Afr. J. Med. Med. Sci. (2024) **5**3(4) 231-237

Detection of biofilm genes in multi-drug resistance *Staphylococcus* species and its relevance in drug resistance

Cajethan O. Ezeamagu[a*], Oluwafemi M. Adeogun[a] and Toyosi F. Osisami[a]
[a]Department of Microbiology, Babcock University, Ilishan, Ogun Statem Nigeria

Abstract
Objectives: Staphylococcus species are notorious pathogens associated with significant morbidity and mortality in healthcare institutions. Studies have shown that biofilm-producing *Staphylococci* are more difficult to control with higher resistance to antibacterial agents than those not embedded in biofilm. The study was aimed to evaluate the prevalence of biofilm genes in multidrug resistance *Staphylococcus* species and its effect on drug resistance.
Methods: A total of 53 Staphylococcal isolates were obtained from two teaching hospitals (include name of the hospitals), with the sources comprising of urine [6] and feaces [1]; hospital door handles [31], hospital walls [5], and hospital bed [10]. Identity of *Staphylococcus* species were confirmed by sequencing its tuf gene. Antibiotic susceptibility was performed using the diffusion method. Biofilm genes (*fnbA*, *cna*, *icaA*, *icaD* and *fnbB*) were assayed by multiplex polymerase chain reaction. The data were analyzed by descriptive statistics and effect on resistance by Chi square at pd"0.05.
Results: Staphylococcus species identified were *Staphylococcus epidermidis* [38], *Staphylococcus scuiri* [7], *Staphylococcus xylosus* [5], *Staphylococcus saprophyticus* [2] and *Staphylococcus arlettae* [1]. The isolates were highly resistant to all the antibiotics tested except ofloxacin, ciprofloxacin and levofloxacin. The biofilm genes (*fnbA*, *icaD* and *icaA*) were found in 35%, 17% and 1% isolates respectively and had no effect on antibiotic resistance (p>0.05).
Conclusion: This study revealed that some *Staphylococcus* species irrespective of the sources produced biofilms and were highly resistant to different antibiotic classes regardless of the biofilm status. Therefore, regular surveillance system is important to monitor and mitigate the spread of antimicrobial resistance in our community.

Keywords: Biofilm; Multi-drug resistance; PCR; Resistance genes; Staphylococcus species.

Correspondence: Cajethan O. Ezeamahu, Department of Microbiology, Babcock University, Ilishan, Ogun State, Nigeria
Email: onyezecajeth@yahoo.com

Abstrait
Objectifs: Les espèces de Staphylococcus sont des agents pathogènes notoires associés à une morbidité et une mortalité importantes dans les établissements de santé. Des études ont montré que les staphylocoques producteurs de biofilm sont plus difficiles à contrôler et présentent une résistance plus élevée aux agents antibactériens que ceux qui ne sont pas intégrés dans le biofilm. L'étude visait à évaluer la prévalence des gènes de biofilm dans les espèces de Staphylococcus multirésistantes aux médicaments et son effet sur la résistance aux médicaments.
Méthodes: Un total de 53 isolats de staphylocoques ont été obtenus dans deux hôpitaux universitaires (inclure le nom des hôpitaux), les sources comprenant l'urine [6] et les matières fécales [1] ; les poignées de porte d'hôpital [31], les murs d'hôpital [5] et le lit d'hôpital [10]. L'identité des espèces de Staphylococcus a été confirmée par séquençage de son gène tuf. La sensibilité aux antibiotiques a été réalisée à l'aide de la méthode de diffusion. Les gènes de biofilm (fnbA, cna, icaA, icaD et fnbB) ont été analysés par réaction en chaîne par polymérase multiplex. Les données ont été analysées par des statistiques descriptives et l'effet sur la résistance par le Chi carré à (p>0.05).
Résultats: Les espèces de Staphylococcus identifiées étaient Staphylococcus epidermidis [38], Staphylococcus scuiri [7], Staphylococcus xylosus [5], Staphylococcus saprophyticus [2] et Staphylococcus arlettae [1]. Les isolats étaient très résistants à tous les antibiotiques testés à l'exception de l'ofloxacine, de la ciprofloxacine et de la lévofloxacine. Les gènes du biofilm (fnbA, icaD et icaA) ont été trouvés dans 35%, 17% et 1% des isolats respectivement et n'avaient aucun effet sur la résistance aux antibiotiques (p>0.05).
Conclusion: Cette étude a révélé que certaines espèces de Staphylococcus, quelle que soit leur source, produisaient des biofilms et étaient très

résistantes à différentes classes d'antibiotiques, quel que soit le statut du biofilm. Par conséquent, un système de surveillance régulier est important pour surveiller et atténuer la propagation de la résistance aux antimicrobiens dans notre communauté.

Mots-clés: Biofilm ; Résistance multiple aux médicaments ; PCR ; Gènes de résistance ; Espèces de Staphylococcus.

Introduction

The human pathogens *Staphylococcus* species are a major contributor to nosocomial infections as well as community- and healthcare-associated illnesses. Skin infections, invasive infections such as pneumonia, infections of bone, soft tissues, heart valves, and even fatal septicemia in humans are associated with *Staphylococcus* species [1]. The presence of biofilm development and virulence factors such as toxic shock syndrome, hemolysins, and enterotoxins, corresponds with the severity of a Staphylococcal infection [2].

A biofilm is described as a population of bacteria that attaches to a biotic or abiotic surface and is encased in a self-produced exopolysaccharide matrix [3]. A bacterial formation called biofilm covers surfaces and is typically mixed with inorganic and organic contaminants. Antimicrobial agents, bacteriophage infection, and dehydration are all protected by bacterial biofilm. Because of their propensity to withstand antimicrobial treatments, biofilms pose a serious threat to the food business by promoting bacterial contamination and food deterioration [3] In essence, biofilms are extracellular polymeric substances (EPS) that give bacterial cells specific niches to occupy. They help pathogens survive for a long time by shielding the embedded bacterial cells from the host immune cells [4].

Staphylococcus species generally has the potential for biofilm production particularly on living and non-living surfaces. High percentage cases of human infections have been linked to biofilm-associated bacteria which are more difficult to treat due to reduced susceptibility to antibiotics [5,6]. This leads to increase in morbidity and mortality as previous study reported that approximately 671,689 cases were associated with antibiotic resistance [7]. Globally, 495 mortality rate was reported [8] and a number of mechanisms are employed by bacteria to achieve this resistance fit; alteration of drug target, impermeability to drug as well as mutations to drug targets [9]. Technically, biofilm formation by bacteria has been one of the effective strategies employed to evade the action of antibiotics [10]. A literature report shows that bacteria can exhibit an astronomical increase in resistance of order of 1,000 times greater in biofilm strains than strains with no potential for biofilm formation [11]. *Staphylococcus* species are common opportunistic pathogens associated with human infection [12] and potential of biofilm production with these organisms confer them the ability to evade antibiotic treatment especially in patient with infections [13]. Most studies associated with biofilm formation in *Staphylococcus* species were limited to isolates obtained from clinical samples. However, in this study, an investigative comparison was made between strains obtained from clinical samples and those not directly isolated from human patients. In addition, the effect of biofilm with respect to drug resistance was evaluated.

With increasing threat of antibiotic resistance in microorganisms globally, understanding the role biofilm plays in the development of resistance will help in proper treatment of infections arising as a result of *Staphylococcus* spp. and surveillance system. The aim of this present study was to evaluate the prevalence of biofilm in multidrug resistance in *Staphylococcus* spp. isolated from clinical and fomites samples as well its relation in drug resistance.

Materials and Methods

Sources and Isolation procedure

A total of 53 isolates were collected from one teaching hospital in Oyo (Adeoyo marternity Hospital) and Ogun (Babcock University) States. The isolates were of both clinical and non-clinical sources. Clinical sources include urine [6] and feaces [1]. While non-clinical sources (equipment and environment) include Hospital door handles [31], hospital walls [5], and hospital bed [10]. Isolates obtained from clinical samples were collected in a sterilized nutrient broth while those from fomites were obtained by swabbing using commercial cotton swab and transported to the Microbiology laboratory, Babcock University, for further analysis. All isolates were sub-cultured using mannitol salt agar. Purity was ensured on nutrient agar (HiMedia Laboratories, India). Preliminary screening such as Gram staining and catalase tests were performed on the isolates.

Molecular identification of the isolates

DNA was extracted from the representative bacterial isolates according to the protocol described in Quick-DNA miniprep plus kit (Zymo Research, Biolab, USA). The tuf gene of *Staphylococcus* spp and or 16S rRNA was amplified by standard PCR protocol.

The amplicons were purified and sequenced using Sanger sequencing method by Inquaba Biotech, South Africa commercially. The low-quality reads were trimmed with BioEdit ((version 7.2.5.0). The reads were then blasted on Basic Local Alignment Search Tool (http:blst.ncbi.nilm.nih.gov/blast.cgi) for species identification. The sequenced data were then deposited in GenBank under the accession numbers OR435824-OR435829, and OR431750-OR431751.

Antimicrobial susceptibility test and Biofilm gene detection

All isolates were subjected to antimicrobial susceptibility testing using the following antibiotics (CelTeCh Diagnostic, Belgium); amoxicillin-clavulanate, (30 µg), cefotaxime (25 µg) ceftriaxone sulbactam (45 µg), cefexime (5 µg), levofloxacin (5 µg), ciprofloxacin (10 µg), imipenem/clastatin (30 µg), cefuroxime (30 µg), ofloxacin (5 µg), erythromycin (15 µg), gentamycin (10 µg), azithromycin (30 µg) according to standard disk diffusion technique (modified Kirby–Bauer method) and the results interpreted according to Clinical and Laboratory Standards Institute standards [14]. Multiplex PCR was done to detect the target genes in the isolates. The target biofilm genes were *fnbA*, *cna*, *icaA*, *icaD* and *fnbB*. DNA amplification was achieved with aid of GeneAmp PCR 9700 system (Applied Biosystems) as described previously [15]. About 10 µL of amplicon was resolved by 1.5% agarose gel electrophoresis

Statistical analysis of data and Ethical consideration

Susceptibility and prevalence of biofim data was analysed by descriptive statistics. The drug resistance and biofilm data was analysed by Chi-Square and the level of significance was set at 0.05.

The ethical clearance for this study was obtained from Babcock University Health Research Committee (BURHEC)

Results

The sequencing analysis revealed five different species of *Staphylococcus* encountered: *S. epidemidis* [38], *S. scuiri* [7], *S. xylosus* [5], *S. saprophyticus* [2] and *S. arlettae* [1]. The distribution revealed that *S. epidemidis* was more frequently isolated compared to other species (Figure 1).

The susceptibility report indicated that virtually all the isolates tested were resistant against the antibiotics with the exception of ciprofloxacin (CIP), ofloxacin (OFX) and levofloxacin (LBC). The most resistant drug against the isolates was imipenem. However, LBC exerted greatest activity against the isolates (Figure 2). Of the five biofilm genes assayed, only *fnbA*, *icaD* and *icaA* were found in 35%, 17% and 1% isolates respectively. There was no significant difference between the isolates producing biofilms and non-producers with respect to drug resistance ($p>0.05$) (Table 2). *Staphylococcus epidermidis* isolates were the prominent biofilm producers among the isolates screened.

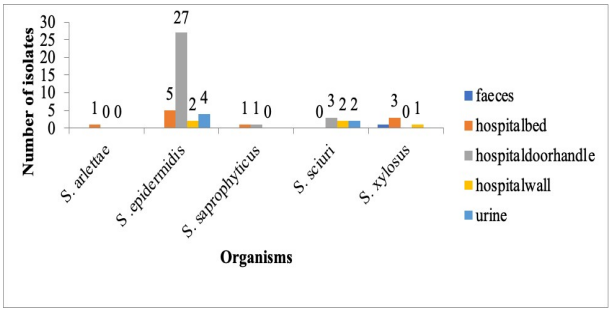

Figure 1: Distribution of Organisms according to sources

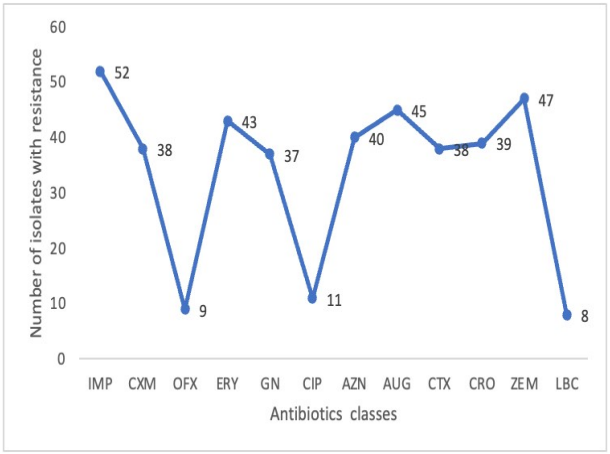

Figure 2: Antibiotics Resistance Pattern

Key: Iimepenem (IMP), cefuroxime (CXM), amoxicillin/clavulanate (AUG), cefixime (ZEM), cefotaxime (CTX), erythromycin (ERY), gentamicin (GN), azithromycin (AZN), ceftriaxone (CRO), ciprofloxacin (CIP), ofloxacin (OFX) and levofloxacin (LBC)

Table 1: Primers properties used in this study

S/N	Primers	Sequences 5'-3'	Tm (°C)	Molecular weight (bp)	Reference
1	fnbAF	CATAAATTGGGAGCAGCATCA	48	128	[16]
2	fnbAR	ATCAGCAGCTGAATTCCCATT			
3	fnbBF	GTAACAGCTAATGGTCGAATTGATACT	52	523	[16]
4	fnbBR	CAAGTTCGATAGGAGTACTATGTTC			
5	cnaF	AAAGCGTTGCCTAGTGGAGA	48	192	[16]
6	cna R	AGTGCCTTCCCAAACCTTTT			
7	icaA F	CCT AAC TAA CGA AAG GTA G	43	1315	[17]
8	icaA R	AAG ATA TAG CGA TAA GTG C			
9	icaD F	AAA CGT AAG AGA GGT GG	43	381	[17]
10	icaD R	GGC AAT ATG ATC AAG ATA C			
11	Tseq271	AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	55	884	[18]
12	Tseq1138	CCI ACI GTI CKI CCR CCY TCR CG			
13	341F	CCTACGGGAGGCAGCAG	49	466	[19]
14	R806	GGACTACHVGGGTWTCTAAT			

Table 2: Chi Square Analysis of Biofilm- Producing and Non-Producing Isolates with Respect to Drug Resistance

	N=53		P	
Antibiotics	**X^2**	**df**	***icaD***	***fnbA***
IMP	1.982	1	0.159	0.488
CXM	0.004	1	0.952	0.237
OFX	0.666	1	0.414	0.383
ERY	0.086	1	0.769	0.097
GN	0.128	1	0.721	0.468
CIP	0.36	1	0.85	0.701
AZN	1.142	1	0.285	0.138
AUG	0.337	1	0.561	0.642
CTX	0.004	1	0.952	0.596
CRO	0.026	1	0.872	0.743
ZEM	0.776	1	0.378	0.391
LBC	0.053	1	0.819	0.721

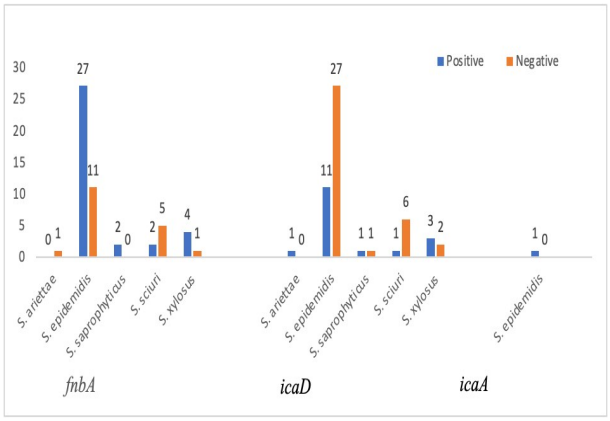

Figure 3: Distribution of Biofilm Producers according to the species

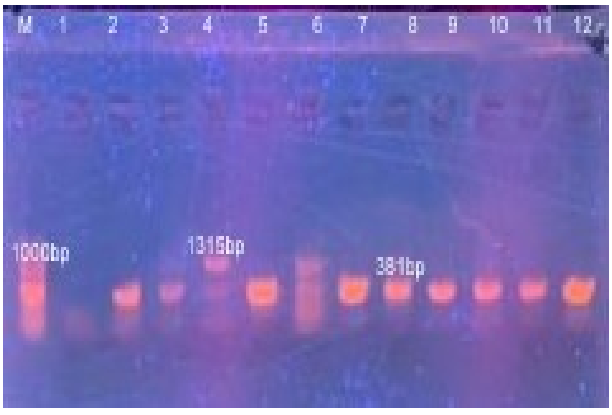

Figure 4: Multiplex Detection of Biofilm Gene
Lane M: 100bp ladder; lane 2, 3, 5, 7-12: *icaD* and lane 4: *icaA* positive isolates, Lane 1: Negative control (pcr mixture without DNA).

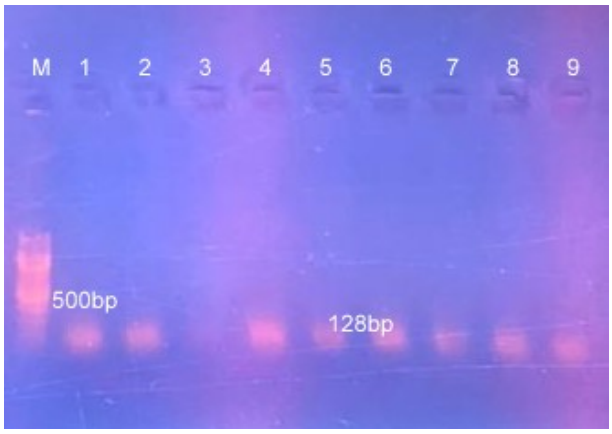

Figure 4: Electrophoregram of Biofilm fnbA gene
Lane M: 100bp ladder; lane 1,2, 4-9 (*fnbA* positive); Lane 3: Negative control (PCR mixture without DNA).

Discussions

Among the five *Staphylococcus* species obtained in this study, the most predominant was *Staphylococcus epidermidis* (73%) while another study [20] revealed that *Staphylococcus aureus* (43%) was predominant in addition to other species as *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lentus*, *S. lugdunensis*, *S. schleiferi*, *S. sciuri*, and *S. xylosus* with *S. saprophyticus* and *S. warneri*. From these studies, it is shown that abundance of *Staphylococcus* species is evidently diverse in clinical environment where they can be associated with opportunistic infections. In this study, 58.5% of the isolates were recovered from handles of hospital doors and this was in line with finding from another study which classified formites as microbial reservoirs [21]. The study further maintained that door handles and other related surfaces in clinical settings harbor diversity of microorganisms like *Staphylococcus* species which could be source of infection among caregivers and patients [21].

It is important to note that the low prevalence of *Staphylococcus* species on the hospital beds in this study does not preclude its potentials as reservoir for bacterial infections. An earlier report indicated that hospital beds can be a source of contamination through which bacterial infection can occur including the species of *Staphylococcus* [22]. Another report elsewhere further proved the existence of *Staphylococcus* on the hospital beds as well as other surfaces and maintain that hygiene is important to reduce the level of bacterial load [23]. Therefore, proper hygiene is an inevitable tool in the control of bacterial contamination and infection in both clinical and community settings.

Hospital-associated bacteria are typically resistant to various types of antibiotics. Since staphylococcal strains are common, their remarkable capacity to develop multi-drug resistance is a public health problem [24]. One serious issue affecting human health is antibiotic resistance. The bulk of isolates (Figure 2) were shown by the current findings to be resistant to the tested drugs. However, the study also showed that most isolates were susceptible to levofloxacin, ofloxacin, and ciprofloxacin. Since these antibiotics are inexpensive, they could be used to treat coagulase negative staphylococcus (CNS) infections caused by CNS in a resource-constrained nation like Nigeria. The high frequency of susceptibility pattern to certain antibiotics is consistent with the earlier study by [25, 26].

The prevalence of biofilm genes (fnbA, icaD, and icaA) in *Staphylococcus* species indicate varying levels of biofilm formation potential. A considerably higher tendency for biofilm formation, possibly via fibronectin-binding mechanisms, is indicated by the presence of the fnbA gene in 35% of the isolates in this study. Intercellular adhesion (encoded by ica genes) may not be as common in biofilm formation as other mechanisms, as evidenced by the presence of icaD in 17% and icaA in just 1% of isolates from our study. The multi-factorial character of biofilm production in *Staphylococcus* species is consistent with this pattern. As evidence of the complexity of biofilm formation across various strains and species, a study that examined the prevalence of biofilm-related genes in *Staphylococcus* strains from implant-associated illnesses showed varied gene distributions [27]. This study revealed the capacity of *Staphylococcus* species to form biofilms was relatively similar to that of staphylococcal strains isolated from medical implants [25].

In this study, there was no significant difference in resistance phenotype among biofilm producing *Staphylococci* and the non-biofilm producing counterpart (Figure 2). This agrees with a study on *S. epidermidis* isolated from hospital in Italy [28], but in contrast to earlier report that biofilm-producing organisms are more resistant to antibiotics than the non-biofilm formers [29]. In another study using *S. epidermidis* as a model to investigate the relationship between drug resistance and biofilm formation, it was found that although, correlation exist between biofilm formation and resistance, but, that this relationship was not seldom linear [30]. This means that somehow other factors may be mediating in the resistance phenotype. This could probably explain the non-correlation of biofilm formers with

drug resistance as obtained in this present study. Thus, this gives an avenue to explore other genetic and enzymatic principles that permit bacteria to exhibit resistance. Otto [31] suggested that real-time evaluation of infection due to biofilm using experimental animal with bioluminescent bacteria can give an insight on the potentials of biofilm factors in the event of infection.

Conclusion

The results revealed that *Staphylococcus* species irrespective of the sources have potentials for biofilm formation and are highly resistant to different antibiotic classes regardless of the biofilm status. Therefore, regular surveillance system is important to monitor and mitigate the spread of antimicrobial resistance in our community. Creating awareness among all age groups on the proper use of antibiotics and the dangers of antibiotic abuse would help the society greatly. Also, strict regulations and monitoring on antibiotic use should be ensured by the appropriate regulatory bodies.

Source of funding: This study received no funding from any funding agency.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The study was conducted with ethical approval obtained from University Health Research Ethics Committee (BUHREC), under the authorization number: BUHREC100/22.

Authors contributions: COE conceived and designed the study, OMA and TFO carried out the study, COE and OMA analysed the data. All authors were involved in draft and approval of the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript

References

1. Chambers H F, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. ***Nat Rev Microbiol.*** **2009**;7: 629-641.
2. Waters EM, Rowe SE, O'Gara JP, Conlon BP. Convergence of *Staphylococcus aureus* Persister and Biofilm Research: Can Biofilms Be Defined as Communities of Adherent Persister Cells? **PLoS Pathog**. **2016**;12(12): e1006012.
3. Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. Biofilms in the Food Industry: Health Aspects and Control Methods. **Front. Microbiol. 2018**; 9:898.
4. Ghasemian A, NajarPeerayeh S, Bakhshi B, Mirzaee M. *Biofilm formation between Methicillin-resistant and methicillin-susceptible isolates of Staphylococcus aureus.* ***Iran Biomed.*** **2016**; 20: 175-181.
5. Sharma D, Misba L, Khan AU. (2019). Antibiotics versus biofilm: an emerging battleground in microbial communities. **Antimicrob Resist Infect Control. 2019**; 8:76.
6. Andersson DI, Hughes D. Peristence of antibiotic resistance in bacterial populations. **FEMS Microbiol Rev. 2011** 35:901–911.
7. Tacconelli E, Pezzani MD. Public health burden of antimicrobial resistance in Europe. The **Lancet Infect Dis. 2019**; 19(1): 4-6.
8. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. **Lancet. 2022**; 399: 629-55.
9. Bharadwaj A, Rastogi A, Pandey S, Gupta S, Sohal JS. Multidrug-Resistant Bacteria: Their mechanism of action and prophylaxis. **BioMed res int. 2022**; 2022: 5419874
10. Silva V, Capelo JL, Igrejas G, Poeta P. Molecular mechanisms of antimicrobial resistance in *Staphylococcus aureus* biofilms. In: Emerging Modalities in Mitigation of Antimicrobial Resistance. Springer; 2022. pp. 291-314.
11. Thöming JG, Häussler S. *Pseudomonas aeruginosa* is more tolerant under biofilm than under planktonic growth conditions: a multi-isolate survey. **Front cell infect microbiol. 2022**; 12:851784.
12. Denissen J, Reyneke B, Waso-Reyneke M, Havenga B, Barnard T, Khan S, Khan W. Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. **Int. J of hyg Environ Health. 2022**; 244:114006.
13. Sasirekha B, Usha MS, Amruta JA, Ankit S, Brinda N, Divya R. Incidence of constitutive and inducible clindamycin resistance among hospital associated *Staphylococcus*. **FEMS Microbiol. Lett. 2014**; 2: 85-89.

14. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI 2020; Seventeenth Informational Supplement. Document M100-S17. Wayne, PA.
15. Ezeamagu C, Imanatue I, Dosunmu M, Odeseye A, Baysah G, Aina D, Odutayo F, Mensah-Agyei G. Detection of Methicillin Resistant and Toxin-associated Genes in *Staphylococcus aureus*. **Beni-Suef Univ J Basic Appl Sci. 2018**; 7(1): 92-97.
16. Shahmoradi M, Faridifar P, Shapouri R, Mousavi, SF, Ezzedin M, Mirzaei B. Determining the biofilm forming gene profile of *Staphylococcus aureus* clinical isolates via multiplex colony PCR method. **Rep Biochem Mol Biol. 2019**; 7(2): 181-188.
17. Demira C, Demirci M, Yigin A, Tokman HB, Yildiz SC. Presence of biofilm and adhesin genes in *Staphylococcus aureus* strains taken from chronic wound infections and their genotypic and phenotypic antimicrobial sensitivity patterns. **Photodiagnosis Photodyn Ther. 2020**; 29: 101584.
18. Martineau F, Picard FJ, Ke D, Paradis S, Roy PH, Ouellette M, Bergeron MG. Development of a PCR Assay for Identification of *Staphylococci* at Genus and Species Levels. **J Clin Microbiol. 2001**; 39(7):2541-2547.
19. Ogbodogbo FO, Ezeamagu CO, Barns JN. Diversity and susceptibility pattern of medically important bacteria isolated from intestinal tract of Hemidactylus frenatus in Ilishan Remo, Ogun State. **J Appl Biol Biotech. 2021**; 9(2): 131-141.
20. Socohou A, Sina H, Degbey C, Nanoukon C, Chabi-Sika K, Ahouandjinou H, Lehmane H, Baba-Moussa F, Baba-Moussa L. Antibiotics Resistance and Biofilm Formation Capacity of *Staphylococcus spp*. Strains Isolated from Surfaces and Medicotechnical Materials. **Int J Microbiol. 2020**; 10(6): 1-6.
21. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. **Infect Control Hosp Epidemiol. 2013**; 34(5): 508-515.
22. Mokracka J, Koczura R, Kaznowski A. High prevalence of *Staphylococcus aureus* (MRSA-MSSA) in environmental samples from hospital wards. **Polish J Microbiol. 2017**; 66(3): 363-370.
23. Bhargava HN, Leonard PA. Challenges in Hospital Environmental Hygiene and the Need for Preemptive Measures. **J Healthc Eng. 2014**; 5(2):185-195.
24. Rebiahi SA. Caract´erisation de souches de *Staphylococcus aureus* et ´etude de leur antibioresistance au niveau du centre hospitalo-universitaire de Tlemcen, 2012; Ph.D. thesis, Universit´e Tlemcen, Tlemcen, Algeria.
25. Shrestha LB, Bhattarai NR, Rai K, Khanal B. Antibiotic resistance and *mecA* gene characterization of coagulase-negative staphylococci isolated from clinical samples in Nepal. Infect Drug Resist. 2020;13:3163–9.
26. Manandhar S, Singh A, Varma A, Pandey S and Shrivastava N. Phenotypic and genotypic characterization of bioflm producing clinical coagulase negative staphylococci from Nepal and their antibiotic susceptibility pattern. **Ann Clin Microbiol Antimicrob (2021)** 20:41.
27. Arciola CR, Campoccia D, Gamberini S, Baldassarri L, Montanaro L. Prevalence of cna, fnbA and fnbB adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant. **FEMS Microbiol Lett. 2015**; 245(1): 87-93.
28. Carcione D, Leccese G, Conte G, Rossi E, Intra J, Bonomi A, Sabella S, Moreo M, Landini P, Brilli M, Paroni M. (2022). Lack of Direct Correlation between Biofilm Formation and Antimicrobial Resistance in Clinical *Staphylococcus epidermidis* Isolates from an Italian Hospital. **Microorganisms. 2022** 10: 1163.
29. Mah TF. Biofilm-specific antibiotic resistance. **Future Microbiol. 2012**; **7:**1061-1072.
30. Subbiahdoss G, Kuijer R, Grijpma DW, van der Mei HC, Busscher H J. Microbial biofilm growth vs. tissue integration: "The race for the surface" experimentally studied. **Acta Biomaterialia. 2012**; 8(2): 397-403.
31. Otto, M. (2008). Staphylococcal Biofilms. Infect Immun, 76(5): 2048-2057.

Received = 23/10/2023
Accepted = 29/11/2023