DOI: 10.4274/jarem.galenos.2022.51423
J Acad Res Med 2022;12(2):55-60

Investigation of *in vitro* Biofilm Formation and Its Correlation with Antibiotic Resistance Pattern Among Clinical Isolates of *Staphylococcus aureus*: A Cross-sectional Study in Northern Cyprus

Ahmed Hassan İbrahim[1], Emrah Güler[2,3], Cemile Bağkur[3], Kaya Süer[4], Eşref Çelik[1]

[1]Near East University Faculty of Medicine, Department of Medical Microbiology and Clinical Microbiology, Nicosia, Cyprus
[2]Near East University Faculty of Health Sciences, Department of Nutrition and Dietetics, Nicosia, Cyprus
[3]Near East University, DESAM Research Institute, Nicosia, Cyprus
[4]Near East University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Nicosia, Cyprus

Cite this article as: İbrahim AH, Güler E, Bağkur C, Süer K, Çelik E. Investigation of *in vitro* Biofilm Formation and Its Correlation with Antibiotic Resistance Pattern Among Clinical Isolates of *Staphylococcus aureus*: A Cross-sectional Study in Northern Cyprus. J Acad Res Med 2022;12(2):55-60

ABSTRACT

Objective: *Staphylococcus aureus* (*S. aureus*), including methicillin resistant *S. aureus* (MRSA), can produce biofilm leading to increased morbidity and mortality in hospital infections. Antibiotic resistance is an inherent feature of bacterial biofilms, and the formation of biofilms is more widespread in MRSA. This study aimed to reveal the phenotypic biofilm-forming abilities of *S. aureus* isolates and to investigate the relationship of antibiotic resistance of biofilm-forming *S. aureus* with biofilm formation.

Methods: A cross-sectional descriptive study was carried out in the microbiology laboratory at the Near East University Hospital in the Turkish Republic of Northern Cyprus. A total of 67 non-duplicative samples (wound/pus, sputum, aspirate, blood and urine) for the study were collected between January 2020 and April 2021 from samples of inpatients and outpatients from various hospital departments. VITEK 2 system was used for bacterial identification and antibiotic susceptibility testing, biofilm formation was evaluated using Congo red agar (CRA).

Results: It was observed that 56 (84.3%) of 67 *S. aureus* isolates cultured on CRA produced biofilm, while the remaining 11 (15.7%) were not biofilm producers. A statistically significant relationship was found between methicillin resistance and biofilm formation in *S. aureus* isolates. Accordingly, a significantly higher biofilm formation was observed in MRSAs compared to those with negative methicillin resistance (92.1% vs. 72.4%, p=0.034). A high proportion of isolates of *S. aureus* showed susceptibility towards tigecycline (100%) and gentamycin (100%).

Conclusion: The findings of this study indicated that methicillin-resistant strains produced more biofilms and exhibited a high degree of resistance to most antibiotics.

Keywords: *Staphylococcus aureus*, MRSA, biofilm, Congo red agar, Northern Cyprus

ORCID IDs of the authors: A.H.İ. 0000-0001-9303-4872; E.G. 0000-0002-1635-0051; C.B. 0000-0003-0998-4176; K.S. 0000-0002-2565-3425; E.Ç. 0000-0003-1873-1218.

Corresponding Author/Sorumlu Yazar: Emrah Güler,
E-mail: emrah.guler@neu.edu.tr

Received Date/Geliş Tarihi: 01.02.2022 **Accepted Date/Kabul Tarihi:** 09.05.2022

J Acad Res Med 2022;12(2):55-60

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a gram-positive commensal opportunistic pathogen which poses a threat to public health. It is responsible for bacteremia acquired in hospitals with a mortality rate of 20-30%. Other infections caused by *S. aureus* are bloodstream infections, surgical site infections, skin, and soft tissue infections, infectious endocarditis, osteomyelitis, device-related infections and pneumonia (1).

S. aureus can colonize and spread by attaching to the host's extracellular matrix components and serum proteins. In the pathogenesis of *Staphylococcal* infection, it has been observed that biofilm production has a very active role in protecting the colony from environmental factors, antibacterial therapy, and immune reactions of the host (2). Biofilms are complex assemblages of bacteria embedded in an extracellular matrix of exopolysaccharides, proteins, and macromolecules like DNA. They can grow on both living and non-living surfaces. Studies using molecular methods and scanning electron micrographs have shown that biofilms colonize on wounds. They shield the microorganisms from host immunity and prevents antibiotics from reaching the site of infection, causing wound healing to be hindered (3).

In developed countries, methicillin resistant *S. aureus* (MRSA) is now endemic in nearly all medical centers (4). The *mecA* or *mecC* genes are found on the *Staphylococcal* chromosomal cassette and encode penicillin-binding protein 2A (PBP2A), an enzyme that crosslinks the peptidoglycans in the bacterial cell wall, which confers methicillin resistance. Beta lactam antibiotics are ineffective against these enzymes, which results in resistance. Vancomycin has been used as a first-choice antibiotic to treat MRSA infections for years. Outbreaks of multidrug-resistant, medium and high-level vancomycin-resistant *S. aureus* (VRSA) have occurred over the last two decades, posing an important public health risk (5). *S. aureus* can be easily transmitted between individuals in the healthcare and in the community settings, owing to its commensal presence with immunocompetent individuals. Furthermore, it is a growing matter of concern due to its relation to hospital-acquired infections and antibiotic resistance (6). MRSA is a serious and widespread problem due to its capacity to colonize and cause disease in humans and animals (7). Therefore, the aim of our study was to investigate the phenotypic biofilm forming abilities of *S. aureus* isolates and the relationship of antibiotic resistance of biofilm forming *S. aureus* with biofilm formation.

METHODS

Design of Study

A cross-sectional descriptive study was carried out in the microbiology laboratory at the Near East University Hospital in the Turkish Republic of Northern Cyprus. A total of 67 non-repeated samples for the study were collected between January 2020-April 2021 from hospitalized patients from various hospital departments.

Samples Collection

Collected samples were cultured on blood agar (Merck, KgaA, Germany) and Eosin Methylene Blue agar (Becton Dickinson, Sparks, MD 211 52, USA) and incubated at 35 °C for 24-48 hours to obtain pure colonies. Only colonies that grew on blood agar media were loaded into the VITEK 2 (bioMérieux SA, F-69280 Marcy l'Etoile, France) system for bacterial identification and antibiotic susceptibility patterns; then, when the VITEK 2 device identified *S. aureus*, the bacterial colonies were transferred and stored in bacteria storage tubes (OR-BAK, Ankara, Turkey) at -30 °C until used.

Samples Isolation and Culturing

To revive the stored samples, *S. aureus* strains were inoculated on blood agar for growth and incubated for 24-48 hours at 35 °C to get pure colonies, then Congo red agar (CRA) was prepared and pure colonies from blood agar were inoculated on CRA for biofilm detection and subsequently incubated for 24-48 hours at 35 °C, colonies that were black were considered biofilm positive whereas colonies that showed red were considered biofilm negative. Both blood agar and CRA were prepared as per the manufacturer's directions.

Antibiotic Susceptibility Testing

For bacterial identification and antibiotic susceptibility, VITEK 2 system was employed. All *S. aureus* isolates were tested for their sensitivity against 16 commonly used antibiotics which were as follows: benzylpenicillin, cefoxitin, gentamicin, ciprofloxacin, levofloxacin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, tetracycline, tigecycline, fosfomycin, fusidic acid, mupirocin and cotrimoxazole. Vancomycin resistant strains were confirmed by the E-test method using Vancomycin MIC Test Strip (Liofilchem s.r.l., Italy).

Detection of Biofilm Production

For the formation of biofilm in *S. aureus* clinical isolates, CRA method was utilized. In CRA method, *S. aureus* was inoculated in CRA comprising Blood Base 2 media (40 gr/L supplemented with 10 gr/L glucose and Congo red (0.4 gr/L). It was incubated at 37 °C for 24-48 hours. The biofilm produced was observed and interpreted; a positive result indicated black color colonies (Figure 1) with a dry crystalline consistency and negative result indicated red color colonies (Figure 2).

To ensure quality control of test organisms, three bacterial strains were used as controls for the experiment: *S. aureus* ATCC29213 was used as the positive control for biofilms, while *S. aureus* ATCC6538 and *S. epidermidis* ATCC11047 were used as negative biofilm controls, respectively. They were then incubated on CRA plates to determine whether they produced black colonies. For growth and biofilm formation, all control species were cultured on both blood agar and CRA. The isolates were then incubated at 37 °C for 24-48 hours.

J Acad Res Med 2022;12(2):55-60

Statistical Analysis

All data acquired were statistically analyzed with a computer-based SPSS 22 software package. Frequency and cross-tabs analysis were used to test the totals. To discover an association between two variables, a Pearson chi-square test was utilized with a significance level of $p<0.05$.

Ethical Approval

This study was approved by the Scientific Research Ethics Committee of Near East University on 25.02.2021 (2021/88-1194). Patient consent was not required because the samples sent to the routine laboratory were examined. The names of the patients were covered, and the privacy of data was maintained.

RESULTS

A total of 67 samples for the study were collected between January 2020 and April 2021 from hospitalized patients from various hospital departments and subjected to microbiological analysis to isolate *S. aureus* strains. The mean age of the patients with MRSA isolated was 63.32±26.10 (between 3-97 years), while the average age of patients isolated with methicillin-sensitive *S. aureus* (MSSA) was 44.24±28.50 (between 1-92 years). According to the data obtained, the frequency of MRSA infection increased significantly as the age got older (p=0.006) as shown in Table 1. Among a total of 67 *S. aureus* isolates, 38 (56.7%) were identified to be MRSA by using VITEK 2 antibiotic susceptibility testing system with cefoxitin performed and the remaining 29 (43.3%) were identified to be MSSA.

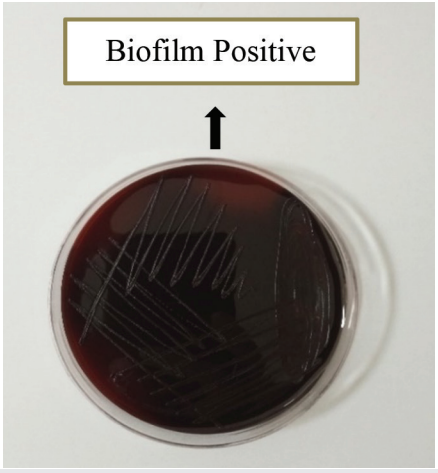

Figure 1. Black colonies positive biofilm

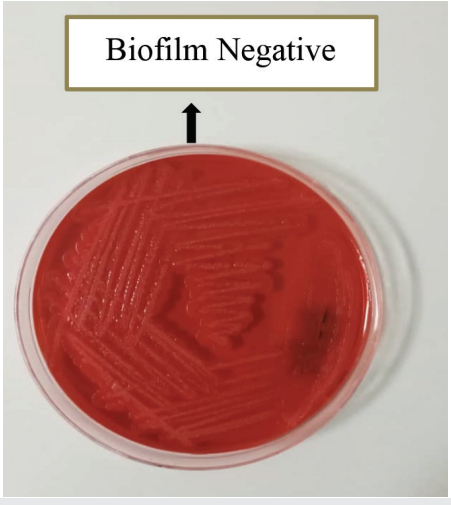

Figure 2. Red colonies negative biofilm

Out of 38 MRSAs, 29 (76.3%) of them were recovered from inpatients and 9 (23.7%) from outpatients. The association between MRSA occurrences in inpatients was statistically significant (p=0.018), which demonstrated the fact that the possibility of finding MRSA in admitted patients was high as compared to the outpatients as shown in Table 2.

Among 67 *S. aureus* isolates, 29 were MSSA and 38 were MRSA. Of those 35 were male and 32 were female patients. However, no significant relationship between gender and growth of MRSA was identified (p=0.675). Among 67 *S. aureus* strains recovered, 52.2% were MRSA, 43.3% were MSSA, while 4.5% were VRSA.

A total of 67 *S. aureus* isolates undergoing CRA method demonstrated 56 (84.3%) as biofilm producer and the rest 11 (15.7%) as non-biofilm producer. A statistically significant relationship was found between methicillin resistance and biofilm formation in *S. aureus* isolates. Accordingly, significantly higher biofilm formation was observed in MRSA (92.1%) compared to MSSA (72.4%) isolates (p=0.034) as shown in Table 3.

All *S. aureus* isolates were tested for their sensitivity against 16 commonly used antibiotics. Resistance rates of the MRSA isolates were significantly higher towards benzylpenicillin 33 (97.1%), clindamycin 27 (75%) and tetracycline 17 (47.2%) compared to MSSA isolates. Of the MSSA isolates 22 (81.5%) were resistant to benzylpenicillin, 10 (34.5%) to clindamycin, and 5 (17.2%) to tetracycline. Lower rate of resistance was observed in 1 (3.7%) MSSA against linezolid, however MRSA showed no resistance against linezolid, making it the most effective antibiotic for severe MRSA infections and it could be used as empiric therapy. On the other hand, both MRSA and MSSA showed less resistance against ciprofloxacin, levofloxacin, daptomycin, mupirocin and trimethoprim/sulfamethoxazole. Furthermore, the MRSA isolates showed a statistically significant resistance pattern against the following antibiotics: clindamycin and tetracycline compared to MSSA (p=0.001 and 0.011, respectively). Almost all isolates were sensitive to tigecycline and gentamycin. Interestingly, MRSA isolates were even resistant to vancomycin and teicoplanin [3 (8.3%) and 2 (5.7%), respectively]. On the other hand,

Table 1. Distribution of MRSA due to age

	No of patients	Mean age of patients	Standard deviation	p-value
MSSA	29	44.24	28.50	0.006
MRSA	38	63.32	26.10	

MSSA: methicillin-sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

no MSSA was found resistant to vancomycin, but it was resistant to teicoplanin 1 (3.6%) as shown in Table 4.

DISCUSSION

Bacteria in biofilms are of considerable concern as they represent up to 65% of human infections and they have high resistance (10-1000 times) to normal antibiotics (2). Nosocomial infections are a severe and persistent issue in the hospital settings. MRSA is a significant human pathogen which causes various diseases in humans, ranging from skin infections to severe infections like pneumonia, soft tissues, bones, heart valves, and even fatal septicemia (8). In this study, the CRA method was used to detect biofilm production of *S. aureus*. A total of 67 *S. aureus* isolates were incorporated, together with two control strains of *S. aureus* and *S. epidermidis*, as positive and negative biofilm controls, respectively.

This study discovered that patient age was a risk factor for MRSA infection in admitted patients. The mean age of patients with MRSA infection was 63.32±26.10 (range: 3-97 years), whereas the average age of patients with MSSA infection was 44.24±28.50 (between 1-92 years). According to the data obtained, the prevalence of MRSA infection increased significantly with age (p=0.006), which correlated with the findings of a previous study by Kshetry et al. (9), who found that 29 strains of MRSA were isolated from adults and 18 strains were isolated from pediatric patients, with the difference being statistically significant. In our current study, the prevalence of MRSA was found to be high (n=38, 56.7%) compared to MSSA (n=29, 43.3%), with a similar rate reported by Belbase et al. (10). The numbers and rates of and MSSA were 36 (47.4%) and 17 (22.4%), respectively in the study by Piechota et al. (8). However, a lower prevalence of MRSA was reported as 26.12% by Pandey et al. (11). Of the 38 (56.7%) MRSA strains, 3 (4.5%) were resistant to vancomycin which was comparable to the results of Jahanshahi et al. (12). In our study, substantial proportion of MRSA isolates were obtained from hospitalized patients (n=29, 76.3%). Colonized health care workers in hospitals are the primary source of MRSA infection in hospitalized patients, resulting in increased infection rates. However, the isolation rate of MRSA among outpatients was low, (n=9, 23.7%). Additionally, admitted patients who became colonized during their hospital stay might act as secondary sources of community-acquired MRSA infections. The higher rate of MRSA infection in admitted patients was statistically significant (p=0.018), which was consistent with the findings of Belbase et al. (10), 54.5% and 41.9% in inpatients and in outpatients, respectively. This difference could be explained by a prolonged hospital stay, instrumentation, and other invasive devices, as well as the fact that *S. aureus* was mostly associated with nosocomial infections.

Table 2. Distribution of MRSA and MSSA in outpatients and inpatients

Patient's type	Number of MRSA (%)	Number of MSSA (%)	Total number (%)	p-value
Inpatients	29 (76.3)	14 (48.3)	43 (64.2)	0.018
Outpatients	9 (23.7)	15 (51.7)	24 (35.8)	
Total	38 (100)	29 (100)	67 (100)	

MSSA: methicillin-sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

Table 3. Correlation between biofilm production and methicillin-resistance

Biofilm	MRSA (%)	MSSA (%)	p-value
Producer	35 (92.1)	21 (72.4)	0.034
Non-producer	3 (7.9)	8 (27.6)	
Total	38 (100)	29 (100)	

MSSA: methicillin-sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

Numerous studies have been conducted on producing biofilms by *Staphylococcus* species using various methods (13,14). It was revealed in this study that the technique used could detect the formation of biofilms between isolated strains. The current study evaluated the production of biofilms/ESPs by 67 *S. aureus* strains on CRA. Out of 67 cultures inoculated on CRA, 56 (84.3%) were identified as *S. aureus* producing biofilm, which was comparable to the results of Sharma et al. (15), (2021), which identified 53 (80%) as biofilm producing *S. aureus*. However, Haghi Ghahremanloi Olia et al. (16) reported a higher rate of biofilm production (n=57, 95%), which could be explained by the imprecision with which this method identified moderate biofilm-producing strains (17).

Because biofilms are protective, bacteria growing in them are intrinsically resistant to a wide variety of antibiotics. Positive biofilm producers were detected in 92.1% of MRSA samples and 72.4% of MSSA samples. A statistically significant relationship between methicillin resistance and biofilm formation in *S. aureus* isolates (p=0.034) was observed, consistent with the results of Khasawneh et al. (18), indicated that 90.9% of MRSA and 71.4% of MSSA isolates were resistant to methicillin. According to a study conducted by Grinholc et al. (19), only 45-47% of MRSA strains and 66-69% of MSSA strains could form biofilms *in vitro*. Certain strains have been reported to produce no biofilm despite the presence of a locus. Biofilm formation is widely regarded as a significant factor in the virulence of antibiotic-resistant bacteria, particularly MRSA. Further phenotypic and genotypic characterization of the *ica* locus genes is required to better understand the mechanism of biofilm production in *Staphylococcal* infections (20).

The development of MRSA among *S. aureus* strains leads to problems in the treatment of these infections. Monitoring *S. aureus'* antimicrobial susceptibility patterns is of prime significance to understand new emerging resistance trends and to treat infections in hospitals and in community (21). This study found that commonly used antibiotics were more resistant to MRSA than to MSSA; the highest resistance rates were observed against benzylpenicillin (n=33, 97.1%), clindamycin (n=27, 75%), and tetracycline (n=17, 47.2%). In this study, a high proportion of

J Acad Res Med 2022;12(2):55-60

Table 4. Resistance pattern of *S. aureus* from different clinical specimens (n, %)

Antibiotics	MSSA	MRSA	p-value	Biofilm-producer	Non-biofilm producer	p-value
Benzylpenicillin	22/27 (81.5)	33/34 (97.1)	0.055	46/51 (90.2)	9/10 (90.0)	0.676
Gentamicin	0/29 (0)	0/35 (0)	-	0/54 (0)	0/10 (0)	-
Ciprofloxacin	1/29 (3.4)	7/36 (19.4)	0.054	5/52 (9.6)	0/10 (0)	0.402
Levofloxacin	1/29 (3.4)	5/38 (13.2)	0.174	4/54 (7.4)	0/10 (0)	0.498
Clindamycin	10/29 (34.5)	27/36 (75.0)	0.001	30/54 (55.6)	7/11 (63.6)	0.441
Linezolid	1/27 (3.7)	0/35 (0)	0.435	1/50 (2.0)	0/11 (0)	0.823
Daptomycin	0/28 (0)	2/35 (5.7)	0.305	2/53 (3.8)	0/10 (0)	0.706
Teicoplanin	1/28 (3.6)	2/35 (5.7)	0.584	3/52 (5.8)	0/11 (0)	0.557
Vancomycin	0/28 (0)	3/36 (8.3)	0.171	3/54 (5.6)	0/10 (0)	0.595
Tetracycline	5/29 (17.2)	17/36 (47.2)	0.011	20/54 (37.0)	2/11 (18.2)	0.199
Tigecycline	0/29 (0)	0/35 (0)	-	0/54 (0)	0/10 (0)	-
Fosfomycin	0/29 (0)	3/34 (8.8)	0.151	3/53 (5.7)	0/10 (0)	0.590
Fusidic acid	0/27 (0)	3/34 (8.8)	0.166	3/52 (5.8)	0/9 (0)	0.614
Mupirocin	1/27 (3.7)	0/32 (0)	0.458	1/50 (2.0)	0/9 (0)	0.847
SXT	3/29 (10.3)	2/38 (5.3)	0.372	5/56 (8.9)	0/11 (0)	0.396

SXT: trimethoprim/sulfamethoxazole, MSSA: methicillin-sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

isolates (97.1%) were penicillin-resistant. This was expected, as only a minority of *S. aureus* strains did not produce beta-lactamases. In a study carried out by Ansari et al. (21), a comparable rate of resistance to penicillin was observed (94.7%).

The MRSA is commonly treated with clindamycin. Other types of antibiotics, like macrolides, can also lead to macrolide-resistant strains of *S. aureus*. Resistance to macrolides, on the other hand, can occur due to mutation of the 23S rRNA encoded by the *erm* gene, known as MLSB resistance, and is also referred to as clindamycin resistance or MLSB resistance (due to efflux mechanism encoded by the *msrA* gene). Failure can occur if the treatment is applied to a strain of bacteria that contains an *erm* gene, which can induce resistance (21). In our study, we identified 27 (75%) MRSA resistant strains and 10 (34.5%) MSSA resistant strains against clindamycin which were in line with the findings of Horváth et al. (22), indicating that clindamycin resistance was present in 79.1% of patients.

Study Limitations

This study was done only phenotypically. Therefore, molecular analyses of genes responsible for biofilm formation and antibiotic resistance are needed. Also, this study was cross-sectional with small size of 67 samples from a single center and therefore, did not represent an overall prevalence of biofilm forming MRSA in hospitals in Northern Cyprus. Multicenter studies with large number of samples collected from patients are required to estimate the overall prevalence of biofilm forming MRSA in hospitals across the country. In addition, the fact that teicoplanin-resistant strains were nor confirmed by a different method (such as the E-test) was another limitation of our study.

CONCLUSION

According to the findings of this study, *S. aureus* formed biofilms and this finding was clinically significant as biofilm formation was associated with the pathogenicity of organisms that caused device-related infections and exhibited high resistance to antibiotics. The CRA method used in the study to detect biofilm was reliable and the prevalence rate of MRSA isolated from hospitalized patients with *S. aureus* was high.

In the hospital setting, the wound/pus was the primary source of *S. aureus* and MRSA. Tigecycline and gentamycin (100%) were the prior drugs of choice for the treatment of *S. aureus* infections, including MRSA, followed by linezolid, mupirocin, and daptomycin. MRSA strains exhibited multidrug resistance and were unusually resistant to vancomycin, the drug of choice, indicating that MRSA was a vibrant organism. As a result, this threat can be mitigated through the implementation of sound infection control policies, regular surveillance of the antibiotic profile of *Staphylococcus* isolates to establish antibiotic policies, and the reasonable use of antimicrobial agents. Additionally, as this study only qualitatively presents biofilm in isolates, additional research is recommended that further research be conducted on the molecular mechanisms involved. There is a need for detailed information on the molecular mechanisms underlying biofilm formation and its relationship to other microbial processes such as virulence and antibiotic resistance.

Ethics Committee Approval: This study was approved by the Scientific Research Ethics Committee of Near East University on 25.02.2021 (2021/88-1194).

Informed Consent: Patient consent was not required because the samples sent to the routine laboratory were examined.

J Acad Res Med 2022;12(2):55-60

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - A.H.İ., E.G.; Concept - E.G., K.S.; Design - E.G., E.Ç.; Data Collection and/or Processing - A.H.İ., E.G.; Analysis and/or Interpretation - E.G., K.S., E.Ç.; Literature Search - A.H.İ., C.B.; Writing - A.H.İ., C.B.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Parastan R, Kargar M, Solhjoo K, Kafilzadeh F. Staphylococcus aureus biofilms: Structures, antibiotic resistance, inhibition, and vaccines. Gene Reports 2020; 20: 100739.
2. Haney EF, Trimble MJ, Cheng JT, Vallé Q, Hancock REW. Critical Assessment of Methods to Quantify Biofilm Growth and Evaluate Antibiofilm Activity of Host Defence Peptides. Biomolecules 2018; 8: 29.
3. Neopane P, Nepal HP, Shrestha R, Uehara O, Abiko Y. In vitro biofilm formation by Staphylococcus aureus isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. Int J Gen Med 2018; 11: 25-32.
4. Kobayashi SD, Malachowa N, DeLeo FR. Pathogenesis of Staphylococcus aureus abscesses. Am J Pathol 2015; 185: 1518-27.
5. Cong Y, Yang S, Rao X. Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features. J Adv Res 2019; 21: 169-76.
6. Ward AC, Hannah AJ, Kendrick SL, Tucker NP, MacGregor G, Connolly P. Identification and characterisation of Staphylococcus aureus on low cost screen printed carbon electrodes using impedance spectroscopy. Biosens Bioelectron 2018; 110: 65-70.
7. Papadopoulos P, Angelidis AS, Papadopoulos T, Kotzamanidis C, Zdragas A, Papa A, et al. Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in bulk tank milk, livestock and dairy-farm personnel in north-central and north-eastern Greece: Prevalence, characterization and genetic relatedness. Food Microbiol 2019; 84: 103249.
8. Piechota M, Kot B, Frankowska-Maciejewska A, Grużewska A, Woźniak-Kosek A. Biofilm Formation by Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus Strains from Hospitalized Patients in Poland. Biomed Res Int 2018; 2018: 4657396.
9. Kshetry AO, Pant ND, Bhandari R, Khatri S, Shrestha KL, Upadhaya SK, et al. Minimum inhibitory concentration of vancomycin to methicillin resistant Staphylococcus aureus isolated from different clinical samples at a tertiary care hospital in Nepal. Antimicrob Resist Infect Control 2016; 5: 27.
10. Belbase A, Pant ND, Nepal K, Neupane B, Baidhya R, Baidya R, et al. Antibiotic resistance and biofilm production among the strains of Staphylococcus aureus isolated from pus/wound swab samples in a tertiary care hospital in Nepal. Ann Clin Microbiol Antimicrob 2017; 16: 15.
11. Pandey S, Raza MS, Bhatta CP. Prevalence and antibiotic sensitivity pattern of methicillin-resistant-Staphylococcus aureus in Kathmandu Medical College-Teaching Hospital. Journal of Institute of Medicine Nepal 2012; 34: 13-7.
12. Jahanshahi A, Zeighami H, Haghi F. Molecular Characterization of Methicillin and Vancomycin Resistant Staphylococcus aureus Strains Isolated from Hospitalized Patients. Microb Drug Resist 2018; 24: 1529-36.
13. Croes S, Deurenberg RH, Boumans ML, Beisser PS, Neef C, Stobberingh EE. Staphylococcus aureus biofilm formation at the physiologic glucose concentration depends on the S. aureus lineage. BMC Microbiol 2009; 9: 229.
14. Metzler A. Developing a crystal violet assay to quantify biofilm production capabilities of Staphylococcus aureus (dissertation). The Ohio State University; 2016.
15. Sharma S, Bhandari U, Oli Y, Bhandari G, Bista S, Gc G, et al. Identification and detection of biofilm producing Staphylococcus aureus and its antibiogram activities. Asian Journal of Pharmaceutical and Clinical Research 2021; 14: 150-6.
16. Haghi Ghahremanloi Olia A, Ghahremani M, Ahmadi A, Sharifi Y. Comparison of biofilm production and virulence gene distribution among community- and hospital-acquired Staphylococcus aureus isolates from northwestern Iran. Infect Genet Evol 2020; 81: 104262.
17. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 2011; 15: 305-11.
18. Khasawneh AI, Himsawi N, Abu-Raideh J, Salameh MA, Al-Tamimi M, Al Haj Mahmoud S, et al. Status of Biofilm-Forming Genes among Jordanian Nasal Carriers of Methicillin-Sensitive and Methicillin-Resistant Staphylococcus aureus. Iran Biomed J 2020; 24: 386-98.
19. Grinholc M, Wegrzyn G, Kurlenda J. Evaluation of biofilm production and prevalence of the icaD gene in methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains isolated from patients with nosocomial infections and carriers. FEMS Immunol Med Microbiol 2007; 50: 375-9.
20. Darwish SF, Asfour HA. Investigation of biofilm forming ability in Staphylococci causing bovine mastitis using phenotypic and genotypic assays. Scientific World Journal 2013; 2013: 378492.
21. Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, Upadhyay G, et al. Threat of drug resistant Staphylococcus aureus to health in Nepal. BMC Infect Dis 2014; 14: 157.
22. Horváth A, Dobay O, Sahin-Tóth J, Juhász E, Pongrácz J, Iván M, et al. Characterisation of antibiotic resistance, virulence, clonality and mortality in MRSA and MSSA bloodstream infections at a tertiary-level hospital in Hungary: a 6-year retrospective study. Ann Clin Microbiol Antimicrob 2020; 19: 17.