Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

Study of the Resistance of Biofilms Found in Liquid Effluents from the Dentistry Department

Zineb Haouari [1], Chafik Mhamdia [2], Salheddine Khaldi [3], Ahmed Reda Belmamoun [2], Abdelkader Ammam [4], Imene Berrabeh [5], Wissam Khelifi [2], Khadidja Larik [2]

[1] Faculty of Medicine, TALEB Mourad, Djillali Liabes University, 22000, Sidi Bel Abbes, Algeria.
[2] Faculty of Nature and Life Sciences, Djillali Liabes University, 22000, Sidi Bel Abbes, Algeria.
[3] Proximity Public Health Establishment, Chellala, El bayadh, 32000, Algeria.
[4] Moulay Tahar University, 20000, Saida, Algeria.
[5] University Hospital Center, 22000, Sidi Bel Abbes, Algeria.

(Received: 11 June 2024 Revised: 16 July 2024 Accepted: 10 August 2024)

KEYWORDS

Biofilm;
Antibiotic Resistance;
Staphylococcus;
Dental Practices;
Infection Control

ABSTRACT:

Our examination of biofilms and antibiotic resistance within bacterial populations derived from dental unit effluent in Sidi-Bel-Abbès, Algeria, produced significant revelations. We adeptly isolated and characterized four distinct bacterial strains. All isolated strains tested positive for free and bound coagulase, with the latter verified through the Staphytect Plus diagnostic assay. Notably, upon cultivation on Congo red agar, three strains exhibited morphological characteristics indicative of biofilm formation.

Antibiotic susceptibility assessments unveiled a concerning pattern of multi-drug resistance. These isolates showed an impressive degree of resistance to penicillin, oxacillin, erythromycin, and nalidixic acid. This resistance profile raises significant apprehensions regarding the efficacy of conventional antibiotic treatments for infections stemming from these sources. While our research did not explore the precise genetic foundations of this resistance, it is broadly acknowledged that staphylococci develop resistance through various mechanisms. Subsequent studies investigating the genetic foundations of resistance in these isolates would be highly beneficial.

Detecting biofilm-forming, antibiotic-resistant bacteria within dental unit waterlines highlights the imperative for enhanced infection control measures in dental practices. Biofilms create a defensive layer for bacteria, thus lessening their antibiotic exposure and complicating the host's immune reaction. The conclusions drawn from this study strongly advocate for establishing preventive protocols meant to hinder biofilm creation in dental practices, which are critical for diminishing the proliferation of antibiotic-resistant bacteria and preserving patient health in the long run. Future investigations should concentrate on formulating effective biofilm prevention and eradication methodologies within dental settings.

INTRODUCTION

Staphylococci, pervasive Gram-positive microorganisms, are acknowledged as prominent pathogens accountable for many human infections, presenting a substantial challenge to public and clinical health (Harris *et al.*, 2002). Hospital-acquired infections are increasingly alarming, emphasizing the necessity of comprehending and addressing the pathogenicity of these microorganisms (Hygis, 1998). *Staphylococcus aureus* and related species attribute a considerable portion of their resilience and pathogenic capabilities to their proficiency in biofilm formation (Roux and Ghigo, 2006). This characteristic markedly increases their resistance to therapeutic interventions and potential to induce chronic infections. This trait significantly enhances their resistance to treatment and capacity to cause persistent infections. Biofilms, complex communities of microorganisms adhering to surfaces and enveloped in a protective extracellular matrix, confer

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

enhanced resistance to conventional antibiotic treatments and host immune defense mechanisms(Hughes, 1997). Biofilm production by staphylococci diminishes antibiotic effectiveness, resulting in persistent infections and heightened patient morbidity (Roux and Ghigo, 2006). This study explores biofilm formation in **S. aureus** isolated from dental office mouth rinse samples, focusing on the correlation between biofilm formation and antibiotic resistance. Bacterial characterization detected * *Staphylococcus warneri**, **Staphylococcus warneri**, and **Micrococcus spp** within the analyzed specimens. Antibiotic susceptibility tests were carried out, examining the activity of 12 antibacterial agents commonly used in human medicine in Algeria. The research seeks to elucidate essential data concerning the prevalence, traits, and antibiotic resistance of biofilm-forming staphylococci within dental environments, thereby facilitating the development of enhanced infection prevention and control strategies.

MATERIALS AND METHODS

Sampling and isolation

Three samples of oral rinsing fluid were taken from two dental practices in Sidi-Bel-Abbès, Algeria. After culture in nutrient agar and Heart-Brain broth, bacterial strains were isolated on Chapman medium and incubated at 37°C for 24 to 48 hours. Four strains with distinct morphological characteristics were purified based on cultural traits and Gram staining and preserved on inclined nutrient agar. A series of biochemical tests was used to identify the strain. Initially, a macroscopic study of the colonies was carried out. This was followed by a microscopic study (gram staining) to determine cell morphology (Belmamoun *et al.*, 2022).

1. Catalase test

To assess the existence of the enzyme catalase, bacterial colonies were subjected to interaction with hydrogen peroxide (Batabyal, 2016). The liberation of oxygen bubbles signifies a positive outcome (Chaturvedi, 2022).

2. Free coagulase test

The free coagulase test was performed by mixing 0.5 ml bacterial suspension from overnight culture broths with 0.5 ml rabbit plasma incubated at 37°C and observing clot formation every 30 minutes, followed by a final reading after 24 hours for negative cases at 4 hours (Bello and Qahtani, 2005).

3. DNase assay

A DNase test was performed after subculturing the strain to be studied in BHIB, incubating for 18 hours at 37°C, inoculating using a heavy inoculum, and performing a central sting to detect the production of deoxyribonuclease, an enzyme that hydrolyzes DNA. The formation of a clear zone around the due colony identified DNase-producing colonies (Cheesbrough, 2006).

The API Staph gallery

Complete biochemical identification was done using the API STAPH system (bioMérieux). This standardized system comprises 20 microtubes containing dehydrated substrates, enabling 19 biochemical tests (Camille, 2014).

After inoculation with a bacterial suspension adjusted to 0.5 McFarland standard, the gallery was incubated at 37°C for 24 hours. Biochemical reactions were interpreted according to the manufacturer's instructions, generating a seven-digit code number. This code was then compared with the API database to identify the bacterial species (Gahrn-Hansen *et al.*, 1987).

Staphytect plus test

The Staphytect Plus (Oxoid), a latex agglutination test, was used to detect bound coagulase (clumping factor), protein A, and capsular polysaccharides, surface markers specific to *Staphylococcus aureus* (Stiles and Holzapfel, 1997).

Five colonies of each strain, grown on nutrient agar for 18 to 36 hours, were emulsified with one drop of test reagent (blue latex sensitized to porcine fibrinogen and rabbit IgG) and one drop of control reagent (non-sensitized latex) on a reaction card. After rotation for 20 seconds, agglutination of the sensitized latex was considered a

as a positive result (Leboffe and Pierce, 2021).

Antibiotic susceptibility test

The susceptibility of strains to antibiotics was determined by the diffusion method on 4 mm thick Mueller-Hinton agar, by the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM). Pre-incubated cultures were diluted in the sterile buffer. Twelve antibiotics were tested:

Penicillin (PG-10 IU), Oxacillin (OX- 1 µg), Erythromycin (E-15 µg), Trimethoprim + Sulfamethoxazole (SXT- 1.25 / 23.75 µg), Tetracyclines (Te -30 µg), Vancomycin (Va-30 µg), Clindamycin (Cm-2 µg), Ciprofloxacin (CIP-5ug), Cephalotin (KF 30 ug), Nalidixic acid (Na 30ug), Gentamicin (GN-10ug), Chloramphenicol (C-30ug)

After incubating the antibiotic discs at 37°C for 18-24 h, measure the precise area around each disc in millimeters (Akbar and Anal, 2013).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

Biofilm formation test

Evaluate strains' ability to form biofilms. After cultivation of staphylococcal isolates on Congo red agar (CRA) and incubation for 24 hours at 37°C (Mustapha *et al.*, 2020).

The formation of black colonies indicates a positive result for biofilm production, while the formation of red colonies indicates a negative result.

RESULTS & DISCUSSION

This section presents the results of the bacteriological analysis carried out on the oral rinse samples.

Sampling and isolation of strains

Seven strains were purified from the three oral rinse samples collected from two dental practices. Four of them were positive on Chapman agar, indicating the presence of staphylococci.

Macroscopic examination

After 24 hours of incubation, suspected staphylococci colonies on Chapman agar had the following characteristics: a yellow appearance, a smooth, round shape, a diameter between 1 and 2 mm, and yellow pigmentation (Fig. 1).

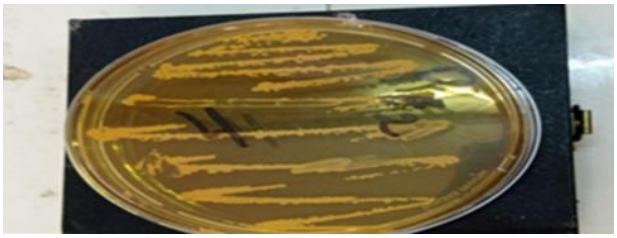

Fig. 1: Staphylococcus on Chapman medium

Microscopic examination

Microscopic observation confirmed the presence of Gram-positive cocci grouped in grape clusters, characteristic of staphylococci (Fig.2).

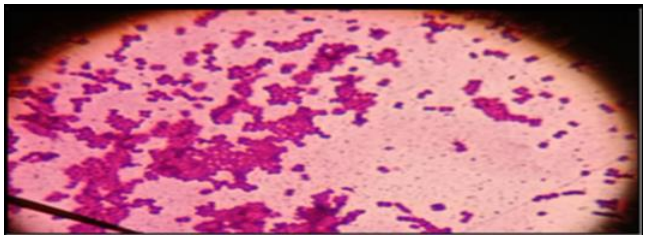

Fig. 2: Microscopic examination of Staphylococci (light microscope observations, magnification ×100)

Catalase test

All four isolated strains tested positive in the catalase test (Fig.3), confirming their membership of the Staphylococcus genus.

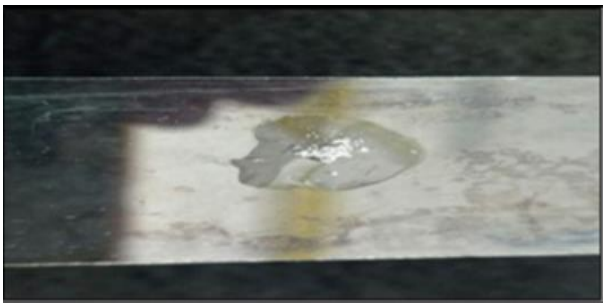

Fig. 3: Positive catalase test

Coagulase test

All four strains also tested positive for coagulase, resulting in the appearance of a clot (Fig. 4). It should be recognized that the coagulase gene is not exclusive to S. *aureus*. This gene can also be detected in other Staphylococcus species, including S. *intermedius*, S. *delphini*, and *S. hyicus* (Woo *et al.*, 2001) (Fernandes Queiroga Moraes *et al.*, 2021).

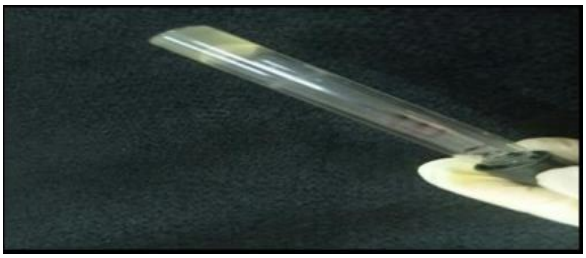

Fig. 4: Positive coagulase test

All the strains isolated (4/4) showed positive DNase activity (Fig. 5). Coagulation-negative staphylococci utilize DNase to break down DNA in the extracellular environment, enhancing their capacity to avoid immune system detection (Chon *et al.*, 2020).

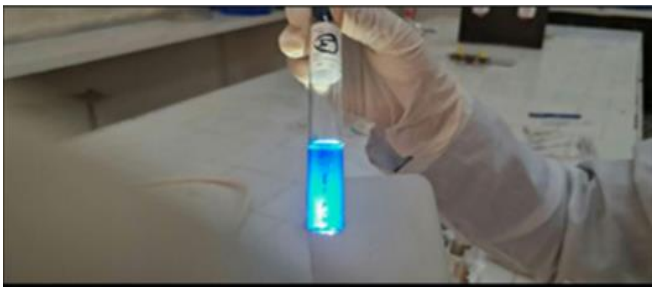

Fig. 5: Dnase test

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

Identification by API Staph

The API STAPH system identified four distinct biotypes among the strains isolated (Fig. 6):

4104100: *Staphylococcus capitis*
6310111: *Staphylococcus warneri*
0004101: *Micrococcus spp.*
6104100: *Staphylococcus capitis*

Fig. 6: API Staph galleries results

Staphytect Plus test

Three of the four strains isolated showed a positive result in the Staphytect Plus test, indicating the production of bound coagulase (Fig. 7). It is essential to highlight that the Staphytect Plus assay, despite being refined to minimize cross-reactivity with various staphylococcal species, may yield positive outcomes for species beyond *Staphylococcus aureus*. (PLUS)

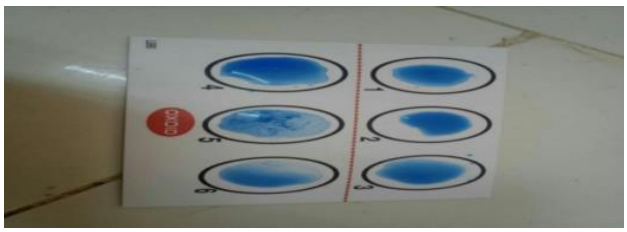

Fig.7: Staphytect test

Antibiotic susceptibility testing

The emergence of bacterial Resistance to antibiotics is a significant public health issue. A dual strategy is being implemented to combat this phenomenon: reducing antibiotic prescribing to limit selection pressure (GAGNAIRE *et al.*, 2015).

The study selected 12 antibiotics commonly used in human medicine in Algeria. To evaluate their effectiveness against bacteria, these substances were examined in laboratory conditions on staphylococci strains obtained from wastewater discharged by two dental offices.

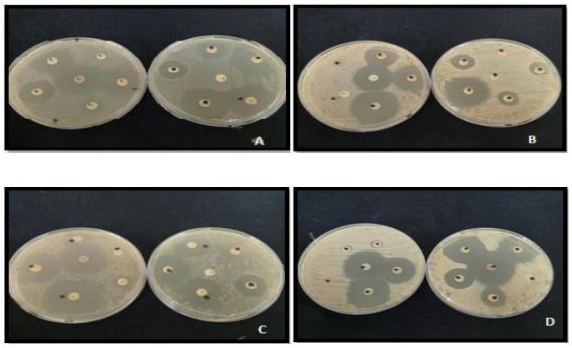

Fig.8: Antibiogram results: A: strain 01, B: strain 02, C: strain 03D: strain 01

as illustrated in Table 03, the phenomenon of antibiotic resistance was observed in our four isolated strains.

Table 03: Resistance and sensitivity of Staphylococcus strains to different antibiotics

Antibiotiques Testés	Code	Charge du Disque	Diamètres critiques (mm)			1	2	3	4
			R	I	S				
Pénicilline	PG	10 UI	≤28	-	≥29	R	R	R	S
Oxacilline S. aureus S.C.N	OX	1 µg	≤19	-	≥20	R	R	R	R
Erythromycine	E	15 µg	≤13	14-22	≥23	I	R	I	R
Triméthoprime+ Sulfaméthoxazole	SXT	1.25/23.75 µg	≤10	11-15	≥16	S	S	S	S
Tétracyclines	Te	30 µg	≤14	15-18	≥19	S	R	S	S
Vancomycine**	Va	30 µg	-	-	≥15	S	R	S	S
Clindamycine	Cm	2 µg	<14	15-20	≥21	R	S	I	I
Ciprofloxacine	CIP	5ug	≤15	16-20	≥21	S	S	S	S
Cephalothin	KF	30ug	<14	15-17	>18	R	R	R	R
Acide nalidixic	NA	30ug	<13	14-18	>19	R	R	R	R
Gentamicine	GN	10ug	≤12	13-14	≥15	S	S	S	S
Chloramphénicol	C	30ug	≤12	13-14	≥18	S	S	S	S

Our study showed Resistance, varying proportions depending on the antibiotic family (Fig. 9).

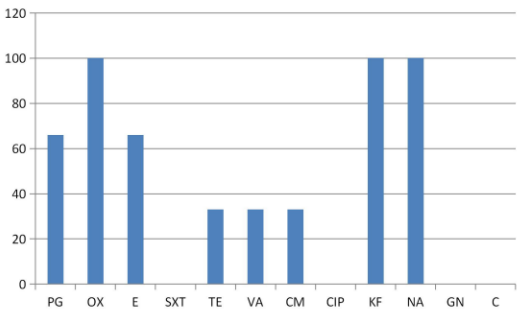

Fig.9: Antibiograms of isolated staphylococci

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

Resistance to oxacillin (meticillin), cephalothin, and nalidixic acid was high, with a resistance rate of 100%. Significant Resistance to penicillin and erythromycin was observed, both at 66%. In contrast, Resistance to clindamycin, vancomycin, and tetracycline was low (33%). No resistance was detected to gentamycin, trimethoprim + sulfamethoxazole, ciprofloxacin, and chloramphenicol. Resistance to one antibiotic confers Resistance to another, known as cross-resistance. Bacteria are considered multi-resistant when, due to an accumulation of natural and acquired resistances, they are only sensitive to a small number of antibiotics. They are then resistant to several antibiotics or pharmacological classes of antibiotics (Carle, 2009).

Table 04: Multiple antibiotic resistance among staphylococcal strains

Les biotypes	Les souches Résistantes	Nombre de souches résistantes par rapport au nombre d'antibiotiques						
		1	2	3	4	5	6	7
4104100	1					*		
6310111	1							*
0004101	1				*			
6104100	1				*			

Resistance to meticillin remains confined to hospital and institutional settings. The spread of Resistance outside these environments is a threat that cannot be ruled out (Leclercq, 2002). MRSA has remained confined to the hospital environment or, more rarely, to subjects in contact with the hospital environment (nursing staff, home care). This lasting compartmentalization between "hospital" and "community" has not been observed with *S. aureus* resistance to other classes of antibiotics (Tattevin, 2011). MRSA, which accounts for almost 30% of hospital staphylococci, has become multi-resistant, limiting therapeutic possibilities to use glycopeptides. To maintain the possibility of using new therapeutic molecules, it is essential to anticipate the extent to which they will affect bacterial populations by creating new resistances (Temime, 2013). Staphylococci exhibiting Resistance to penicillin, having acquired a plasmid-mediated penicillinase (an enzymatic agent that degrades penicillin), first manifested in clinical settings but swiftly disseminated to the broader populace. This Resistance is observed in over 90% of *S. aureus* strains.

The sequencing of the *S. aureus* genome has partially unlocked the secret of this power of adaptation. The *S. aureus* genome is made up of two distinct functional domains. The central part of the chromosome contains the genes that maintain the bacterium. The second part of the genome comprises accessory and mobile genetic elements such as plasmids, transposons, prophages, or pathogenesis islands carrying most of the genes associated with virulence factors and antibiotic resistance. Therefore, in addition to spontaneous mutations, *S. aureus* enhances its genetic variability through the horizontal transfer of genetic material with other bacterial species. *Staphylococcus aureus* demonstrates exceptional flexibility and has developed various strategies for Resistance. Oxacillin continues to exhibit efficacy against these particular strains; however, staphylococci acquired in hospital settings and, more recently, those sourced from the community present a growing concern. (present outside the hospital) have developed cross-resistance between M penicillins (meticillin, oxacillin) and other β-lactams through the production of a penicillin-binding protein (PLP2a) with low affinity for these compounds. The gene encoding PLP2a, mecA, is carried by a chromosomal element containing other genes to resist heavy metals and other antibiotics (Dumitrescu *et al.*, 2010).

Biofilm formation test

Our results showed that of the 04 strains, 03 were slim-producing strains with black colonies and a dry crystalline consistency (Fig.10).

Fig.10 : Biofilm test

The organization of cells into biofilms compromises the ability of antimicrobials to penetrate bacterial cells, preventing the accumulation of antibiotic concentrations (Asante *et al.*, 2022). Biofilm-producing cells are resistant to antibiotics and the immune system, leading to the recurrence of infection (Li *et al.*, 2020). The dye congo red interacts directly with specific polysaccharides, forming colored complexes so that slime-producing strains produce black colonies on congo red medium and non-producing strains red colonies (TERKI *et al.*, 2022). Methicillin-resistant S. capitis has been reported to form a viscous biofilm on medical devices. In addition, *S warneri* can form a biofilm

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

carrying more antibiotic-resistance genes than negative isolates (Yong *et al.*, 2019).

CONCLUSIONS:

This investigation elucidated the existence of bacterial biofilms, particularly *Staphylococcus capitis* and *Micrococcus spp*, within the liquid effluent of two dental clinics in the wilaya of Sidi-Bel-Abbès. Furthermore, these organisms demonstrated resistance to a range of antibiotics.

The results emphasize the critical danger that the dissemination of antibiotic-resistant bacteria represents in dental care, which may threaten public health and environmental integrity.

Additional investigations are necessary to examine the underlying resistance mechanisms and identify innovative strategies for preventing and eradicating biofilms, which would curtail the dissemination of resistant bacterial strains.

Reference

1. Akbar, A. & Anal, A. K. 2013. Prevalence and antibiogram study of Salmonella and Staphylococcus aureus in poultry meat. *Asian Pacific journal of tropical biomedicine,* 3**,** 163-168.
2. Asante, J., Abia, A. L., Anokwah, D., Hetsa, B. A., Fatoba, D. O., Bester, L. A. & Amoako, D. G. 2022. Phenotypic and genomic insights into biofilm formation in antibiotic-resistant clinical coagulase-negative Staphylococcus species from South Africa. *Genes,* 14**,** 104.
3. Batabyal, B. 2016. *Oral Carriage & Suffering of Staphylococcus Aureus: Oral Infection & Staph. Aureus*, Educreation Publishing.
4. Bello, C. & Qahtani, A. 2005. Pitfalls in the routine diagnosis of Staphylococcus aureus. *African Journal of Biotechnology,* 4**,** 83-86.
5. Belmamoun,A.R. Ammam,A. Chadli,R. Madouni,N. Ouali,I. 2022. Study of Colonization by Antibiotic-Resistant Staphylococci in Hospital Liquid Waste in the Southern Region of Sidi-Bel-Abbès. I*ndian Journal of Novel Drug Delivery, 14,105-110.*
6. Camille, D. 2014. *Pratique en microbiologie de laboratoire? Recherche de bactéries et de levures-moisissures*, Lavoisier.
7. Carle, S. 2009. La résistance aux antibiotiques: un enjeu de santé publique important. *Pharmactuel,* 42**,** 6-21.
8. Chaturvedi, A. 2022. *STAPHYLOCOCCUS AUREUS: CASES REPRESENTING MRSA WITH THEIR COMPLICATIONS & DIAGNOSTIC TESTS*, Booksclinic Publishing.
9. Cheesbrough, M. 2006. *District laboratory practice in tropical countries, part 2*, Cambridge university press.
10. Chon, J.-W., Lee, U. J., Bensen, R., West, S., Paredes, A., Lim, J., Khan, S., Hart, M. E., Phillips, K. S. & Sung, K. 2020. Virulence characteristics of mecA-positive multidrug-resistant clinical coagulase-negative staphylococci. *Microorganisms,* 8**,** 659.
11. Dumitrescu, O., Dauwalder, O., Boisset, S., Reverdy, M.-É., Tristan, A. & Vandenesch, F. 2010. Résistance aux antibiotiques chez Staphylococcus aureus-Les points-clés en 2010. *médecine/sciences,* 26**,** 943-949.
12. Fernandes Queiroga Moraes, G., Cordeiro, L. V. & De Andrade Júnior, F. P. 2021. Main laboratory methods used for the isolation and identification of Staphylococcus spp. *Revista Colombiana de Ciencias Químico-Farmacéuticas,* 50**,** 5-28.
13. Gagnaire, J., Verhoeven, P., Denis, C., Grattard, F., Carricajo, A., Pozzetto, B. & Berthelot, P. 2015. Prise en charge des bactéries multirésistantes aux antibiotiques dans les établissements de santé. *Feuillets de Biologie,* 322.
14. Gahrn-Hansen, B., Heltberg, O., Rosdahl, V. T. & Søgaard, P. 1987. EVALUATION OF A CONVENTIONAL ROUTINE METHOD FOR IDENTIFICATION OF CLINICAL ISOLATES OF COAGULASE-NEGATIVE STAPHYLOCOCCUS AND MICROCOCCUS SPECIES Comparison with API-Staph and API-Staph-Ident. *Acta Pathologica Microbiologica Scandinavica Series B: Microbiology,* 95**,** 283-292.
15. Harris, L. G., Foster, S. & Richards, R. G. 2002. An introduction to Staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review. *Eur Cell Mater,* 4**,** 100-20.
16. Hughes, K. A. 1997. Bacterial biofilms and their exopolysaccharides. *KB thesis scanning project 2015*.
17. Hygis, N. 1998. *Hygiène hospitalière*, Presses Universitaires Lyon.
18. Leboffe, M. J. & Pierce, B. E. 2021. *A photographic atlas for the microbiology laboratory*, Morton Publishing Company.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1451-1457 | ISSN:2251-6727

19. Leclercq, R. Résistance des staphylocoques aux antibiotiques. Annales francaises d'anesthesie et de reanimation, 2002. Elsevier, 375-383.
20. Li, Y., Xiao, P., Wang, Y. & Hao, Y. 2020. Mechanisms and control measures of mature biofilm resistance to antimicrobial agents in the clinical context. *ACS omega,* 5**,** 22684-22690.
21. Mustapha, A., Bello, H., Ibrahim, M., Wakil, S. & Bello, R. 2020. Evaluation of biofilm formation by Staphylococcus aureus recovered from clinical samples of patients attending a tertiarycare hospital in North-Eastern Nigeria. *Arch Clin Microbiol,* 11**,** 125.
22. Plus, D. S. S. Diagnostic Reagents.
23. Roux, A. & Ghigo, J.-M. 2006. Les biofilms bactériens. *Bulletin de l'Académie vétérinaire de France,* 159**,** 261-268.
24. Stiles, M. E. & Holzapfel, W. H. 1997. Lactic acid bacteria of foods and their current taxonomy. *International journal of food microbiology,* 36**,** 1-29.
25. Tattevin, P. 2011. Les infections à Staphylococcus aureus resistant à la méticilline (SARM) d'acquisition communautaire. *Médecine et maladies infectieuses,* 41**,** 167-175.
26. Temime, L. 2013. La diffusion des staphylocoques en milieu hospitalier. *Les cahiers de la Recherche: Santé, Environnement, Travail***,** 37-39.
27. Terki, I. K., Hassaine, H., Terki, A. K. & Bellifa, S. 2022. EFFET DE CERTAINS DESINFECTANTS ET ANTIBIOTIQUES SUR LE BIOFILM A STAPHYLOCOCCUS AUREUS ISOLEES DE DISPOSITIFS MEDICAUX AU CHU DE SIDI BEL ABBES. *SARAHMED Editions***,** 15.
28. Woo, P., Leung, A., Leung, K. & Yuen, K. 2001. Identification of slide coagulase positive, tube coagulase negative Staphylococcus aureus by 16S ribosomal RNA gene sequencing. *Molecular Pathology,* 54**,** 244.
29. Yong, Y. Y., Dykes, G. A. & Choo, W. S. 2019. Biofilm formation by staphylococci in health-related environments and recent reports on their control using natural compounds. *Critical reviews in microbiology,* 45**,** 201-222.