were MRSE among the *icaA* negative strains. Methicillin-Resistant *Staphylococcus epidermidis* is common and the majority of the biofilm-producing strains were highly resistant to methicillin. This suggests a close association between biofilm-formation in *S. epidermidis* with increased methicillin resistance.

Keywords: *Staphylococcus epidermidis*; Bacteremia; Methicillin-resistance; Biofilm; Intercellular adhesion gene

Introduction

Staphylococcus epidermidis is a member of the skin microflora and plays a commensal part of the human host. It is the most abundant microbial flora found on the human skin and mucous membrane with about 10-24 different strains seen on an average individual [1,2]. In immuno-compromised patients, *S. epidermidis* is becoming a formidable pathogen especially with the increased use of indwelling prosthetic medical devices [3,4].

These bacterial species are genetically flexible with the ability to harness different mechanisms in adapting to a dynamic environment [5]. It adapts to various environmental conditions of the human skin such as temperature, moisture content, pH range and nutrients. With the high salt content on the dermal surface, *S. epidermidis* has some osmoprotective systems [4].

The ability of *S. epidermidis* to colonize the mucosal membranes and skin is enhanced by the presence of arginine catabolic mobile element, which is present in most of the strains. It can withstand harsh conditions enabling it to survive in hospitals and medical devices [6,7]. *Staphylococcus epidermidis* is a prominent cause of hospital-acquired infections especially on medical devices such as indwelling catheters. It is the dominant pathogen in neonatal late-onset sepsis of very low birth weight infants [5].

Staphylococcus epidermidis produces a family of toxins having the potential to destroy red and white blood cells, and it is associated with the aggressive form of *S. epidermidis* infection. These proinflammatory cytolysins exert selective antimicrobial activity against other organisms and help *S. epidermidis* to overshadow other microbes by dominating the skin [7].

The frequent coagulase-negative *Staphylococcus* isolated in bloodstream infection is *S. epidermidis* [8,9]. It can colonize and adhere to implanted medical devices and venous catheters through its ability to develop an organized structure of micro-colonies or multicellular structures known as biofilm [6]. *Staphylococcus epidermidis* can transform from a human commensal to an infectious organism through their ability to form biofilm [10].

Several proteins, adhesion factors and exopolymers associated with biofilm production and immune evasion are produced by *S. epidermidis* [11]. The mechanism of biofilm production depends on specific accumulation-associated proteins, extracellular DNA and polysaccharide intercellular adhesin [6].

Methicillin resistance depicted by the presence of *mecA* gene in *S. epidermidis* is common. *Staphylococcal* strains possessing the *mecA* gene are either homogenous in resistance expression or heterogeneous

with occasional borderline minimal inhibitory concentrations that could be misinterpreted as susceptible [12]. This structural gene encodes the penicillin-binding protein-2a with decreased affinity to β-lactam antibiotics [13].

This study determined the methicillin resistance and biofilm formation of *S. epidermidis* isolates in pediatric patients under five years with bacteremia.

Materials and Methods

Clinical background

The clinical cases ranged from sepsis, early and late-onset neonatal sepsis, cerebral meningitis, bronchial and congenital pneumonia, hyperbilirubinemia, respiratory tract infection, pharyngotonsillitis, hemorrhagic disease of the newborn, perinatal asphyxia, anemia, neonatal jaundice, and retroviral disease.

Inclusion criteria

The criteria met were hyperthermia above 38°C, fast breathing, cough, difficulty in breathing, muscle pain, chills, vomiting, hypotension, severe malnutrition and irritability amongst others. Due to the stringent process before recruiting the children into the study, only one blood culture sample was collected.

Ethical statement

Consent was obtained from the International Foundation against Infectious Disease in Nigeria (IFAIN), Abuja to gain access to the stored coagulase-negative *Staphylococci* isolates obtained between 2009 and 2016. IFAIN has the written approval by the Research Ethics Committee of all the hospitals in this study.

Subjects

All the patients were children under five years having positive blood culture using the BACTEC 9050 and 9240 automated blood culture device (Becton Dickson Diagnostic Instrument Systems, Sparks, MD, USA).

Microbiology

The clinical isolates were obtained from the positive blood culture by subculturing on 5% sheep blood agar (Oxoid, Basingstoke, Hants, UK), chocolate blood agar (Becton Dickson, Sparks, USA) and Mac Conkey agar (Remel TM Thermo Fisher Scientific, USA). Identification of the isolates includes Gram staining to classify as Gram-positive bacteria. Catalase test was done to differentiate *Staphylococci* from another Gram-positive organism while coagulase test differentiated the coagulase-positive and coagulase-negative *Staphylococci* (CoNS). The CoNS isolates were stored without species identification.

In this study, the stored clinical isolates were sub-cultured on 5% sheep blood agar (Oxoid, Basingstoke, Hants, UK) and tryptic soy agar (BBLTM, Becton Dickson and Company, Sparks, USA) to obtain fresh colonies. The API Staph-20 (bioMerieux, Marcy-I'Etiole, France) was used for species identification of *S. epidermidis*. Control strains used are *S. epidermidis* ATCC 29213 and *S. aureus* ATCC 25923.

Bacterial isolates

This study was carried out on 102 *Staphylococcus epidermidis* isolates from bacteraemic children under five (Table 1) attending the neonatal and paediatric units of Nyanya General Hospital, Abuja (n=4); University of Abuja Teaching Hospital, Abuja (n=61); National Hospital, Abuja (n=2), Federal Medical Center, Keffi (n=25); Aminu Kano Teaching Hospital, Kano (n=3); Hasiya Bayero Hospital, Kano (n=4) and Murtala Mohammed Specialist Hospital, Kano (n=3). These hospitals are located in north-central and north-west Nigeria.

Sites	No. of Children	Percentage
Nyanya General Hospital, Abuja	4	3.90%
University of Abuja Teaching Hospital, Abuja	61	59.80%
Federal Medical Center, Keffi	25	24.50%
National Hospital, Abuja	2	2.00%
Aminu Kano Teaching Hospital, Kano	3	2.90%
Hasiya Bayero Paediatric Hospital, Kano	4	3.90%
Murtala Mohammed Specialist Hospital, Kano	3	2.90%
Total	102	

Table 1: Distribution of the *Staphylococcus epidermidis* strains from the various sites.

Detection of methicillin resistance

This was analyzed with the cefoxitin (30 µg) disc agar diffusion test on Mueller Hinton agar.

Phenotypic biofilm formation

The Christensen biofilm assay method was used for the quantitative analysis of biofilm formation [14]. Fresh culture from tryptic soy agar plates were inoculated in 10 ml of trypticase soy broth containing 1% glucose and incubated at 37°C for 18-24 hrs. Using a colony suspension in 50 µl of normal saline, a 1:100 dilution of the culture was done by dispensing 2 µl of colony suspension into the 198 µl of Tryptic Soy Broth (TSB) in the 96-well optically clear flat-bottom microtitre plate (Thermo Scientific-NunclonTM Delta Surface). The control strains used were *S. epidermidis* 1457 (PIA-positive), *S. epidermidis* CSF41498 (PIA-positive) and *S. epidermidis* 1457-M10 (PIA-negative).

The samples were run in triplicates and incubated for 24 hrs at 37°C without agitation. Following incubation, the content of each well was removed gently by tapping and wells washed thrice with 200 µl of 1% phosphate buffer saline (pH 7.2) to remove free flowing bacteria before drying the microtitre plate inverted in the 45°C incubator for 1 hour.

Biofilm produced by remaining adherent bacteria was stained with 150 µl of crystal violet for 15 minutes. Excess stain was dumped carefully and the 960-well plate rinsed with running water. According to Christensen, et al., samples with optical density (OD>0.11) should be considered as positive [14]. The optical density quantitation of biofilm produced was done using the Multilabel Plate Counter (PerkinElmer VICTOR 3TM V 1420-041).

Molecular detection of *icaA* gene

The detection of the biofilm-forming *icaA* gene in the *S. epidermidis* isolates was performed by conventional polymerase chain reaction (Thermal Cycler, PCR System 2700, GeneAmp®, Applied Biosystems). The forward primer 5'-GCTACAATGAAGTGAAAC-3' and reverse primer 5'-GGCACTAACATCCAGCATAG-3' were used to detect a 597-bp fragment of *icaA* genes in this study [15].

The amplification was performed at 50°C annealing temperature using 25 µl of PCR mixture containing 12.5 µl of Midas mix, 9.5 µl of sterile free RNase/DNase water, 1 µl of *icaA*-F and 1 µl of *icaA*-R. A 0.8% agarose gel was used to visualize the *icaA* DNA template (Figure 1).

Figure 1: PCR amplification of *icaA* gene in the *S. epidermidis* strains.

Results

Among the 102 *Staphylococcus epidermidis* strains, 74 (72.5%) were Methicillin-Resistant *S. epidermidis* (MRSE). Phenotypic biofilm formation was detected in 20 (19.6%) *S. epidermidis* strains (Table 2). The *icaA* gene of the intercellular adhesion locus was detected in 23 (22.5%) of the 102 *S. epidermidis* strains. In correlating the biofilm formation using MTP method and *icaA* gene detection, 20 (19.6%) were biofilm and *icaA*-positive while 3 (2.9%) carried the *icaA* gene but were not expressed using phenotypic MTP method.

Parameters	Total	Percentage
Methicillin-resistance		
MSSE	28	27.50%
MRSE	74	72.50%
Phenotypic Biofilm Formation		
Biofilm positive	20	19.60%
Biofilm negative	82	80.40%
Molecular Detection		
icaA positive	23	22.50%
icaA negative	79	77.50%

MSSE: Methicillin-Sensitive *Staphylococcus epidermidis;* MRSE: Methicillin-Resistant *Staphylococcus epidermidis; icaA*: intercellular adhesin A gene

Table 2: Methicillin resistance and biofilm formation in *S. epidermidis* (n=102).

The *icaA* negative *S. epidermidis* were 79 (77.5%). Out of the 23 *icaA* positive *S. epidermidis* strains, 21 (91.3%) were MRSE while the *icaA* negative *S. epidermidis* had 55 (69.6%) strains that were MRSE (Figure 2).

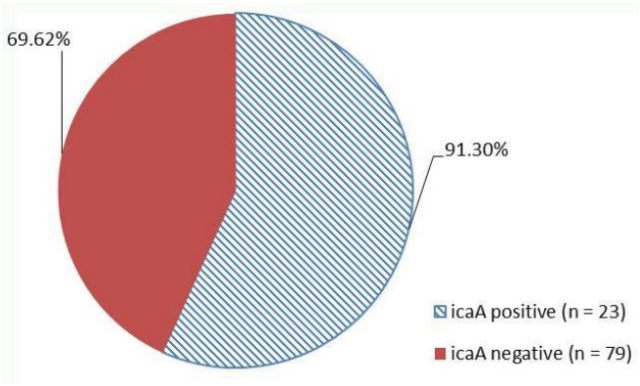

Figure 2: Methicillin-resistant *Staphylococcus epidermidis* among the *icaA* positive and negative strains.

Discussion

Staphylococcus epidermidis easily accumulate multiple determinants of antibiotic resistance and its frequency of methicillin resistance reaches up to 80% globally with a few exceptions like Iceland and Denmark having a lower frequency of about 30% -40% [3]. In this study, MRSE was 72.5%. This correlates with a study by Havaei, et al., having methicillin-resistant *S. epidermidis* as 87.1% [16]. The methicillin resistance (*mecA*) gene that codes for the altered penicillin-binding protein in *staphylococcal strains* is integrated into the genome of the mobile genetic element *Staphylococccal* cassette chromosome *mec* [17].

Biofilm formation among the *S. epidermidis* isolates were 19.6% and 22.5% using the microtitre plate method and molecular detection of *icaA* gene. It is known that the pathogenesis of *S. epidermidis* is linked to its presence as a normal flora on the human skin and the ability to adhere to biomedical materials and produce biofilm [18]. These sticky agglomerations enable *S. epidermidis* to inhibit most host defense mechanism and it is implicated in multiple antibiotic resistance [6,19]. This can justify the 91.3% methicillin resistance observed among the biofilm-producing *S. epidermidis* strains having the *icaA* gene. According to Fey, et al., differentiating *S. epidermidis* as a true pathogen or contaminant in bacteremia poses a challenge especially with the formation of biofilm and increased rate of antibiotic resistance [7]. Biofilms are known to protect bacteria from the host immunity and antimicrobial agents [20].

It is believed that there is a complication in treatment due to the emergence of MRSE [1]. *Staphylococcus epidermidis* easily accumulate multiple determinants of antibiotic resistance and its frequency of methicillin resistance reaches up to 80% globally [3]. It is associated with biofilm-mediated life-threatening infections [20,21]. Since the detection of methicillin resistance and biofilm production alone cannot be used to treat *S. epidermidis* bacteremia, other factors such as clinical evidence of bacteremia and the use of inflammatory markers is required.

Conclusion

Methicillin-resistance is significant among the *S. epidermidis* isolates and the majority of the strains having the *icaA* gene were highly resistant to methicillin. This suggests a close association between biofilm-formation in *S. epidermidis* strains and increased methicillin resistance.

References

1. Nguyen TH, Park MD, Otto M (2017) Host response to Staphylococcus epidermidis colonisation and infections. Front Cell Infect Microbiol 7: 90.
2. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, et al. (2009) Topographical and temporal diversity of the human skin microbiome. Science 324: 1190-1192.
3. Rolo J, de Lencastre H, Miragaia M (2012) Strategies of adaptation of Staphylococcus epidermidis to hospital and community: amplification and diversification of SCCmec. J Antimicrob Chemother 67: 1333-1341.
4. Otto M (2009) Staphylococcus epidermidis-The accidental pathogen. Nat Rev Microbiol 7: 555-567.
5. Dong Y, Speer CP (2014) The role of Staphylococcus epidermidis in neonatal sepsis: Guarding angel or pathogenic devil? Int J Med Microbiol 304: 513-520.
6. Dong Y, Speer CP, Glaser K (2018) Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity. Virulence 9: 621-633.
7. Fey PD (2014) Staphylococcus epidermidis: Methods and Protocols. New York: Humana Press.
8. Qin L, Da F, Fisher EL, Tan DC, Nguyen TH (2017) Toxin mediates sepsis caused by methicillin-resistant Staphylococcus epidermidis. PLoS Pathogens 13: e1006153.
9. Koksal F, Yasar H, Samasti M (2009) Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol Res 164: 404-410.
10. Kaiser TD, Pereira EM, Dos Santos KR, Maciel EL, Schuenck RP (2013) Modification of the Congo red agar method to detect biofilm production by Staphylococcus epidermidis. Diagn Microbiol Infect Dis 75: 235-239.
11. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, et al. (2001) Survey of infections due to Staphylococcus specie: Frequency and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and Western pacific region for the SENTRY antimicrobial surveillance program, 1997-1999. Clin Infect Dis 32: S114-S132.
12. Giormezis N, Kolonitsiou F, Foka A, Drougka E, Liakopoulos A (2014) Coagulase-negative staphylococcal bloodstream and prosthetic-device-associated infections: The role of biofilm formation and distribution of adhesin and toxin genes. J Med Microbiol 63: 1500-1508.
13. Anand KB, Agrawal P, Kumar S, Kapila K (2009) Comparison of cefoxitin disc diffusion test, oxacillin screen agar and PCR for mecA gene detection of MRSA. Indian J Med Microbiol 27: 27-29.
14. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, et al. (1985) Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 22: 996-1006.
15. Schaeffer CR, Hoang TN, Sudbeck CM, Alawi M, Tolo IE (2016) Versatility of biofilm matrix molecules in Staphylococcus epidermidis clinical isolates and importance of polysaccharide intercellular adhesin expression during high shear stress. mSphere 1: e00165-16.
16. Havaei SA, Namvar AE, Moghim S, Lari AR (2015) Evaluation of various staphylococcal cassette chromosome mec (SCCmec) types in Staphylococcus epidermidis invasive strains from hospitalised patients in Iran. Infez Med 1: 18-22.
17. Monecke S, Coombs GW, Pearson J, Hotzel H, Slickers P, et al. (2015) A clonal complex 12 methicillin-resistant Staphylococcus aureus strain, West Australian MRSA-59, harbours a novel pseudo-SCCmec element. Antimicrob Agent Chemother 59: 7142-7144.
18. Madhusoodanan J, Seo KS, Remortel B, Park JY, Hwang SY, et al. (2011) An enterotoxin-bearing pathogenicity island in Staphylococcus epidermidis. J Bacteriol 193: 1854-1862.
19. Juárez-Verdayes MA, Ramón-Peréz ML, Flores-Páez LA, Camarillo-Márquez O, Zenteno JC (2013) Staphylococcus epidermidis with the icaA-/icaD-/IS256- genotype and protein or protein/extracellular-DNA biofilm is frequent in ocular infections. J Med Microbiol 62: 1579-1587.
20. Pinheiro L, Brito CI, Oliveira Ad, Pereira VC, Cunha Mde L (2016) Staphylococcus epidermidis and Staphylococcus haemolyticus: detection of biofilm genes and biofilm formation in blood culture isolates from patients in a Brazilian teaching hospital. Diagn Microbiol Infect Dis 86: 11-14.
21. Nasr RA, AbuShady HM, Hussein HS (2012) Biofilm formation and presence of icaAD gene in clinical isolates of staphylococci. The Egyptian J Medic Human Genet 83: 269-274.