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Biofilm Formation in MRSA Staphylococcus aureus Isolated from Diabetics′ UTI

Weam S. Al-Hamadany*
Biology Department / College of Science/ Muthanna University/ Iraq

Abstract:
Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are of medical concern especially if they are able to form biofilm. The present study investigated the ability for biofilm formation in Diabetic UTI clinical isolates, since those patients are classified as immunocompramised and the bacteria *S. aureus* are opportunists. A total of (20 isolate) were tested using microtiter dish biofilm formation assay. Results confirmed that (60%, 12/20) of isolates had another virulence factor rather than Methicillin resistance, biofilm formation ability; those two influencers cooperated to perform invasion and push forward UTI infection success in most isolates included in this study.
Key Words: *S.aureus*, biofilm formation, MRSA, antibiotics resistance, Diabetes and UTI.

INTRODUCTION

Staphylococcus aureus constitute the most causative pathogen responsible of Urinary Tract Infections UTI in diabetic patients, since those patients are classified as immunocompramised and the bacteria *S. aureus* are opportunists. Moreover; the numerous virulence factors featured by *S. aureus* they have the ability to resist the most common antibiotics used to treat UTI as drug of choice pointing to beta lactam group of antibacterial antibiotics, they usually called Methicillin-resistant *S. aureus* (MRSA) [1 and 2].

Staphylococcus aureus do not grow individually, they usually exist in intimate and complex communities communicating among themselves called biofilm during colonization and infection. This growth pattern begins by a single bacterium nucleating a surface followed by replicating themselves or recruiting other bacteria into a forming colony. The biofilm matrix components, contain polysaccharides, proteins, and DNA, they play a major role in its general structure and contribute to its conservation and resistance phenotype [3and 4].

The recognition of this growth pattern has been increasingly appreciated. The conceptual strategy of biofilm formation is by forming layer upon layer of bacterial growth, the initial bacterium are less susceptible to immune clearance by the host, and penetration by anti-therapeutics is shielded by external growth. The study of biofilms formation ability in the laboratory (*in vivo*) is an evolving science since it was considered as one of the most important virulence factors of wild isolates which recently interested, moreover, many environmental factors and elements affect biofilm formation and its composition, mentioning glucose which enhances biofilm formation [5].

Biofilm formation increases the pathogenicity of *S. aureus* because this complex polymer pushes forward bacterial growing by sharing materials and shielding from host defenses suggesting that these isolates are more virulent than others [6].

METHODS AND MATERIALS

1. **Isolates:** Twenty clinical *S. aureus* isolates were obtained from the laboratory of Diabetic Center of Al-Hussein Hospital they were isolated from diabetic patients with recurrent UTI resultant from MRSA *S. aureus* during their periodic checkup during spring 2017. *S. aureus* isolates then were identified microbiologically using standard bacteriological procedures and biochemical tests as [7 and 8].
2. **Methicillin-Resistance Confirmation:** it was the first step of the work and accomplished for all isolates. The method used was disc diffusion method on Muller Hinton agar plates. Two antibiotics discs were used, Methicillin (5 Mcg) and Carbencillin. (100 Mcg), Conda /Spain both. After inoculation, discs were plated, and all dishes were incubated aerobically at 37 ^{o} C for overnight. Antiseptic conditions were considered during all work steps [7].
3. **Biofilm Formation Test:**
   The method used was microtiter dish biofilm formation assay according to the protocol [9] the most widely applied assay. Each isolate was cultured overnight in Brain Heart Infusion BHI containing 1% glucose, then cultures were diluted 1:100 with fresh medium using polystyrene 96 well microtitration plates (Dragon /China). After incubation, staining was applied with (0.1%) crystal violet (Sigma/ USA) and then fixation step was with Sodium acetate (0.2%) (B.D.H/ England).
   Biofilm formation was considered as positive result when a visible film lined the wall and the bottom of the well. The ability of biofilm formation was detected by *in vitro* slime production and scored as (no, moderate and high). Negative control wells contained sterile broth [10]. This method was repeated three times for each isolate and modified according to [11].
4. **Statistical Analysis:**
   Percentages and diagrams were performed using Microsoft 2010.
5. **Ethical Approval:**
   The authorization and patients' ethical approval for samples were obtained before work starts.

RESULTS

Methicillin resistance was confirmed for all isolates tested before investigation for biofilm formation ability, figure (1). The figure (2) showed the results of biofilm formation abilities recorded for the 20 isolates involved in the present study, whereas; 12 (60%) of tested isolates were able to form biofilm and distributed as 4 (20%) isolates with moderate ability and 8 (40%) isolates with high ability for biofilm formation. The others 8 (40%) isolates were unable for formation (negative result) when compared with the control negative wells, figure (3).

DISCUSSION

The present study demonstrated that there was an association between the resistance for methicillin and biofilm formation ability, since most of the present study isolates included in the present study were Methicillin-resistant and able to form biofilm. Both characteristics are considered as virulence factors and exhibited by wild clinical isolates, giving a clue that they may work synergically during colonization and infections induction. These outcomes need more investigations and wider studies for the conditions that may affect bacterial abilities in forming biofilm and to find the environmental factors that may differ between *in vivo* and *in vitro* bacterial growing and effect on biofilm formation character of clinical isolates.

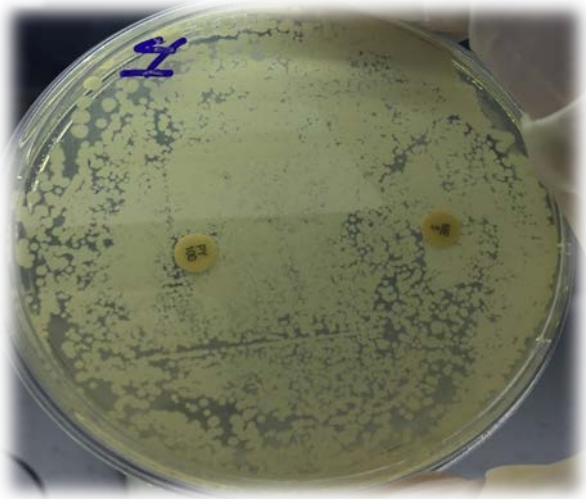

Figure (1): Methicillin resistance Confirmation Test. Picture shows a resistant isolate for both Methicillin (5 Mcg) and Carbencillin. (100 Mcg) during disc diffusion method.

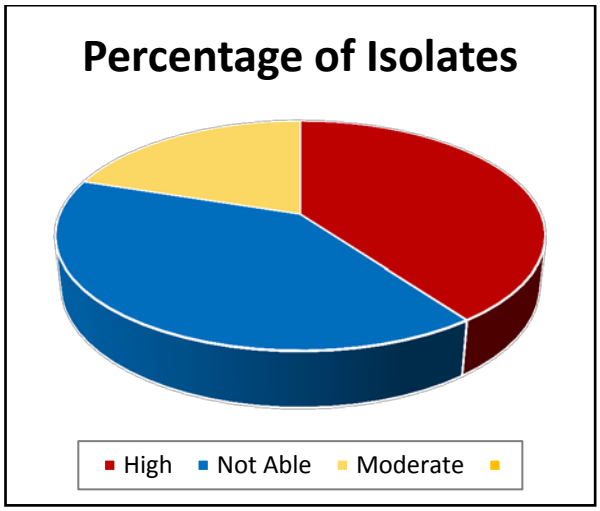

Figure (2): Biofilm Formation abilities for MRSA (Methicillin-resistant) *Staphylococcus aureus* Isolates. Red area for High ability isolates, Yellow Area for moderate ability isolates and blue area for unable isolates for biofilm formation.

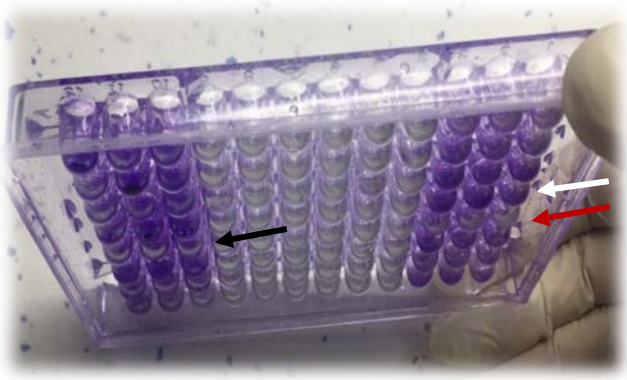

Figure (3): Microtitration Plate with Biofilm Formation Test Results. White arrow shows positive result, *S. aureus* is non-motile and forms the biofilm on the bottom of the well. Red arrow shows negative result and black arrow shows control negative, sterile broth well.

Accordingly, Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen which includes, moreover its many virulence factors, the ability to produce biofilm. This ability allows *S. aureus* to persistently colonize mucosal and inanimate surfaces alike, thus making its eradication from hospital settings very difficult. One of the many characteristics of the biofilm-associated bacteria in clinical medicine is the markedly enhanced resistance to antimicrobial agents, sometimes leading to multidrug resistance and therapeutic failure [12].

The authors [10] stated that growth in microtitre plate for testing biofilm production could be used to determine the phenotype of an isolate and had the potential for biofilm production when compared with the genotype, since the sensitivity and specifity of this method can reach between (88.9%-100%) as recorded by the authors.

Those two characteristics, antibiotics resistance and biofilm formation were recently under investigations of many researchers to find if there is a genetic relationship between those virulence factors, [13] stated that antibiotic resistance and biofilm-forming ability contribute to the success of *S. aureus* as a human pathogen, these virulence factors do not function independently of each other and the biofilm phenotype expressed by clinical isolates of *S. aureus* is influenced by methicillin resistance genes.

The scientists [4] during their research on biofilm formation phenotype in *S. aureus* using different isolates, they found that there was an association between antibiotics resistance and biofilm formation capability despite of the different composition of their biofilm matrix, that opinion supporting our results since 60% of MDR isolates were able to form biofilm despite of the different intensity detected, and that difference in the present study results can be attributed to the matrix composition diversity as seen in the related previous studies.

In another related teamwork study, [14] they investigated for the ability of Methicillin-resistant *S. aureus* isolates, they found that from a total 22 isolates there were 5 (22.7%) isolates were able to form biofilm on polystyrene. These isolates were isolated from cow milk (food borne bacteria). Despite of that this research isolates were not collected from clinical specimens of infected humans, the percentage of positive isolates for biofilm formation was an important issue for food safety from biofilm producing strains of *S. aureus* since this ability was associated to virulence strains. These findings support the idea of the present study that the increasing biofilm formation ability may grow with virulence strains strength and rich media matrix.

In the study of [15] the genetic investigation about the relation between biofilm formation and methicillin resistance genes revealed that biofilm formation was significantly induced when using glucose in culturing isolates while using NaCl induced biofilm gene operon, their research was on staphylococcal device-related infections. These results are in agreement with the present research outcomes since the isolates were collected from diabetic patients' urine samples, pointing to the high levels of glucose sugar in their urine which enhances biofilm formation ability for isolates.

Staphylococcus aureus have numerous virulence mechanisms including enzyme and toxin production, biofilm forming capacity and immune evasion ways. Antibiotic resistance and biofilm-forming capacity, in clinical MRSA *S. aureus* isolates responsible for device-related infections in immuno-compromised patients, this subject was fully investigated and it was found that methicillin resistance has the potential to affect the genes that mediated phenotypes, including altered biofilm expression and virulence as stated by [16].

CONCLUSIONS

The present study confirmed that clinical isolates of MRSA *S. aureus* collected from diabetic patients had additional virulence factor rather than antibiotic resistance, biofilm formation ability; those two influencers cooperated to perform invasion and push forward UTI infection success in most isolates included in this study.

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REFERENCES

1. Martin, E.; Stryjewski,G. and Corey, R. Methicillin-Resistant *Staphylococcus aureus*: An Evolving Pathogen. Clinical Infectious Diseases. J. 2014; 58(1):S10–S19.
2. Budiman, A.; Rusnawan, D. and Yuliana, A. Antibacterial activity of *Piper betle* L. extract in cream forms against *Staphylococcus aureus* and *Propionibacterium acne*. JPSR.2018; 10(3):493-6.
3. Ray, V.; Morris, A. and Visick, K. "A semi-quantitative approach to assess biofilm formation using wrinkled colony development," Journal of Visualized Experiments, 2012; no. 64, Article ID e4035, 2012.
4. Dakheel, K.; Rahim, R.; Neela; V.; Al-Obaidi, J.; Hun, T. and Yousoff, K. Methicillin-Resistant Staphylococcus aureus Biofilms and Their Influence on Bacterial Adhesion and Cohesion. J. BioMed Research International. 2016; Vol 2016:1-14.
5. Archer, N; Mazaitis, M; Costerton, J.; Leid, J.; Powers, M. and Shirtliff, M. *Staphylococcus aureus* biofilms: Properties, regulation and roles in human disease. Virulence. J. 2011; 2(5): 445–59.
6. David, M.; Bamberger, M.; Sarah, E. and Boyd, M. Management of *Staphylococcus aureus* Infections. Am. Fam. Physician. J. 15; 2005; 72(12):2474-2481.
7. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Approved standard M100-S24. National Committee for Clinical Laboratory Standards, Wayne, Pa.U.S.A; 2014; 34(1).
8. Al-Hmadany, W. Isolation and Identification of Gram Positive Cocci Bacterial Pathogens from Sappurative Otitis Media Infections. The Iraqi Journal of Veterinary Medicine,2013; 37(1): 129 – 133
9. O'Toole, G. Microtiter Dish Biofilm Formation Assay. J. Vis.Exp. 2011; (47), e2437.
10. De Castro Melo, P.; Ferreira, L.; Filho, A; Zafalon, L.; Vicente, H. and De Souza, V. Comparison of methods for the detection of biofilm formation by *Staphylococcus aureus* isolated from bovine subclinical mastitis Braz J Microbiol. 2013; 17; 44(1):119-24.
11. Obied, H.; Al-Zobaidy, M. and Hindi, N. An *in Vitro* study of Anti-bacterial, Anti-adherence, Anti-Biofilm and Anti- Motility activities of the aqueous extracts fresh and powdered onion (*Allium cepa*) and onion oil. JPSR. 2018; 10(6):1573-8.
12. Raggi, C.; Filippini, P.; Monaco, M.; Pantosti, A.; Creti. R. and Baldassarri, L. Methicillin Resistance, Biofilm Formation and Resistance to Benzalkonium Chloride in *Staphylococcus aureus* Clinical Isolates. Clin Microbial. 2013; 2:121.
13. McCarthy, H.; Rudkin, J.; Black, N.; Gallahher, L.; O'Neill, E. and O'Gara, J. Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*. Frontiers. Cell. Infect. J. 2015; 5(1):1-9.
14. Vergara, A.; Normanno, G.; Di Ciccio, P.; Pedonese, F.; Nuvoloni, R.; Parisi, A.; Santagada, G.; Colagiorgi, A.; Zanardi; E.; Ghidini, S. and Ianieri, A. Biofilm Formation and Its Relationship with the Molecular Characteristics of Food-Related Methicillin-Resistant *Staphylococcus aureus* (MRSA). J. Food Sci. 2017; 82(10):2364-70.
15. O'Neil, E.; Pozzi, C.; Houston, P.; Smyth, D.; Humphreys, H.; Robinson, D. and O'Gara, J. Association between Methicillin Susceptibility and Biofilm Regulation in *Staphylococcus aureus* Isolates from Device-Related Infections. J. Clin. Microbiol. 2007; 45(5):1379-88.
16. Pozzi, C.; Waters, E.; Rudkin J.; Schaeffer, C.; Lohan, A.; Tong, P.; *et al.* Methicillin Resistance Alters the Biofilm Phenotype and Attenuates Virulence in *Staphylococcus aureus* Device-Associated Infections. PLoS Pathog .2012; 8(4): e1002626.