DETERMINATION THE PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA ISOLATED FROM BURNS AND WOUNDS

Marwah K. Khudhair **Mouruj A. AlAubydi**
Researcher **Prof.**
Dept. of Biotechnology Coll. Scie , University of Baghdad
e-mail *: marwa.kkg92@gmail.Com*

ABSTRACT

The current study conducted to identify the bacterial isolates from burns and wound infections that are responsible for various skin complication in such conditions and determination the development of antimicrobial resistance among these bacterial isolates. This study is carried out Between September 2020 to December 2020, a total number of (103) burns and wound swabs were taken from different patients from different hospitals in Baghdad city, the specimens were collected from; burns (57) and wound (46) swabs, the specimens were included; (51) female and (52) Male, the age average between (2 - 70 years). The positive bacterial containing specimens are (93) swabs, most of them are mixed (82 specimens) with different types of bacterial species and only (11 specimens) are contained with one type of bacterial isolate, while the negative bacterial containing are (10) swabs that showed no aerobic bacterial growth.. Staphylococcus sp. is recorded the most prevalence organisms found in wounds and burns 75/163(46%) included 31(41.3%) and 44(58.7%) isolated from burns and wounds respectively. Whereas Enterobacteriaceae sp. revealed that 57/163 (35%), 25(44%) from wounds and 32(56%) from burns. Also Pseudomonas sp. 23/163(14.1%), Acinetobacter sp. 5/163(3.1%), and Corynebacterium sp. 3/163(1.8%) were isolated from infected wound in a percentage (47.8%,60%,and 33.3%) and from burns (52.2%, 40% and 66.7%) respectively. Another experiment was performed to measure the antimicrobial susceptibility against several antimicrobial agents included; Methicillin, Caphalothin, Tobramycin, Azithromycin, Bacitracin, Novobiocin, Levofloxacin and Sulfamethoxazole + Trimethoprim. The results showed increasing the resistance percentages among different bacterial isolates, and developed multidrug resistant bacteria.

Key words: wounds, burns, Staphylococcus sp. susceptibility test

تحديد مدى تواجد البكتيريا المعزولة من الحروق والجروح و تحسسها للمضادات الميكروبية

مروه كاظم خضير مروج عبد الستار
باحث استاذ
جامعة بغداد / كلية العلوم / قسم التقنيات الاحيائية

المستخلص

اجريت الدراسة الحالية للتعرف على العزلات البكتيرية من الحروق والتهابات الجروح المسؤولة عن مضاعفات الجلد المختلفة في مثل هذه الظروف وتحديد تطور مقاومة مضادات الميكروبات بين هذه العزلات البكتيرية. أجريت الدراسة خلال الفترة من أيلول\ 2020 إلى كانون الأول\ 2020. تم أخذ (103) مسحة من الحروق و الجروح من مختلف المرضى الراقدين في عدد من المستشفيات في مدينة بغداد، تم جمع العينات منها؛ حروق (57) وجروح (46) مسحة، تضمنت العينات على؛ (51) اناث و (52) ذكر متوسط الاعمار ما بين (2 – 70 سنة). العينات الموجبة للتواجد البكتيري هي (93) مسحة معظمها مختلطة (82 عينة) بأنواع مختلفة من الأنواع البكتيرية وفقط (11 عينة) تضمنت على نوع واحد من العزلات البكتيرية بينما السالبة للتواجد البكتري كانت (10) مسحات أظهرت عدم وجود نمو للبكتيريا الهوائية. سجلت انواع بكتريا المكورات العنقودية ألاكثر انتشارًا في الجروح والحروق 163/75 (46٪) متضمنة 31 (41.3٪) و 44 (58.7٪) معزولة من الجرح والحروق على التوالي. في حين أن الانواع التابعه للعائلة المعوية كانت 163/57 (35٪)، 25 (44٪) من الجروح و 32 (56٪) من الحروق. كذالك جنس بكتريا الزائفة الزنجارية 163/23(14.1٪)، وبكتريا الراكدة البومانية 163/5 (3.1٪)، وجنس البكتريا الوتدية 163/3 (1.8٪) من إصابات الجروح بنسبة (47.8٪، 60٪، 33.3٪) ومن الحروق (52.2٪، 40٪، 66.7٪) على التوالي. تم إجراء تجربة أخرى لقياس قابلية الميكروبات ضد العديد من العوامل المضادة للميكروبات كالميثيسيلين، سيفالوثين، توبراميسين، أزيثروميسين، باسيتراسين، نوفوبيوسين، ليفوفلوكساسين وسلفاميثوكسازول + تريميثوبريم. أظهرت النتائج زيادة نسب المقاومة بين العزلات البكتيرية المختلفة، وظهور البكتيريا المقاومة للأدوية المتعددة.

الكلمات المفتاحية: جروح، حروق، المكورات العنقودية، الزائفة الزنجارية، الراكدة البومانية، البكتريا الوتدية، التحسس للمضادات الحيوية
Received:14/5/2021, Accepted:15/8/2021

INTRODUCTION

Burns consider on of the most sever and common form of trauma (8). As for wound, a wound in an organ (such as the skin) may be a simple or significant condition and may extend to other tissues and structures of the body (e.g., muscles, tendons, Vessels, and nerves) (23). Bacterial and fungal infections are now recognized as one of the most common and severe diseases that contribute to severe mortality and morbidity (26). Due to the non-sterile wound and burn environment and the complexity of healing system an effective and targeted cure needed. Thus, researchers are motivated to find an alternative and effective therapy (24). The random and extensive use of antibiotics to treat bacterial infections in humans and animals has increased bacterial strain and has precipitated the development of antibiotic-resistant bacteria (22), also Hospital admissions raise the risk of diseases associated with health treatment and the spread of multidrug-resistant pathogens, resulting in increased use of antimicrobials (19). The wide use of biocidal agents for environmental and personal disinfection, including in non-health-care settings, is another potential threat. Low-level exposure to biocidal agents can select drug-resistant strains and increase the risk of antibiotic cross-resistance (7), particularly those that treat Gram-negative bacteria(17). From all above, this study is aimed to assessment the most prevalence microbial organisms that related particularly with different types of wounds and burns. Simultaneously study the antimicrobial susceptibility for these bacterial isolates against empirical antimicrobial agents used to treat this types of infections.

MATERIALS AND METHODS

Specimen collection: A total of (103) swabs were taken from people who suffered from burns and wounds infections and the specimens were collected from different hospitals in Baghdad city- Iraq which are (Ghazy Al-hariri Hospital for surgical specialties, Baghdad teaching hospital , teaching laboratories in city of medicine , specialized burns hospital , burn center in Al-Yarmouk teaching hospital , special Kadhimiya Hospital), during a period September/2020 to December 2020. Specimens from 103 patients with different wound and burn infection are collected aseptically with the aid of sterile swab of transport media (Cito-swab-China). Each specimen is cultured in a sterilized Brain heart infusion broth and incubated overnight at 37℃.

Isolation and Characterization of isolated bacteria: Afterwards, each activated culture broth was re-cultured using ABC steak plating technique in order to achieve single colony and primary characterization, the bacterial specimens were cultured on four different types of media (all media used in this study are from / Himedia-India except MacConkey agar, Biomark laboratories- india) which are; Mannitol Salt Agar for Gram(+Ve) bacteria, MacConkey Agar for Gram (–Ve) bacteria, Blood Agar for hemolysis detection ability and Brain Heart Infusion Agar for maintenance of bacteria. And then a set of biochemical tests were performed for each obtained bacterial isolates included: Indole, MR, VP, Catalase, Oxidase, coagulase، Urease (18).

Antimicrobial susceptibility test:

All bacterial isolates were activated in a BHI broth for 18-24 hour at 37℃ and the inoculated broth was subjected into centrifuge for 10 min. at 4000g and the supernatant discharged, and the pellet was emulsified by normal saline until the inoculum density was nearly equal to MacFerland turbidity standers ($1.5x\ 10^8$). According to the Kirby-Bauer disk diffusion method (6), incubation of characterized bacterial isolates together with antimicrobial disks by culturing them on Muller Hinton Agar and this method following standard protocol (15). The bacterial isolates susceptibility were tested for several antimicrobial agents including; Methicillin (ME-10 µg), Tobramycin(TOB-10 µg) , Caphalothin (Caphlex) (KF-30 µg), Azithromycin (AZM-15 µg), Bacitracin (B-10 µg) , Novobiocin(NO-5 µg), Levofloxacin(LEV-5 µg), Sulfamethoxazole+ Trimethoprim (SXT-25 µg). The results were read dependent on the zone of inhibition measurement and determined their susceptibility (Resistance, Intermediate and Sensitivity) relies on specific type of antibiotics guide that recommended by CLSI, 2020 guidelines (10)

RESULT AND DISCUSSION

In burns and wound infections, the current study attempts to identify the bacterial isolates that are responsible for various skin complication in such conditions and the antimicrobial resistance of these bacterial isolates. A total number of (103) specimens including; burns (57) and wound (46) swabs were taken from different patients and from different hospitals, the specimens were for (51) female and (52) Male, the age average between (2 - 70 years). The positive specimens are (93) swabs, most of them are mixed (82 specimens) with different types of bacterial species and only (11 specimens) is infected with one type of bacterial isolate, while the negative specimens are (10) swabs that showed no aerobic bacterial growth. The results in table 1 revealed that *Staphylococcus* sp. has the most common isolates 75 (46%) isolates from total bacterial isolates 163. A 44 (58.7%) isolates from burns patients and 31(41.3%) isolates from wounds infection. These isolates numbers included; *S. aureus, S. epidermidis* and *S. saprophyticus 27*(47.4%), 4(25%) and 0(0%) respectively in wound infected patients, compared with that isolated from burned infected patients which are 30(52.6%), 12(75%) and 2(100%) respectively.

Table 1. Number and percentage of *Staphylococcus* species isolated from wounds and burns patients

Staphylococcus sp.		Wound swab		Burn swab	
	Total no.	Number	Percentage	Number	Percentage
S. aureus	57	27	47.4%	30	52.6%
S. epidermidis	16	4	25%	12	75%
S. saprophyticus	2	0	0%	2	100%
Total number	75	31	41.3%	44	58.7%

Ekawati, *et al.,* documented that microbial agents that cause inflammation of the skin surface is considered as a group of pyogenic bacteria. *S. aureus* is classified as one of the pyogenic bacteria(13). Also Gilmara, *et al.,* and coworkers (14) who they concluded that, nasal and skin colonization by *Staphylococcus sp.* may be a source for wound colonization by *S. aureus*, and high proportions of wounds are colonized by *S. aureus* and MRSA. Burns is another type of wound, William, et al., and his colleagues reported that some resistant microorganisms have developed as the maleficent cause of invasive infection in burn patients, including *MRSA* particularly that transmitted from hospitals (25). Other most important family species (57) are related with *Enterobacteriaceae* family. The results in table 2 showed that, the most prevalence *Enterobacteriaceae* sp. found in infected wounds and burns were *Klebsiella sp*., *E. coli, Proteus sp*., *Serratia sp.* and *Enterobacter* at a percentages (54.5% , 33.33%, 52.2%, 33.3%,0%) in wounds respectively compared with that in burns (45.5%, 66.7%, 47.8%, 66.7%, 100%) respectively.

Table 2. Number and percentage of *Enterobacteriaceae* sp. isolated from wounds and burns patients

Enterobacteriaceae Sp.		Wound swab		Burn swab	
	Total no.	Number	Percentage	Number	Percentage
Klebsiella sp.	11	6	54.5%	5	45.5%
E. coli	18	6	33.33%	12	66.7%
Proteus sp.	23	12	52.2%	11	47.8%
Serratia sp.	3	1	33.3%	2	66.7%
Enterobacter	2	0	0%	2	100%
Total number	57	25	44%	32	56%

On another hand, Pseudomonas sp. recorded a 23 isolates 11(47.8%) from wounds and 12(52.2 % from burns) .These results were in agreement with Shama, et al., who they documented that (21), a skin can be infected by a variety of microbial agents, such as bacteria, parasites and fungi. The most prominent of skin infections are caused by bacteria . (G+Ve) bacteria are the most communal causes of infection is hemolytic *S. aureus* and Streptococcus. While (G- Ve) bacteria the rod one that can cause infections in the skin such as *E. coli*, *Enterobacter* sp., *Klebsiella* sp., *Proteus sp*. and *P. aeruginosa* (16), (20). As well as the present results showed that *Acinetobacter* sp. and *Corynebacterium* sp. both are isolated from wounds 3(60%) and 1(33.3%), whereas from

burns were 2(40%) and 2(66.7%) respectively Table 3.

Table 3. Number and percentage of *Acinetobacter* sp. and *Corynebacterium* sp. isolated from wounds and burns patients

Bacterial type		Wound swab		Burn swab	
	Total no.	Number	Percentage	Number	Percentage
Pseudomonas sp.	23	11	47.8%	12	52.2%
Acinetobacter	5	3	60%	2	40%
Corynebacterium	3	1	33.3%	2	66.7%

Acinetobacter sp. is acquisition an importance as one of nosocomial infections, there are a reports of severe necrotizing wound infections and skin and soft tissue infection caused by this pathogen are elevating in frequency as Dubert, *et al.*, elucidated in their compromised analysis study (12). Additionally, *Corynebacterium* sp. is one of skin opportunistic microbiota, wounds infection by this genus is considered as endogenous one which enters into a site of wound. The development of the etiologic agent can be challenging, particularly when opportunistic bacteria are found, signifying contamination of clinical substances as Alina, and Iwona documented (4). Antimicrobial resistance of bacteria is one of worldwide problem. The results in figure1 revealed that, there are increasing in the resistance of bacterial isolates against most empirical antimicrobial agents (Methicillin, Caphalothin, Tobramycin, Azithromycin, Bacitracin, Novobiocin, Levofloxacin and Sulfamethoxazole + Trimethoprim) which are 60 (80%), 56(74.7%),43 (57.3%) , 47 (62.7%), 49(65%), 40(53.3%), 31(41.3%) and 46(61.3%) respectively. Methicillin resistant is obviously increased among Staphylococcus species. In another word, multidrug resistance are developed among Staphylococcus sp. this may due to virulence factors they have. Though, sequence and experimental indications of horizontal transmission of antimicrobial resistance genes among them, preferred by their capacity of generating biofilms. Moreover, the fact that exchange microbiota with our surroundings, such as the relationship between human beings, animals, and the environment as Ciro César, et al., mentioned (9) . As well as the results show that, the drug of choice is Levofloxacin which recorded the lowest resistance for Staphylococcus sp. than other antimicrobials.

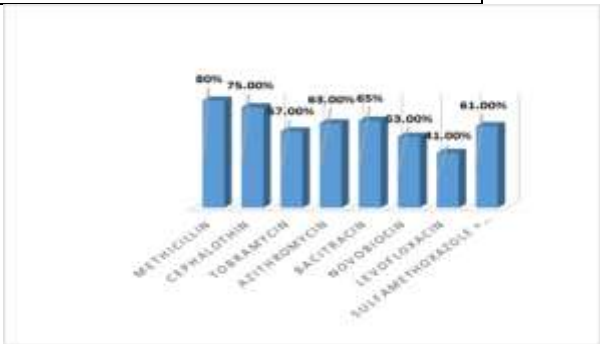


Figure1. Numbers and percentages of antimicrobial resistance for Staphylococcus sp. bacterial isolates

Enterobacteriaceae members also showed increasing resistance against most usable antimicrobials used in this study (Methicillin, Caphalothin, Tobramycin, Azithromycin, Bacitracin, Novobiocin, Levofloxacin and Sulfamethoxazole + Trimethoprim) which are 51(89.5%),49(86%), 22(39%), 27(47%), 44(77%), 33(58%), 25(44%) and 38(66.5%) respectively figure 2. High levels of resistance were observed against beta- lactam group then folate pathway inhibitors due to production of beta-lactamase enzyme and dihydropteroate synthases that encoded by *blaTEM, blaTEM-1, blaSHV-1 and blaCTX-M* and *sul1, sul2 and sul3* genes respectively. Aminoglycoside modifying enzymes due to the carriage of *aac(3)-IIIa, aac(6')-II and aac(6')-Ie-aph(2")* as Anca, et al., reviewed (5) ,(2).

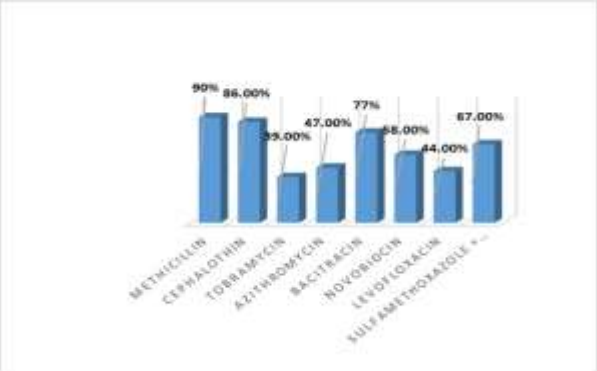
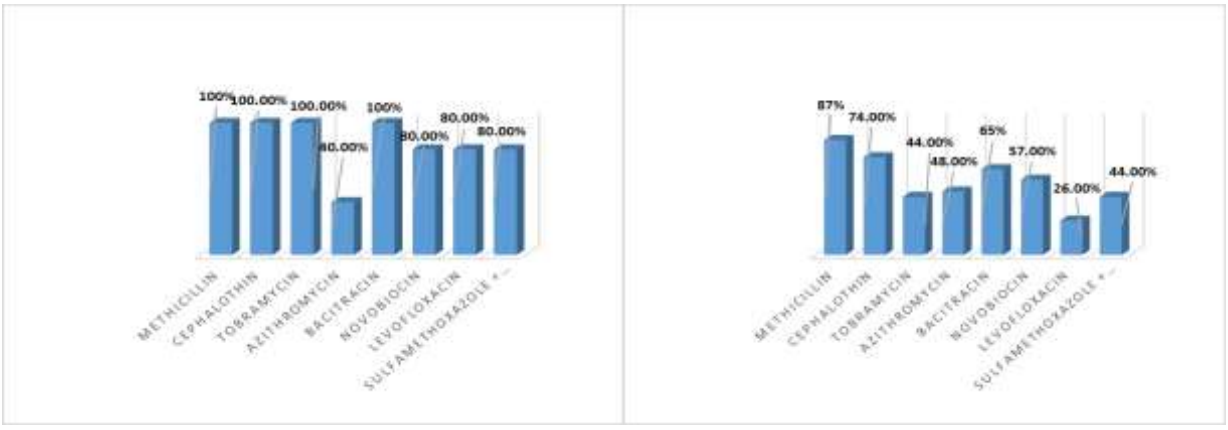


Figure2. Numbers and percentages of antimicrobial resistance for Enterobacteriaceae sp. bacterial isolates

Figure 3 (A and B) showed that Acinetobacteria sp. were resistant in a percentage 100% for Methicillin, Caphalothin, Tobramycin and Bacitracin. Whereas resistant in a percentage 80% for Novobiocin, Levofloxacin and Sulfamethoxazole + Trimethoprim, and 40% for Azithromycin. Some of Acinetobacter sp. is considered as the most important pathogens liable for severe hospital transmitted infections, because of its different resistance mechanisms, such as the decreased membrane permeability, β-lactamases production, efflux pumps, and altered target site of the antibiotic. The massive adaptive capacity of Acinetobacter sp. and the gaining and transfer of antimicrobial resistance factors contribute to the ineffectiveness of most present therapeutic strategies, including new generation or combined antibiotic therapy as Corneliu, *et al.*, and his colleagues documented (11). As well as William *et al.*, demonstrated that several resistant organisms have developed as the maleficent cause of offensive infection in burn patients, including methicillin-resistant *Staphylococcus aureus*, *Pseudomonas*, *Acinetobacter*, vancomycin-resistant *Enterococcus*. Progresses in antimicrobial treatments and the release of new classes of antimicrobials have further armamentarium of healing resources for the clinician (25)



A **B**

Figure3. Numbers and percentages of antimicrobial resistance for Acinetobacter sp.(A) and Pseudomonas sp. (B) bacterial isolates

Whilst Corynebacterium sp. results revealed that increasing resistance in a percentage 100% against 5 empirical antimicrobial agents included; Methicillin, Caphalothin, Bacitracin, Novobiocin and Levofloxacin, whereas each Tobramycin, Azithromycin, and Sulfamethoxazole + Trimethoprim are recorded a percentage of resistance reach 67% figure 4. Corynebacteria is one of skin normal flora, its opportunistic bacteria contaminated wound and developing resistant against antimicrobial in different ways included the following; the mechanism of active efflux of antibiotic from the cell, alteration of the ribosome binding site related with methylation or point mutations within the structural gene region, the gene responsible for resistance in chromosome or plasmid, and the least significant - enzymatic inactivation of the antibiotic as Alina documented (3).

Figure4. Numbers and percentages of antimicrobial resistance for Corynebacteria sp.

Conclusion: In view of the results of the current study, the following conclusions can be presented: Increasing contamination of wound and burn with multidrug resistant bacteria demonstrating the importance of avoiding cross-contamination in hospital ecosystem, particularly among elderly patients. Carefully managed of wounds to prevent microbial spread, thus supporting patient recovery and decreasing healthcare costs. Continuous wound observation with regular testing the specimens of tissues for quantitative culture, Strict infection control methods, and early removal and wound closing remain the principal assistants to counteract of invasive infections in burn patients.

REFERENCES

1. Al-Haideri, H.; H. 2019 . Gene expression of $bla_{oxa\text{-}51\text{-}like}$ and $bla_{oxa\text{-}23}$ in response to β-Lactam antibiotic in clinically isolated acinetobacter baumannii and acinetobacter lowffii from urine samples . Iraqi Journal of Agricultural Sciences. 50(4):1120- 1137 .

2. AL-Hamdani, H. and A. AL- Hashimy . 2020. Molecular detection of urec, hpma, rsba and mrpa genes of proteus mirabilis urinary tract infection in patient with rheumatoid arthritis. Iraqi Journal of Agricultural Sciences. 51:245-251

3. Alina O..2012. Mechanisms of Antibiotic Resistance In *Corynebacterium* Spp. Causing Infections In People, Antibiotic Resistant Bacteria - A Continuous Challenge In The New Millennium, Dr. Marina Pana (Ed.), ISBN: 978-953-51-0472-8, InTech, Available from: http://www.intechopen.com/books/antibiotic-resistantbacteria-a-continuous-challenge-in-the-new-millennium/mechanisms-of-antibiotic-resistance-incorynebacterium-spp-causing-infections-in-people pp: 387-402

4. Alina, O. and L. Iwona, 2010. Wound infections due to opportunistic *corynebacterium* species. *Med. Dosw. Mikrobiol* .62(2):135-40

5. Anca, F.; T. Emma, and B. Anca, 2019. Antibiotic resistance profiling of pathogenic *Enterobacteriaceae* from Cluj-Napoca, Romania. *GERMS* .9(1): 17-27

6. Bauer, A.W.,W.M. Kirby,J.C. Sherris, and M. Turck. (1966).Antibiotic susceptibility testing by a stan-dardized single disc method. Am.J. Clin. Pathol.45:493-496.

7. Caselli, E. H. 2017. Microbial strategies to reduce pathogens and drug resistance in clinical settings. Microb. Biotechnol. 10(5):1079–1083

8. Church, D. ; S. S. Elsayed, .; O. Reid, ; B. Winston, and R. Lindsay. 2006.Burn wound infection . Clin. Microbiol. *Rev*. 19:403-434

9. Ciro César, R.; F.P.Monalessa, and G. Marcia,. 2020. Underrated *Staphylococcus* species and their role in antimicrobial resistance spreading. *Genetics and Molecular Biology*. 43, 1(suppl 2), e20190065

10. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

11. Corneliu Ovidiu Vrancianu, Irina Gheorghe , Ilda Barbu Czobor and Mariana Carmen Chifiriuc..2020. Antibiotic Resistance Profiles, Molecular Mechanisms and Innovative Treatment Strategies of *Acinetobacter baumannii*. *Microorganisms*. 8, 935

12. Dubert M. Guerrero, Federico Perez and A. Robert . 2010 *Acinetobacter baumannii*-Associated Skin and Soft Tissue Infections: Recognizing a Broadening Spectrum of Disease. (Surg Infect Larchmt*)*. 11(1):49-57

13. Ekawati E. R, W. Darmanto, and S. P. A. Wahyuningsih. 2020. Detection of *Staphylococcus aureus* in wound infection on the skin surface. IOP Conf. Series: *Earth and Environmental Science* 456 012038. doi:10.1088/1755-1315/456/1/012038

14. Gilmara Celli Maia Almeida, Marquiony Marques dos Santos, and Kenio Costa Lima.(2014). Prevalence and factors associated with wound colonization by *Staphylococcus spp.* and *Staphylococcus aureus* in hospitalized patients in inland northeastern Brazil: a cross-sectional study. BMC infect Dis. 14:328

15. Hagens, S. and M. J. Loessner, .2014. Phages of Listeria offer novel tools for diagnostics and biocontrol. Front. Microbiol. 5, 159

16. Hussein, N. N. and A.H. Muslim A. H. 2019. Detection of the antibacterial activity of agnps biosynthesized by pseudomonas

aeruginosa. Iraqi Journal of Agricultural Sciences. 50(2):728-716

17. Kampf G. 2018. Biocidal agents used for disinfection can enhance antibiotic resistance in gram-negative species. Antibiotics (Basel). 14;7(4):110

18. MacFaddin, J. F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd Edition, Lippincott Williams & Wilkins, Philadelphia

19. Saleem Z, R. Godman M. A. Hassali I. K. Hashmi F. Azhar and IQ Rehman .2019. Point prevalence surveys of health-care-associated infections: a systematic review. Pathog Glob Health. 06;113(4):191–205

20. Saud, H.; M. and M. A. Alaubydi, .2019. Effect of clinical klebsiellapneumoniae extracted melanin on some immune aspects in mice. Iraqi Journal of Agricultural Sciences . 50(1):241-247

21. Shama Mary, Kulandhaivel Murugesan and Hridhya Vijayan .2018. Isolation identification and antibiotic sensitivity pattern of pyogens from Pyogenic pathogens. *Biomedical & Pharmacology Journal*. 11(1), 463-468.

22. Titilawo Y. and L. Obi, and A. Okoh . 2015. Antimicrobial resistance determinants of Escherichia coli isolates recovered from some rivers in Osun State, South-Western Nigeria: implications for public health. Sci. Total Environ. 523, 82–94 Aug 1

23. van Koppen, C. J. and R.W. Hartmann, 2015.Advances in the treatment of chronic wounds: A patent review. *Expert Opin. Ther. Pat.* 25, 931–937

24. Vyas, K.; and H. C. Vasconez, .2014. Wound healing: Biologics, skin substitutes, biomembranes and scaffolds. Healthcare. 2, 356–400

25. William Norbury, David N. Herndon and Celeste C. Finnerty .2016. Infection in Burns. Surg Infect(Larchmt).1;17(2):250-255

26. World Health Organization. Who Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed. Available online: https://tinyurl.com/kmva5da (accessed on 26 May 2018).