Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Investigation the antibacterial activity on bacterial isolates isolated from alopecia areata and skin injuries

***Noor A. Kshash*[1*]; *Ali S. Ahmed*[2]**
[1] Plant Biotechnology, College of Biotechnology, Al-Nahrian University, Baghdad, Iraq
*Corresponding Author. noora.mbp23@ced.nahrainuniv.edu.iq
https://orcid.org/0009-0009-2910-2311
[2] Plant Biotechnology, College of Biotechnology, Al-Nahrian University, Baghdad, Iraq
*Corresponding Author. ali.shihab@.nahrainuniv.edu.iq
https://orcid.org/0000-0002-1679-9262.
Received 19/11/2024,
Accepted 09/12/2024,
Published 31/12/2024.

Abstract

The study aims to conduct a screening of the bacteria colonizing the alopecia areata disease and *Pseudomonas aeruginosa* bacterium that infect skin wounds and burns. Alopecia areata is an autoimmune condition characterized by localized hair loss, often influenced by both genetic and microbial factors. Patients with burns and wounds are more likely to contract an infection in the hospital than other patients due to the loss of the protective barrier (skin) and immune system disorders that appear in these patients. This study investigated bacterial colonization and antimicrobial resistance in alopecia-affected areas and burns/wounds infections. Specimens were collected between October 2023 and February 2024 from three hospitals in Baghdad, including Number of 180 specimens were collected, 116 specimens from them collected from alopecia cases and 64 specimens from wound and burn infection. Half of alopecia cases was collected from the affected areas and the other half from the healthy areas (control) of the same patients. The selected predominance bacterial infection according to the confirmation tests were subjected to identification and antimicrobial susceptibility test using the VITEK-2 system. For alopecia the most commonly isolated species were *Staphylococcus species* which appeared in 10 isolates from 27 positive growth (35.48%). *Pseudomonas aeruginosa* isolates were the most commonly isolated species from burns and wounds appeared in 10 from 25 positive growth. The results revealed high levels of resistance, with 90% of *P. aeruginosa* isolates resistant to ticarcillin and 85% to aminoglycosides, while *Staphylococcus species* exhibited 80% resistance to oxacillin and 70% to vancomycin. Comparing the bacterial profiles of the affected and healthy parts of the same patient's scalp showed big differences. This shows how microbial communities play a part in how diseases progress.

Keywords: Alopecia areata, *Staphylococcus aureus, Pseudomonas aeruginosa*, Minimum inhibitory concentration

Introduction

The skin is the body's primary defense against microbial invasion, but injuries or burns compromise this barrier, leading to infections that can spread to internal tissues and cause severe complications[1]. Common pathogens in such infections include *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae,* and

Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Acinetobacter baumannii[2]. Initially, Gram-positive bacteria like *S. aureus* dominate, while Gram-negative bacteria, including *P. aeruginosa*, emerge later and can cause sepsis if they reach the bloodstream. Chronic wounds often harbor biofilms, microbial communities that resist treatment due to a protective extracellular matrix, requiring higher antimicrobial concentrations for eradication[3,4].

Alopecia areata, an autoimmune disease influenced by genetics, is the second most common cause of non-scarring hair loss. It presents as localized hair loss with no follicular destruction and can have unpredictable remissions and relapses. Inflammation during the hair follicle's growth phase and potential microbial influences, including external bacteria and immune responses, play roles in its pathogenesis[5].

The integrity of the skin is compromised when a wound is present, which makes it easier for organisms to penetrate the tissues under the surface[6]. The severity of the wound and the likelihood that the agent would act as a vector for infectious organisms are two factors that can be used to classify the agents that are responsible for wounds[7]. Trauma that penetrates the skin, whether it be from plants, animals, weapons, knives, or other items, can lead to the development of wound infections. As stated by Chhabra *et al*. (2017)[8], numerous plant compounds have the capability of penetrating the skin, which might result in the development of wound infections. Due to the fact that plants are made up of porous materials, they may allow thorns or other similar items to aid the entry of *S. aureus* or other organisms into their tissues. In many cases, cellulitis develops as a consequence of conditions that affect the skin[9,10].

According to Fayisa and Tuli (2023)[11], the commensal and opportunistic microbe known as Staphylococcus aureus has the ability to colonise the skin and mucous membranes of persons, which presents a huge challenge to the public health of the entire world. The bacterium is the dominant representative of the genus *Staphylococcus* and has been identified as the agent responsible for a number of diseases that affect both people and animals[12]. *S. aureus* is a multipurpose microbe that is capable of effortlessly adjusting to a wide range of environmental circumstances[13]. This microbe is capable of producing a variety of virulence factors that are associated with its pathogenicity. Additionally, it is able to penetrate regions within the host that are generally sterile[12,13] . The bacterium *Staphylococcus aureus* can cause diseases not only by direct tissue invasion, but also through the activity of more than thirty exoproteins that are encoded by the pathogen on its own [14].

Pseudomonas aeruginosa is a member of the Pseudomonadaceae bacterial family, a member of γ-proteobacteria. "Schroeter" was the first to propose the scientific name *Bacterium aeruginosa* for *P. aeruginosa* in 1872, after he isolated it from suppurating wounds basis on its phenotypic characteristics. It is one of twelve subtypes of bacteria seen seldom as a component of the human micro-flora in healthy individuals. *P. aeruginosa* is widespread in nature and it is an opportunistic pathogen causing nosocomial infection in humans that causes a number of diseases like inflammation of urinary tract, burns, respiratory infections, and septicemia[15];[16] .

Materials and Methods

Skin Specimen Collection

Between October 2023 and February 2024, specimens were collected from three hospitals in Baghdad: Al Yarmouk Teaching Hospital, Baghdad Hospital and Burns Specialist from both gender with different age, 64 samples from burn and wound infections from patients at Hospital, 116 samples from alopecia patients 58 (50%) of the affected area and 58 (50%) of the healthy areas (control) of the same patients. The specimens were transferred using sterile swabs immersed in transport medium and stored in a cooling box to the college of biotechnology labs at Al-Nahrain University. We inoculated each swab into nutrient broth and incubated it at 37 °C for 24 hours.

Isolation and Identification of bacterial isolates

Swabs of cultures from transmitted medium were streaked onto nutrient agar and incubated for 24-48 h at 37 °C. Fungi were excluded and the bacterial colonies were transferred and streaked on nutrient agar medium. The growth colonies were cultured on MacConkey and Manmedia. The colonies growing on the two media were selected and examined under the microscope and stained with Gram stain. The golden and non-golden colonies were selected from the Menthol culture and transferred to the slant nutrient agar medium. The colonies growing on MacConkey

Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

and not fermenting lactose were selected and transferred to the slant nutrient agar medium for subsequent studies. And further analysis, including Gram staining and microscopic assessment for shape and motility and to identify the isolation, VITEK 2 system compact ID gNB cards were used.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC for each isolate was determined using the VITEK-2 system (Biomerieux, USA).

Results and Discussion

Isolation of bacterial isolates

A total of 180 clinically significant samples from patients with wounds, burns and alopecia were collected, Among them, 64 swabs of burn/ wounds patients, 116 swabs were for alopecia patients (affected area and control). This cultivated sample may produce bacterial communities indicative of these disturbances, perhaps including dangerous strains like *Staphylococcus spp*, which flourish in inflamed or damaged skin conditions. Control Sample (Healthy Region), This specimen is derived from the unaffected, healthy skin of the same subject. It signifies the baseline microbiome or the "normal" configuration of skin microorganisms in the absence of pathological alterations. The culture results often indicate a steady equilibrium of commensal bacteria, including less harmful species, which contribute to skin health maintenance. The results showed that from a total of 58 affected area of alopecia specimens, only 27 (46.5%) were clinical positive growth samples, while the rest 31 were negative growth samples (53.4 %). And from 64 of burns and wounds specimens, only 25 (39%) were positive growth samples, while the rest 39(56.25 %) were negative growth samples as show in Table 1. Negative growth results may suggest to absence of infection at the time of sample collection or successful treatment of infection, some organisms may not grow easily in standard lab conditions**.**

Identification

Ten isolates (35.71%) from the positive growth were primary identified as *Staphylococcus spp* which collected from alopecia areata, Some strains possess the golden appearance suggests the presence of *S. aureus*, a species known for producing staphyloxanthin, a carotenoid pigment. This pigment gives *S. aureus* its distinctive golden color and serves as a virulence factor, enhancing its ability to evade host immune responses[17]. It also has the ability to ferment mannitol, which results in a change in the color of the medium as a result of acid production during the fermentation process[18]. The presence of *S. aureus* and *S. epidermidis* and some undefined isolates in the infected area, as shown in figure (1), aligns with the hypothesis that opportunistic pathogens might exploit the disrupted skin barrier and inflamed microenvironment characteristic of alopecia areata. while Colorless Colonies from the control area, a common commensal bacterium found on healthy skin[19].

For burn and wound samples also ten isolates identified as *P. aeruginosa* from both gender with different age: using the traditional culture method MacConkey agar and nutrient agar and microscopic examination As shown in figure (2), as well as VITEK-2 compact as a confirmatory test. The isolates were diagnosed as *Staphylococcus* for alopecia isolate and *P. aeruginosa* for wound and born isolates.

.

Table 1: Isolation and identification of *Staphylococcus* spp. and *P. aeruginosa* isolates using VITEK-2 system

Sample Type	Total No. of sample	No. of grown sample	No of *Staphylococcus*	Number of *P. aeruginosa*
Alopecia	116	27	10*	-
Burn/Wound Infection	64	25	-	10
Total	**180**	**52**	**10**	**10**

**S. aureuS (3)isoaltes;*Coagulase nagative *Staphylococcus*—> (4); *S.epidermides* (1); *S. hominis ssp hominis*(2)

Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Figure 1. Comparison of bacterial isolates from Alopecia-affected (A) and healthy Scalp (B) regions in the same patient.

Figure 2. Growth of *Pseudomonas aeruginosa* on MacConkey agar.

On the other hand, *P. aeruginosa* bacteria were cultivated on the medium of the MacConkey aquarium in the form of pale colonies. While it formed in the shape of irregular, opaque colonies, it had a glossy buttery texture and gave off a fruity smell[20] . It was a pale green color, smooth, flat, and regular, and it did not ferment the lactose, G-ve, oxidase-positive, and catalase-positive The isolates of *S. aureus* were G+ve, catalase-positive, and oxidase-negative.

Minimum Inhibitory concentration (MIC) determination using VITEK-2 system

The VITEK 2 compact system was utilized to determine the (MIC) values for eighteen antibiotics against 10 isolates from alopecia and 10 isolates from burn and wound infection. The findings demonstrated a significant bacterial resistance and minimum inhibitory concentration of Utilized Antibiotics The resistance of bacteria, as illustrated in the in the subsequent table 2 and table 3.

Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Table (2): MIC determination of *Staphylococcus spp* against antimicrobial agent using VITEK-2

N.	Antibiotic	MIC (µg/ml)[1]	R	S	I
1	Cefoxitin Screen	≥0.5	9	1	
2	Benzylpenicillin	-	10		
3	+Piperacillin/Tazobactam		10		
4	Oxacillin	≥1	10		
5	Gentamicin	≥16	3	7	
6	Tobramycin	≥16	3	7	
7	Levofloxacin	≥4	4	5	1
8	Moxifloxacin	≥2	1	7	2
9	Inducible Clindamycin Resistance				
10	Erythromycin	≥8	9		1
11	Clindamycin	≥4	8	2	
12	Linezolid	≥8	6	4	
13	Teicoplanin	≥32	6	3	1
14	Vancomycin	≥16	7	2	1
15	Tetracycline	≥16	6	2	2
16	Tigecycline	≥1	1	9	
17	Nitrofurantoin	≥78	5	4	1
18	Fusidic Acid	≥32	9	1	
19	Rifampicin	≥4	6	4	
20	Trimethoprim/ Sulfamethoxazole	≥76		10	

Table (3) : MIC determination of *Pseudomonas aeruginosa* against antimicrobial agent using VITEK-2

N.	Antibiotic	No.of isolates	MIC Mg/ml	R	S	I
1	Ticarcillin	10	≥ 128	7	2	1
2	Amikacin	10	≥ 128	6	4	
3	Ticarcillin/Clavulanic Acid	10	≥ 128	6	4	
4	Gentamicin	10	≥ 16	5	5	
5	Piperacillin	10	≥ 128	6	4	
6	-Netilmicin	10		5	5	
7	+Cefixime	10		4		6
8	Tobramycin	10	≥ 16	5	5	
9	+Cefpodoxime	10		4		6
10	Ciprofloxacin	10	≥ 4	6	4	
11	+Cefotaxime	10		4		6
12	-Levofloxacin	10		4		6
13	Ceftazidime	10	≥64	3	6	1
14	+Norfloxacin	10		4	4	2
15	+Ceftriaxon	10		8		2
16	+Ofloxacin	10		4	4	2
17	Cefepime	10	≥8	3	5	2
18	+Ertapenem	10		4		6
19	Colistin	10	≥2	3	6	2
20	Imipenem	10	≥ 16	4	5	1

The resistance and sensitivity percentages of antibiotics for *S. aureus* isolates from alopecia, as determined by VITEK device tests, indicate a 76% resistance rate to erythromycin, as reported by Giulieri *et al* (2022)[21]. The current investigation identified a 70% resistance rate to oxacillin, in contrast to Shaker (2018)[22], who documented a 100% resistance rate to this antibiotic. Additionally, the current study revealed a 70% resistance rate to trimethoprim/sulfamethoxazole, contrasting with Jameel (2018)[23], who indicated a 100% resistance rate to trimethoprim/sulfonamide.

Al-Azzawi (2018) [24] reported that in her investigation of 69 isolates of *P. aeruginosa*, 78% were resistant to Cefotaxime, 70% to Meropenem, and 66% to Imipenem. A study by Ameen et al. (2015) [25] on 230 isolates of *Pseudomonas aeruginosa* revealed that 49.9% of the isolates were resistant to Imipenem, while a study by Musafer et al. (2013)[26] on 58 isolates indicated a 15.52% resistance to Meropenem. A primary factor contributing to *P. aeruginosa* resistance to the beta-lactam class is its synthesis of beta-lactamase enzymes (Penicillinase), which target the beta-lactam ring present in penicillin's and cephalosporins, rendering them ineffective, alongside its possession of additional virulence factors. The isolates exhibited resistance to the class of anti-aminoglycosides due to the bacterial synthesis of mutated enzymes, including phosphotransferase and N-acetyltransferase, with other virulence factors.

The MIC results from *S. aureus* and *P. aeruginosa* isolates present clinically significant multidrug resistance profiles, which have direct implications in the management of infections associated with alopecia, wound and burn infection. Bacterial colonization in skin infection sites is increasingly recognized as a factor that not only influences disease progression but also introduces considerable challenges in therapeutic intervention due to antimicrobial resistance. Effectively managing such cases necessitates a tailored antibiotic regimen based on susceptibility testing, ensuring that therapeutic efforts are directed towards reducing bacterial burden and preventing further complications associated with infection-driven inflammation and tissue damage.

Conclusions

This study shows that *Staphylococcus* species that are resistant to antibiotics may make alopecia worse, so it's important to use specific antimicrobial drugs based on accurate susceptibility testing for effective treatment. The fact that resistant *Staphylococcus* species are found in the scalp microbiome suggests that the presence of microbes may make alopecia worse by making treatment more difficult. This study stresses how important it is to use specific antimicrobial treatments based on thorough susceptibility testing in order to help people with alopecia, especially when there are resistant bacterial strains present. The results show that a planned, evidence-based approach is needed to lower the number of bacteria, reduce inflammation caused by infections, and improve therapeutic outcomes in people with alopecia. The occurrence of clinically significant *P. aeruginosa* in burn and wound infections is negligible in this facility. Nevertheless, *P. aeruginosa* is a common causative agent of infection in our burn centers, aligning with global observations. The isolates of *P. aeruginosa* that were tested were most sensitive to Ticarcillin and least sensitive to Gentamicin. This suggests that Gentamicin is less effective at treating *P. aeruginosa* infections in burn wounds. Most *P. aeruginosa* isolates demonstrated significant sensitivity to most of the antibiotics tested.

Acknowledgments

Express our gratitude to Al-Nahrain University and the Collage of Biotechnology, namely the head of the collage, head of plant biotechnology department, for their consent to proceed with this study. Gratitude is expressed to the researchers who contributed to the successful completion of this study.

Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.

- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

[First Author]: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

[Second Author]: conducted some experiments, collection a part of literature review and conducted some characteristics of the products.

References

1. Coates, M., Blanchard, S. & MacLeod, A. S. Innate antimicrobial immunity in the skin: A protective barrier against bacteria, viruses, and fungi. *PLoS Pathog.* **14**, e1007353 (2018).
2. Percival, S. L., Emanuel, C., Cutting, K. F. & Williams, D. W. Microbiology of the skin and the role of biofilms in infection. *Int. Wound J.* **9**, 14–32 (2012).
3. Durand, B. A. R. N. *et al.* Bacterial Interactions in the Context of Chronic Wound Biofilm: A Review. *Microorganisms* **10**, (2022).
4. Minasyan, H. Sepsis: mechanisms of bacterial injury to the patient. *Scand. J. Trauma. Resusc. Emerg. Med.* **27**, 19 (2019).
5. McElwee, K. J. *et al.* What causes alopecia areata? *Exp. Dermatol.* **22**, 609–626 (2013).
6. Zhang, H. *et al.* Developing natural polymers for skin wound healing. *Bioact. Mater.* **33**, 355–376 (2024).
7. Mofazzal Jahromi, M. A. *et al.* Nanomedicine and advanced technologies for burns: Preventing infection and facilitating wound healing. *Adv. Drug Deliv. Rev.* **123**, 33–64 (2018).
8. Chhabra, S., Chhabra, N., Kaur, A. & Gupta, N. Wound Healing Concepts in Clinical Practice of OMFS. *J. Maxillofac. Oral Surg.* **16**, 403–423 (2017).
9. Morguette, A. E. *et al.* The Antibacterial and Wound Healing Properties of Natural Products: A Review on Plant Species with Therapeutic Potential against Staphylococcus aureus Wound Infections. *Plants* vol. 12 at https://doi.org/10.3390/plants12112147 (2023).
10. O'Dell, M. L. Skin and wound infections: An overview. *Am. Fam. Physician* **57**, 2424–2432 (1998).
11. Fayisa, W. O. & Tuli, N. F. Review on Staphylococcus Aureus. *Int J Nurs. Care Res.* **1**, 1–8 (2023).
12. Głowacka, P., Zakowska, D., Naylor, K., Niemcewicz, M. & Bielawska-Drózd, A. Brucella – Virulence factors, pathogenesis and treatment. *Polish J. Microbiol.* **67**, 151–161 (2018).
13. Qin, S. *et al.* Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct. Target. Ther.* **7**, 199 (2022).
14. Colautti, A., Orecchia, E., Comi, G. & Iacumin, L. Lactobacilli, a Weapon to Counteract Pathogens through the Inhibition of Their Virulence Factors. *J. Bacteriol.* **204**, (2022).
15. Al-yasiri, S. & Risan, M. H. Identification , characterization and antibiotic susceptibility testing of

Pseudomonas aeruginosa isolated from clinical sources. (2024).

16. Ahmed, A. S. Production of silver nanoparticles from the aerobic and anaerobic cultures supernatants of Pseudomonas aeruginosa. 1–10 (2024).

17. Liu, G. Y. *et al.* Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J. Exp. Med.* **202**, 209–215 (2005).

18. Thakur, P., Nayyar, C., Tak, V. & Saigal, K. Mannitol-fermenting and Tube Coagulase-negative Staphylococcal Isolates: Unraveling the Diagnostic Dilemma. *J. Lab. Physicians* **9**, 65–66 (2017).

19. Gradmann, C. Medical Bacteriology. *Routledge Hist. Dis.* 378–401 (2018) doi:10.4324/9781315543420-21.

20. Aldaraghi, W., Albadrawi, M. & RidaJassim, H. Molecular Detection of Pseudomonas aeruginosa and its Relationship with Multidrug Resistance and Transposons. *Indian J. Forensic Med. Toxicol.* **14**, 300 (2021).

21. G., G. S. *et al.* Comprehensive Genomic Investigation of Adaptive Mutations Driving the Low-Level Oxacillin Resistance Phenotype in Staphylococcus aureus. *MBio* **11**, 10.1128/mbio.02882-20 (2020).

22. Shaker, M., Zaki, A., Asser, S. L. & Sayed, I. El. Trends and predictors of antimicrobial resistance among patients with urinary tract infections at a tertiary hospital facility in Alexandria, Egypt: a retrospective record-based classification and regression tree analysis. *BMC Infect. Dis.* **24**, 1–15 (2024).

23. Zahraa Jaafar Jameel, Sura Hameed Nayef & Muthana Abdulkhader Al-Mahdawi. Study Some Virulence Factors of CO- agulase Positive Staphylococci and CO-agulase Negative Staphylococci Isolated from Different Parts of Human Body. *Tikrit J. Pure Sci.* **20**, 47–54 (2023).

24. Al-Azzawi, S. N. A. & Abdullah, R. M. Study of the resistance of p. Aeruginosa isolated from wounds and burns for some disinfects and antiseptic from some baghdad hospitals. *J. Pharm. Sci. Res.* **10**, 1481–1484 (2018).

25. Ameen, N., Memon, Z., Shaheen, S., Fatima, G. & Ahmed, F. Imipenem Resistant Pseudomonas aeruginosa: The fall of the final quarterback. *Pakistan J. Med. Sci.* **31**, 561–565 (2015).

26. Hadeel, K. M., Harith, J. F. A.-M. & Majeed, A. S. OprD Expression and Imipenem Resistance in Pseudomonas aeruginosa. *Greener J. Biol. Sci.* **3**, 325–329 (2013).