Characterization of Bacterial Isolates *Klebsiella oxytoca* from Burn Wound Infections

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Abstract

Background: Identifying bacterial pathogens causing wound infections is crucial for early diagnosis and treatment to prevent severe complications. This study aimed to characterize bacterial isolates from burn wound infections to improve diagnostic and therapeutic strategies. Methods: One hundred burn wound swabs were collected from patients admitted to two hospitals in Iraq. Swabs were cultured on nutritional and differential media to isolate bacterial pathogens. Microscopic and biochemical examinations were conducted to identify bacterial cultures. VITEK 2 Compact Automated Systems were used for Gram staining and bacterial identification. Results: *Klebsiella oxytoca* was the most prevalent bacterium, comprising 38% of the samples, followed by Raoultella ornithinolytica at 20%. Staphylococcus epidermidis, Kocuria kristinae, and Kocuria rosea each accounted for 14% of infections. Conclusion: Early identification of bacterial pathogens causing wound infections is essential for effective treatment. Understanding the prevalence and distribution of these pathogens can guide diagnostic and therapeutic interventions to prevent complications and improve patient outcomes.

Significance | Burn injuries are critical due to their severity and high infection rates, contributing to substantial mortality and requiring extensive medical intervention.

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Introduction

Burn injuries are among the most severe traumatic injuries individuals can suffer (WHO, 2011). They account for a significant portion of deaths, with infections from burns contributing to 51% of fatalities (Norbury et al., 2016). Annually, approximately 500,000 individuals in the United States require medical attention for burns, with 40,000 necessitating hospitalization to mitigate bacterial infections (Forson et al., 2017). Despite the initial sterilization of burn wounds by high temperatures, the rapid growth of normal skin flora and existing infections poses a significant challenge (Nielson et al., 2017). Studies indicate that upon admission, 9% and 54% of pediatric burn unit patients are contaminated with Group A Streptococcus (GAS) and Staphylococcus aureus, respectively (Rahim Hateet, 2021). Prolonged stays in intensive care units and the nature of burn injuries contribute to the increased incidence of nosocomial infections among burn patients (Al-Taie et al., 2014). Although burn surfaces are initially sterile post-injury, they eventually become colonized by various microorganisms (Hubab et al., 2020). These microorganisms originate from the patient's endogenous skin flora, gastrointestinal tract, and respiratory tract (Jie Yu et al., 2022). Additionally, external environmental surfaces, water, steam, air, and the hands of healthcare workers can transfer microorganisms to the patient's skin surface (Wang et al., 2022). Burn injuries represent one of the most impactful consequences of severe thermal trauma (WHO, 2011), with infections stemming

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from burns contributing to a staggering 51% of fatalities (Norbury et al., 2016). In the United States alone, approximately 500,000 individuals seek medical assistance for burns annually, with 40,000 patients necessitating hospitalization to mitigate bacterial infection (Forson et al., 2017). The presence of microorganisms on burn wounds is intricately linked to virulence factors. While high temperatures theoretically sterilize burn wounds, normal skin flora and existing infections can proliferate rapidly (Nielson et al., 2017). Routine cultures have revealed that upon admission, 9% and 54% of pediatric burn unit patients are contaminated with Group A Streptococcus (GAS) and Staphylococcus aureus, respectively (Rahim Hateet, 2021). Prolonged stays in intensive care units and the nature of burn injuries contribute to a heightened incidence of nosocomial infections among burn patients (Al-Taie et al., 2014). Despite burn surfaces being sterile immediately post-injury, they eventually become colonized by numerous microorganisms (Hubab et al., 2020), originating from the patient's endogenous skin flora, gastrointestinal tract, and respiratory tract (Jie Yu et al., 2022). Additionally, microorganisms can be transferred to the patient's skin surface through contact with contaminated external environmental surfaces, water, steam, air, and the hands of healthcare workers (Wang et al., 2022).

Klebsiella species stand as significant culprits in systemic infections, harboring various resistance genes against conventional antibiotics (Adeleke et al., 2022, Betty Fitriyasti et al. 2024, Farah M. Muhammad et al. 2024), Hiba Ahmed Jawade et al. 2024, Hussein Abdulkadhim Hasan et al. (2024). Mostafa Alamholo (2024).). Gram-negative and non-motile, these organisms are lactose-fermenting, facultative anaerobic rod-shaped bacteria, typically appearing as mucosal lactose fermenters on MacConkey agar (Osagie et al., 2017). While part of the normal flora in the mouth, skin, and intestines, their inhalation into human and animal lungs can lead to severe complications (Osagie et al., 2017). Klebsiella pneumoniae, a clinically vital member of the Enterobacteriaceae family, is a well-known cause of community-acquired infections and is closely related to *Klebsiella oxytoca*, differentiated by its indole-negative nature (Tavares et al., 2020). K. oxytoca, increasingly isolated, was initially classified as Bacterium oxytocum by Flugge in 1886, sharing characteristics with other Klebsiella species such as indole formation, positive Voges–Proskauer reaction, and gelatin liquefaction (Hagiwara et al., 2013). Typically acquired from environmental sources, K. oxytoca poses a notable health concern (Hagiwara et al., 2013).

Raoultella ornithinolytica, a Gram-negative, oxidase-negative, lactase-positive, aerobic, non-motile rod within the Enterobacteriaceae family, was initially classified as Klebsiella ornithinolytica until the establishment of the Raoultella genus in 2001 (Hajjar et al., 2020). Commonly inhabiting aquatic and soil environments, R. ornithinolytica has garnered attention due to its ability to convert histidine to histamine, leading to histamine toxicity in humans (Hajjar et al., 2020). This bacterium has emerged as a significant pathogen, particularly affecting co-morbid patients, with symptoms ranging from skin redness to vomiting, diarrhea, headache, or itching, depending on the extent of infection (Buyukcam et al., 2019).

Despite advancements in topical treatments and immediate transverse eradication techniques, pathogens continue to pose a significant challenge in treating burn victims (Gonzalez et al., 2016). Furthermore, overcrowding in burn hospitals contributes to infections spreading among patients (Singh et al., 2017). Despite the widespread use of broad-spectrum antibiotics, colonization by these bacteria may often be asymptomatic yet serve as a source of infectious agents leading to severe illness and mortality. Therefore, this study aimed to assess the infective bacteria present in burn wounds at the burn units of Imam Sadiq Teaching Hospital in Babylon Governorate and Al Diwaniyah Hospital in Al-Qadisiyah Governorate, Iraq.

Materials and methods

Preparation of the culture media:

The culture media used for isolating and diagnosing bacteria, which is a general medium for all types of bacteria, were prepared according to the instructions of the supplying companies. They were sterilized in an autoclave at a temperature of 121 °C for 15 minutes under a pressure of approximately 1.5 kg / cm2 and according to the following:

Solid agar medium (Nutrient agar)

Prepare the medium according to the manufacturer's instructions by dissolving (28g) of the medium in a liter of water distilled and sterilized in an autoclave at a temperature of 121 °C and a pressure of 15 pounds / inch 2 for 15 minutes, and after sterilization it was cooled. The medium was reduced to (45-50 m) and distributed in Petri dishes. This medium was used for the purpose of bacteria development.

Medium MacConkey:

This medium was prepared according to the instructions of the supplying company, by dissolving 51.5 gm in a liter of distilled water and sterilized by autoclave. This medium was used to isolate dye-negative bacteria Gram and diagnosed in terms of its ability to ferment the sugar lactose.

Medium Blood Agar base:

This medium was prepared according to the instructions of the supplying company, by dissolving 40 g in a liter of distilled water, and sterilized by autoclave. After completing the sterilization process, it was cooled to a degree (45-50) C, and human blood was added to it at a rate of (5-10)%. This medium was used as a rich medium to isolate and bacterial diagnosis.

Table 1. Phenotypic and Gram stain tests for bacterial isolates

Isolation symbol	Gram stain	Phenotypic characteristics
38 isolates	Negative	cream to pink-yellow colored colonies Cylindrical rod shaped which appear singly, in pairs or in short chains
20 isolates	Negative	Gram-negative, oxidase-negative, catalase-positive, aerobic, non-motile rod
14 isolates	Positive	Medium sized, white, opaque and flat colonies Non-motile cocci
14 isolates	Positive	smooth, small moist, slightly convex, pale-pink colonies
14 isolates	Positive	whitish, small, round, raised, convex colonies

Table 2. Percentage of diagnosed bacterial isolates

Samples of bacterial isolates	Percentage of diagnosed bacteria %
Klebseilla oxytoca	38
Raoultella ornithinolytica	20
Staphylococcus epidermidis	14
Kocuria kristinae	14
Kocuria rosea	14

Sample collection:

Samples were collected from Imam Al-Sadiq Hospital (Turkish) and from Al-Diwaniyah General Hospital, Department of Burns, from people who were subjected to burns of different degrees. Swab media swabs were taken and placed in clean and sterile refrigerated containers during transportation to the laboratories of the Musayyib Technical Institute, Department of Medical Laboratories. The sample collection period was for a period of Two months, during which (100) samples were obtained.

Transplant samples:

The solid nutritious culture medium was prepared according to the manufacturer's instructions and sterilized with the sterilizer (autoclave). After cooling the medium to 45-55 degrees Celsius, it was distributed in clean Petri dishes in the hood room. After the medium hardened in the dishes, we transferred the soap media to the surface of the solid medium and under sterile conditions and labeled The dishes are then incubated in the incubator at 35°C for 48 hours.

After the incubation period and obtaining growth, we purified the obtained colonies by re-cultivation of each obtained bacterial colony, transplanting it, or transferring it to new petri dishes containing a solid nutrient medium to obtain pure cultures and incubate the Petri dishes under the same previous conditions in the incubator at a temperature of(35 c0) for a period of 48 hours. After the incubation period, obtaining bacterial colonies, they are kept in the refrigerator for the purpose of conducting the rest of the other diagnostic tests..

Phenotypic examinations of bacterial colonies:

After obtaining the bacterial colonies, they were classified according to the morphology of the colonies, the color of the colony, and the nature of the colony. Thus, the numbers of isolates were listed according to the mentioned characteristics. The Gram stain technique, pioneered by Danish physician Hans Christian Gram in 1880, stands as a cornerstone in bacterial identification within hospital settings. This method, developed during Gram's tenure at the anatomy laboratory of Berlin Hospital, facilitates the differentiation of bacteria based on their cell wall composition. Gram-negative bacteria appear red, while Gram-positive bacteria appear blue. The staining process involves several sequential steps: stabilization of the bacterial suspension, application of violet crystal dye followed by rinsing, addition of iodine solution and subsequent rinsing, treatment with alcohol and rinsing, application of Safranin and final rinsing. The coloration observed in a Gram stain is contingent upon the chemical makeup of the bacterial cell wall. Following staining, the slide is dried and then examined microscopically.

Diagnosis of biochemical characteristics using the Vitek 2 Compact System:

The diagnostic process was carried out using the Vitek2 device for the selected isolates, which were selected based on the qualitative screening of the isolates for positive bacteria as well as for negative bacteria McConkey Agar culture medium and blood agar medium were used. (Anonymous, 2010).

Statistical Analysis

Data analysis was performed using the SPSS program (IBM Corp., 2017) to compare averages statistically at a significance level of $p \leq 0.05$.

Results and Discussion

Diagnosis of the selected isolates

The selected isolates were subjected to culture and phenotypic tests, and the results of microbial culture on the nutrient broth medium after a 24-hour incubation period at a temperature of 37 °C revealed the appearance of bacterial colonies, as shown in Table 1. As for the microscopic characteristics, after staining with a gram stain, the bacterial isolates appeared after 24 hours of incubation. There are some bacterial isolates that are Gram-negative and others that are Gram-positive as previosuly publsihed study (Abdulah Y. Al-Mahdi et al., 2024).

Diagnosis of biochemical characteristics using the Vitek 2 Compact System

The results of the examination with the Vitec 2 device (data not shown) showed different types of bacteria, and in total 100 isolates were studied. It was found that Klebseilla oxytoca was the most common pathogen [38%], followed by Raoultella ornithinolytica [20%], finally, Staphylococcus epidermidis, Kocuria kristinae & Kocuria rosea which had the same percentage [14%] (Table 2). Differences in bacteria species can be explained by many environmental factors or may be due to contamination during treatment (Rahim Hateet, 2021).

Burned surface area in addition to the length of duration in hospital is a risk factor for infection problems, according to SOARES, (2007), which is consistent with study findings. This study also was performed by Zambar, (2017) witch was find that majority of patients were seriously injured and had a long hospital admission. In Turkey it was done Study by Yildirim et al. (2005) for burn intensive care unit patients and they found that Klebseilla spp. was the common pathogen witch was the percentage of isolation 42.4%.

Infection complications in individuals with severe burns are caused by thermal damage of the stratum corneum and simultaneous suppression of local or systemic patient humoral immune mechanisms however, based on several investigations Klebsiella species was the most common organism that colonized burns wounds (Adeleke et al., 2022)

From the above, we note that Klebseilla oxytoca bacteria are the most affected by burn patients in hospitals, and this may be due to

their resistance to many antibiotics, Adeleke et al., (2022)found that Klebsiella spp are opportunistic pathogens the bacterium appears to rapidly develop resistance to many antimicrobials, and it is frequently involved in outbreaks in hospital settings.

It was noticed from the comparison of the results obtained with the local and international results that there is a convergence and difference in the percentage of bacteria isolation from the different samples, and this is due to reasons including: places of isolation such as hospitals and intensive care centers, and the difference in percentages may be due to the number of samples collected by the researcher and also It is due to the degree of attention to cleanliness and the type of sterilizers and various disinfectants in hospitals, because bacteria are resistant to most sterilization materials and antibiotics, being a major cause of hospital- acquired infections (Tsolakidis et al., 2022, Muntaha R. Ibraheem et al. 2024, Nurul Azila Abdul Razak et al., 2024, Rana S. Hasan et al., 2024, Shaima H. Abdullah et al. 2024).

Conclusion

In conclusion, infection stands as a significant contributor to morbidity and mortality among hospitalized burn patients, with approximately 75% of deaths attributed to infection rather than osmotic shock and hypovolemia. The prevalence of antibiotic-resistant isolates, particularly *Klebsiella oxytoca* and Raoultella ornithinolytica, underscores the indiscriminate use of antibiotics in treating infections caused by these bacteria. To mitigate the emergence of resistant strains, consideration should be given to employing histidine-free antibiotics. Moving forward, it is recommended to conduct additional surveys across various hospital locations to identify the sources of these bacteria. Furthermore, antibiotic susceptibility testing should be performed on the bacterial strains identified in this study to guide appropriate treatment strategies.

Author Contributions

A.A.A., A.K.J. drafted the manuscript and made substantial contributions to the design of the study. D.S.N. reviewed and drafted the paper.

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Competing financial interests

The author has no conflict of interest.

References

Abdulah Y. Al-Mahdi, Alabed Ali A. Alabed, Mohammed Faez Baobaid et al., (2024). Antibacterial Activity of Herbal Essential Oils against Gram-positive and Gram-negative Bacteria with a Potential for Multidrug Resistance, Journal of Angiotherapy, 8(2), 1-7, 9517

Adeleke, O. E., John-Mese, O. J., Afolabi, A. T., Ohunayo, A. S., & Dauda, O. S. (2022). Comparative Antibacterial Activity of Honey and Gentamicin against Klebsiella Species. Journal of Advances in Biology & Biotechnology, 25(8), 28-34.

Al-Taie, L. H., Hassan, S., Al-Mayah, K. S., & Talib, S. (2014). Isolation and identification of bacterial burn wound infection and their sensitivity to antibiotics. Al-Mustansiriyah J Sci, 25(2), 17-24.

Betty Fitriyasti, Siska Ferilda, Widia Sari et al. (2024). In vitro Antibacterial Activity of Seribu kuman Leaf (Rhinacanthus nasutus (l.) Kurz) Extracts Against Staphylococcus aureus and Pseudomonas aeruginosa. Journal of Angiotherapy, 8(1), 1-7, 9433

Buyukcam A, Liste U, Bicakcigil A, Kara A, Sancak B.(2019). A case of Raoultella ornithinolytica urinary tract infection in a pediatric patient. J Infect Chemother.;25(6):467–469.

Farah M. Muhammad, Bushra A. Khairallah, K. A. Albadrany. (2024). Synthesis, characterization and Antibacterial Evaluation of Novel 1,3-Oxazepine Derivatives Using A Cycloaddition Approach, Journal of Angiotherapy, 8(3), 1-5, 9506

Forson OA, Ayanka E, Olu-Taiwo M, Pappoe-Ashong PJ, Ayeh-Kumi PJ.(2017). Bacterial infections in burn wound patients at a tertiary teaching hospital in Accra, Ghana. Ann Burns Fire Disasters. 30(2):116–20.

Gonzalez, M. R., Fleuchot, B., Lauciello, L., Jafari, P., Applegate, L. A., Raffoul, W. & Perron, K. (2016). Effect of human burn wound exudate on Pseudomonas aeruginosa virulence. Msphere, 1(2), e00111-15.

Hagiwara S., Murata M., Aoki M., Kaneko M., Oshima K.(2013). Septic shock caused by K. oxytoca: an autopsy case and a survival case with driving Extracorporeal Membrane Oxygenation. Hippokratia, 17(2):171–173.

Hajjar, R., Ambaraghassi, G., Sebajang, H., Schwenter, F., & Su, S. H. (2020). Raoultella ornithinolytica: emergence and resistance. Infection and Drug Resistance, 1091-1104.

Hiba Ahmed Jawade, Zahraa Yosif Motaweq, Hawraa Dheyaa Rasool et al. (2024). Study of Antibiotic Resistance in ESKAPE Bacteria Using β-lactamase and ESBL Genes, Journal of Angiotherapy, 8(3), 1-8, 9618

Hubab M, Maab H, Hayat A, Ur Rehman M.(2020) Burn Wound Microbiology and the Antibiotic Susceptibility Patterns of Bacterial Isolates in Three Burn Units of Abbottabad, Pakistan. J Burn Care Res. 41(6):1207–11.

Hussein Abdulkadhim Hasan, Saad M. Mahdi, Hanaa Addai Ali. (2024). Synthesis, Charecterization, Antibacterial and Cytotoxicity of Novel Metal Complexes Derived from Azomethine Ligand (Bis Azo-Schiff Base) In Vitro and In Silico, Journal of Angiotherapy, 8(3), 1-12, 9531

Jie Yu, Yan Liu & Zhen Xu (2022) Hand carriage, antimicrobial resistance and molecular characterisation of methicillin-resistant coagulase- negative staphylococci isolated from gynaecological surgical staff, Journal of Obstetrics and Gynaecology, 42:8, 3605-3610.

Mostafa Alamholo. (2024). Determination of Active Biomarkers and the Antioxidant and Antibacterial Potential of Standardized Zygophyllum spp Extract. Journal of Angiotherapy, 8(1), 1-10, 9369

Muntaha R. Ibraheem, Dhafar N. Al-Ugaili. (2024). Nanoparticle-Mediated Plasmid Curing in Combating Antibiotic Resistance in Pathogenic Bacteria, Journal of Angiotherapy, 8(3), 1-8, 9495

Nielson CB, Duethman NC, Howard JM, Moncure M, Wood JG.(2017) Burns: Pathophysiology of Systemic Complications and Current Management. J Burn Care Res. 38(1):469–81.

Norbury W, Herndon DN, Tanksley J, Jeschke MG, Finnerty CC.(2016). Infection in Burns. Surg Infect (Larchmt) ;17(2):250–5.

Nurul Azila Abdul Razak, Ang Chung Huap, Vivien Jong Yi Mian, Siti Nor Ain Seri Masran. (2024), Black Soldier Fly Larvae Feed with Fermented Bamboo Culms Enriched with Lactic Acid Bacteria, Journal of Angiotherapy, 8(1), 1-10, 9359

Osagie RN, Eyaufe AA, Iserhienrhien O, Okodua M, Unuabonah F, Daibo OO.(2017). Antibiotic susceptibility profile of Klebsiella pneumoniae isolated from sputum samples amongst hospitalized adults in parts of Edo State, South-South, Nigeria. Merit Res J Med Med Sci. 5(8):378- 83.

Rahim Hateet R.(2021). Isolation and Identification of Some Bacteria Contemn in Burn Wounds in Misan, Iraq. Arch Razi Inst.76(6):1665- 1670. doi: 10.22092/ari.2021.356367.1833.

Rana S. Hasan, Sawsan M. Alomari, Roaa A. Thamer et al., (2024). Risks, Identification, and Antibiotic Susceptibility Against High Prevalence of Bacterial Infection in Contact Lens Solutions, Journal of Angiotherapy, 8(3), 1-8, 9538

Shaima H. Abdullah, Mahmoud M. Salih, Khalid, A. Al-Badrany. (2024). Synthesis, characterization and Antibacterial Evaluation of Novel Thiazolidine Derivatives, Journal of Angiotherapy, 8(3), 1-6, 9501

Singh, N. P., Rani, M., Gupta, K., Sagar, T., & Kaur, I. R. (2017). Changing trends in antimicrobial susceptibility pattern of bacterial isolates in a burn unit. Burns, 43(5), 1083-1087.

Soares JL, Macedo SCR, Santos JB.(2007). Complicações infecciosas e fatores preditivos de infecção em pacientes queimados. J Rev Soc Bras Cir Plást. 22(1):34-8

Tavares, T. D., Antunes, J. C., Padrão, J., Ribeiro, A. I., Zille, A., Amorim, M. T. P.& Felgueiras, H. P. (2020). Activity of specialized biomolecules against gram-positive and gram-negative bacteria. Antibiotics, 9(6), 314.

Tsolakidis, S., Freytag, D. L., Dovern, E., Alharbi, Z., Kim, B. S., Houschyar, K. S., ... & Grieb, G. (2022). Infections in burn patients: a retrospective view over seven years. Medicina, 58(8), 1066.

Wang J, Zhang H, Yan J, Zhang T.(2022). Literature review on the distribution characteristics and antimicrobial resistance of bacterial pathogens in neonatal sepsis. J Matern Fetal Neonatal Med. 35(5):861-870.

WHO.(2011).Burn prevention: success stories and lessons learned. World Health Organization. https://apps.who.int/iris/handle/10665/97938

Zampar, E. F., Anami, E. H. T., Kerbauy, G., Queiroz, L. F. T., Carrilho, C. M. D. M., Cardoso, L. T. Q., & Grion, C. M. C. (2017). Infectious complications in adult burn patients and antimicrobial resistance pattern of microorganisms isolated. Annals of burns and fire disasters, 30(4), 281.