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Research Article

ISOLATION AND IDENTIFICATION OF SOME PATHOGENIC AND CONTAMINATED BACTERIAL SPECIES FOR BURNS IN THE CITY OF HILLA

***Zahraa A. Al-Ajeeli**

Dairy Technology Science /Nutritional Science, Al-Qasim Green University,51013, Babil, Iraq

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Abstract

The objective of this study was to gather samples of burns and wounds from patients who were admitted to Al-Sadiq Hospital. A total of 25 samples of burns and wounds were obtained from individuals of diverse racial backgrounds. This study spanned from June 11, 2022, to March 30, 2023. The specimens were cultivated on several culture mediums, including Nutrient agar, Mannitol salt agar, and MacConkey agar. The bacterium Pseudomonas aeruginosa was identified and separated from other microorganisms. By culturing them on a Nutrient agar medium Upon cultivating, a distinct shift in the medium's colour was seen, transitioning from a pale yellow hue to a vibrant phosphorous green shade. This alteration serves as an indicator of the bacteria's pigment-producing capability, which is a defining trait of this pathogenic strain. Staphylococcus aureus was additionally obtained by cultivating the burn sample on a medium called Mannitol salt agar. Upon incubation, the media underwent a noticeable colour shift from pink to yellow, indicating that the bacteria successfully fermented mannitol sugar and consequently altered the pH of the medium from alkaline to acidic. Bacteria have the choice to utilise this media. Staphylococcus aureusan additional harmful bacterium, Klebsiella, was cultured on MacConkey's medium. The object was observed in a pale violet hue. This bacterium possesses the capacity to undergo lactose fermentation, making it a fundamental microbe in MacConkey's medium. The mucous texture was also used to diagnose it. Additional biochemical assays were conducted. The diagnosis of the previously isolated samples was verified with the tests for Indol, Urea, Catalase, Oxidase, and Coagulase. A test to determine the sensitivity of an antibiotic was conducted. A pseudomonas bacterium antibiotic susceptibility test was performed on the samples using a panel of seven different antibiotics. Following a 24-hour incubation period, it was determined that the sample exhibited resistance to all antibiotics employed in the test, hence indicating the bacteria's resistance.

Keywords: Burns samples, pathogenic bacteria, Antibiotic.

INTRODUCTION

The skin typically harbours about 200 distinct bacterial species (Benbow, 2010). An exposed wound creates an ideal environment, characterised by moisture, warmth, and nutrients, which facilitates the growth and spread of microorganisms (Young, 2012). When there is an increase in the number of microorganisms in the wound, the host's body responds both locally and systemically. This might result in an infection and a subsequent slowing down of the healing process (Angel *et al.,* 2011). According to Cutting (2010), it is crucial to keep the bacteria at a level that allows the host to maintain control in order to prevent wound infection. In 2001, Bowler *et al.* discovered that chronic wounds with inadequate blood flow are more vulnerable to infection. This is because blood supplies oxygen, nutrition, and immune cells, which limits the chances for germs to establish and multiply. Burn wounds are highly susceptible to infection, as they are easily inhabited by several types of potentially harmful microbes, such as Pseudomonas aeruginosa and Staphylococcus sp. (Mayhall, 2003). According to Martineau and Dosch (2007), invasive infection leading to systemic sepsis is still the primary cause of death in patients with thermal injuries. The skin acts as a defensive shield against the infiltration of bacteria, fungi, and viruses. However, any hole in this shield allows for the simple entry of microorganisms (Liwimbi and Komolafe, 2007). Undamaged skin serves as an effective barrier against bacterial infiltration, whereas skin injuries create opportunities for bacteria, fungus, and yeasts to penetrate (Young, 2012).

Corresponding Author:** ***Zahraa A. Al-Ajeeli
Dairy Technology Science /Nutritional Science, Al-Qasim Green University, 51013, Babil, Iraq.

Rashid *et al.* (2000) have provided evidence that confirms the existence of biofilms on the surface of chronic human wounds and burns. Mah and O'Toole (2001) stated that biofilms pose a greater challenge for eradication compared to their planktonic counterparts. This is because the sessile bacteria in biofilms have a higher ability to withstand unfavourable conditions by forming aggregates, adapting their characteristics, and/or undergoing metabolic changes to avoid a hostile environment and the immune responses of the host. According to Bjarnsholt (2013), when bacteria are able to establish a biofilm inside the human body, the resulting illness is frequently resistant to treatment and progresses into a chronic condition. Burns are a prevalent and highly destructive type of injury that leads to immunosuppression, making burn victims more susceptible to infection-related complications (Church *et al.,* 2006). Infection is a significant and common consequence of burn injuries, accounting for 50-60% of deaths in burn patients (Absston *et al.,* 2000). The colonisation of microorganisms in the open wound, originating from within the body, often starts within 24 hours and is generally established by the end of the first week after a burn injury (Noronha and Almeida, 2000).

SAMPLES AND METHODS

Samples

A total of 25 samples were collected from male and female patients with burns and wounds at Al-Sadiq Teaching Hospital. Subsequently, these samples underwent cultivation on various culture conditions in order to identify the pathogenic and contaminated bacterial species present in burns and wounds.

Preparation of Reagents

Kovac's reagents: The solution was prepared by dissolving 5 grams of P-dimethylaminebenzylaldehyde in 75 milliliters of amyl alcohol, and then adding 35 milliliters of strong hydrochloric acid. The employment of it was intended to ascertain the existence of Indole production (MacFaddin, 2000).

Solutions

Normal saline solution: The solution was prepared by dissolving 8.5 grams of NaCl in a minimal quantity of purified water. The volume was then adjusted to 1000 millilitres, and the pH was set at 7.2. The solution was sterilised in an autoclave at a temperature of 121 degrees Celsius for 15 minutes. After sterilisation, it was stored at a temperature of 4 degrees Celsius (MacFaddin, 2000).

EDTA Solution: The solution was prepared by dissolving 0.4 grams of EDTA in 12 milliliters of distilled water. The pH was adjusted to 8 by adding 10 N. of sodium hydroxide. The volume was then brought up to 20 millilitres, resulting in a total concentration of 20 milligrammes per millilitre for the solution (Sambrook and Rusell, 2001).

Gram Stain Solutions: The solutions were made in accordance with the specified microbiological techniques. The solutions used in the study were four in total: crystal violet, iodine, pure alcohol, and safranine (Collee *et al.,* 1996).

Preparation of Culture Media: A set of culture media was prepared following the company's instructions and sterilised using autoclaving at a temperature of 121oC for a duration of 15 minutes.

Mac Conkey Agar Medium (pH:7.3): It has been prepared in accordance with the recommended procedure of the manufacturer. The primary purpose of its employment was to isolate the majority of Gram-negative bacteria and distinguish between bacterial isolates that ferment lactose and those that do not (Baron *et al.,* 1994).

Blood Agar Medium (PH:7.1): The blood agar medium was prepared by dissolving 40 grams of blood agar base in 1000 milliliters of distilled water. The media was sterilized using steam sterilization at a temperature of 121oC for a period of 15 minutes. Subsequently, it was cooled down to 50oC. Following this, 5% of fresh human blood was introduced. This medium was employed to culture fastidious bacteria with specific growth needs (Forbes *et al.,* 2007).

Nutrient agar medium

The nutrient agar medium was made in accordance with the manufacturer's instructions, using a concentration of 28 grammes per litre. It was employed for conducting various investigations, fostering growth, and initiating the activity of bacterial isolates as required (MacFaddin, 2000).

Muller-Hinton agar medium: The Muller-Hinton agar medium was made in accordance with the manufacturer's instructions. MacFaddin (2000) employed it for anti-bacterial susceptibility testing.

Urea agar medium: The manufacture of this medium entailed mixing 15 ml of sterilized urea solution (after filtration) with 100 ml of sterilized urea agar base. The urea agar base was sterilized using autoclaving at a temperature of 121oC for 15 minutes, and then cooled to 50oC. The pH was adjusted to 7.1, and the sterilized medium was distributed into test tubes and allowed to solidify in a slanted configuration. This medium was employed to assess the bacteria's capacity to synthesise urease enzyme.

Peptone water medium: The medium was prepared by dissolving 8 grams of peptone in 1000 milliliters of distilled water, then dividing it into test tubes and sterilizing it using autoclaving. The purpose of its employment was to demonstrate the bacterial capacity to break down the amino acid tryptophan into indole (MacFaddin, 2000).

Mannitol salt agar medium: Dissolve 111 grammes of Mannitol Salt Agar in 1000 millilitres of distilled water. Heat the medium until it is fully dissolved. Perform sterilisation by subjecting the material to autoclaving at a pressure of 15 pounds per square inch (121°C) for a duration of 15 minutes. Optionally, one may choose to add sterile Egg Yolk Emulsion (E7899) to the solution after autoclaving, achieving a final concentration of 5% v/v. The citation is from The United States Pharmacopoeia, published in 1985, on page 21.

Eosin Methylene Blue(EMB) Agar Medium: Dissolve 36 grammes of EMB Agar in 1000 millilitres of distilled water. Apply heat until the medium is fully dissolved. Administer and sterilise using autoclaving at a pressure of 15 pounds per square inch (121 °C) for a duration of 15 minutes. Prevent excessive heating. Lower the temperature to 50 °C and agitate the substance to facilitate the oxidation of methylene blue, resulting in the restoration of its blue colour, as well as the suspension of the flocculent precipitate (Cheesbroygh, 2000).

Laboratory diagnosis:

Microscopic examination and colonial morphology: One colony was selected from each primary positive culture and identified based on its morphological characteristics, including colony size, shape, colour, pigmentation, translucency, edge, elevation, and texture. A Gramme stain was performed on a bacterial smear to examine the cellular morphology of bacterial cells, including their Gramme reaction, shape, organisation, presence of capsules, spores, and other characteristics.

Bio chemical tests:

Indole test: The examined bacteria colony was introduced into tubes containing a peptone water medium, which were then placed in an incubator at a temperature of 37ºC for a duration of 18 hours. Subsequently, several drops of Kovac's reagent were added to the broth medium. The red ring's presence on the surface after shaking was considered a favourable outcome (Cruikshank *et al.,* 1975).

Urease test: Urease is an enzyme that catalyses the cleavage of the carbon-nitrogen link in amides, resulting in the production of carbon dioxide, ammonia, and water. The urea base agar was sterilised using an autoclave. It was then cooled to a temperature of 50ºC and supplemented with urea substrate. The mixture was placed into sterile tubes and injected with a bacterial culture. The tubes were incubated at a temperature of 37ºC for a period

of 24 to 48 hours. Upon the decomposition of urea, ammonia was liberated, resulting in an elevation of the pH level of the medium. The alteration in pH was identified through the use of a pH indicator, which exhibited a pink coloration in an alkaline environment. A pink medium signifies a favourable result for urease. The absence of deep pink colour development was indicative of a negative reaction.

Coagulase test: The test involves the utilisation of rabbit's plasma that has been immunised with staphylococcus bacteria. The tube is placed in an incubator set at a temperature of 37 °C for a duration of one and a half hours. The outcome is negative, with a duration of 18 hours. Conversely, if the outcome is positive, the plasma will solidify and manifest as aggregated fragments. The source is a publication by Yrios, J. W. in 1977.

Antibiotic susceptibility testing:

Disk diffusion test

An uncontaminated culture of a preexisting bacterial strain was utilized in the experiment. The most suitable antibiotic for each bacterial isolate was determined based on the standards established by CLSI, (2012).

The inoculum for this test was produced by introducing 5 isolated colonies, cultivated on a blood agar plate, into 5 ml of nutrient broth. The mixture was thereafter placed in an incubator at a temperature of 35 degrees Celsius for a duration of 18 hours, and its properties were then assessed in relation to a tube containing a (0.5) McFarland standard. Aseptic technique was employed to get a sample from the bacterial suspension using a sterile brush. The sample was then evenly spread onto a Mueller-Hinton agar plate and left to dry in the air.

C - The antibiotic discs were placed on the surface of the medium at consistent intervals using sterilized forceps or a disc applicator, and thereafter incubated for a period of 18 hours. The measuring of inhibition zones was performed using a ruler and thereafter compared with the determined zones of inhibition (CLSI, 2012).

RESULTS AND DISCUSSION

Isolation and Identification of pathogenic bacteria

This study comprised 25 samples obtained from burns of patients hospitalised at Imam Al-Sadiq Hospital in Babylon, Iraq. The swabs were introduced into several growth media, including nutritional agar, macConkey agar, and mannitol salt agar. There are numerous research in Iraq that focus on burn pollution. The degree of isolation varied across different studies, potentially attributed to variations in sample collection timing and environmental differences. At the College of Science, University of Baghdad, Staphylococcus isolates were cultivated on mannitol agar in order to determine the specific Staphylococcus species. Several isolates exhibited growth on MacConkey's agar, displaying a pale or pink coloration. The bacteria were cultured on MacConkey's agar, which had a pale hue. The prevalence of P. aeruginosa isolates was 17.78%. The least prevalent bacterial isolates obtained from the infected wound were Staphylococcus aureus and Pseudomonas. In a study conducted in 2017, it was shown that P. aeruginosa was the most prevalent bacterial species identified in burn wounds. Isolating and identifying some harmful microorganisms. In our investigation, many strains of bacteria, such as Staphylococcus aureus, Klebsiella, and Pseudomonas, were isolated and identified from the contaminated burns sample. Gram-negative bacterial species include Klebsiella and Pseudomonas. A bacterium belonging to the Gram-positive group, such as Staphylococcus aureus. Swabs that were exposed to fire were separated and grown on a type of agar called mannitol salt agar. An alteration in the hue of the medium was observed, transitioning from pink to yellow. The cause of this phenomenon is the alteration in the pH of the medium, shifting from a basic value of 9 to an acidic value of 4.5. From this change, the type of bacteria was identified as Staphaureus. As shown in the figure

Figure 1. Staph. aureus on mannitol salt agar

Furthermore, smears were inoculated onto a nutrient agar medium. The colonies exhibited a phosphorescent hue, namely a vibrant shade of green, whereas the medium underwent a transition from a pale yellow colour. This suggests that the bacteria possess the capability to generate pigments, resulting in their manifestation in this particular hue, so confirming their identity as pseudomonas aeruginosa as depicted in the diagram

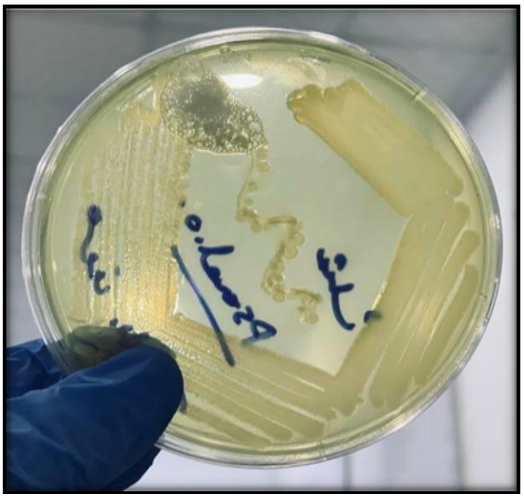

Figure 2. Pseudomonas aeruginosa on nutrient agar

The sample was also cultivated on Macconkey's medium, resulting in the formation of colonies that exhibited a uniform violet hue and had a moist and mucous consistency. These colonies were diagnosed as klebsiella bacteria, which is distinct from colonies of intestinal coli bacteria. As depicted in the diagram

Figure 3. Klebsiella on MaCconkey agar

Conduct biochemical assays to detect the presence of specific pathogenic microorganisms associated with diseases. An indole test is a biochemical analysis performed on a bacterial species to determine its ability to convert tryptophan into indole. The process of division is executed through a series of intracellular enzymes, referred to as 'tryptophanase'. The indole test assesses an organism's ability to metabolize the amino acid tryptophan and produce indole. It is utilized as a constituent of the IMViC protocols, a series of tests devised to distinguish between distinct members of the Enterobacteriaceae family. Tryptophan is an amino acid that can be subjected to deamination and hydrolysis by bacteria that produce the tryptophanase enzyme. Our investigation revealed variations among the isolated bacterial species in their capacity to manufacture the enzyme Tryptophanase. Consequently, we observed that staph.aureus tested positive for the indole test, whereas klebsiella tested negative for the indole test. As shown in figure

Figure 4. Indoletest

- The urease test is a biochemical assay used to identify the presence of microorganisms that may ferment urea in urine, resulting in the creation of ammonia and an alkaline environment.
- The enzymatic process of urea fermentation yields two molecules of ammonia and carbon dioxide.
- The test was established by Christensen in 1946 to distinguish between different types of enteric bacilli. The urea agar base utilised for the assessment of urease activity is referred to as Christensen Urea Agar, named after its developer.
- During the test, the organisms use urea as the only nitrogen source, generating enough ammonia to surpass the medium's buffering capability.
- The alteration in the hue of the medium due to the fluctuation in pH serves as an indication of the test outcome.
- The samples were additionally diagnosed using a urease test, and certain samples, such as staph. aureus and Klebsiella, tested positive for the urease test. As shown in figure

Figure 5. Ureas test

- Coagulase is an enzyme secreted by Staphylococcus aureus that catalyses the conversion of soluble fibrinogen in plasma into insoluble fibrin. Nonetheless, other staphylococci lack the ability to generate coagulase, making this test effective in differentiating S. aureus from other staphylococci. Coagulase exists in two forms: free coagulase and bound coagulase. These two forms can be distinguished using different methods.
- The coagulase that is attached to a surface is referred to as the clumping factor and may be quickly identified using a slide test. The presence of the free coagulase is identified in the test tube due to the formation of a clot. The diagnosis of Staphylococcus aureus was confirmed by the coagulase test, which yielded a positive result for Staphylococcus aureus. Conversely, other samples, such as Klebsiella and Pseudomonas aeruginosa, tested negative for this particular test. As shown in figure

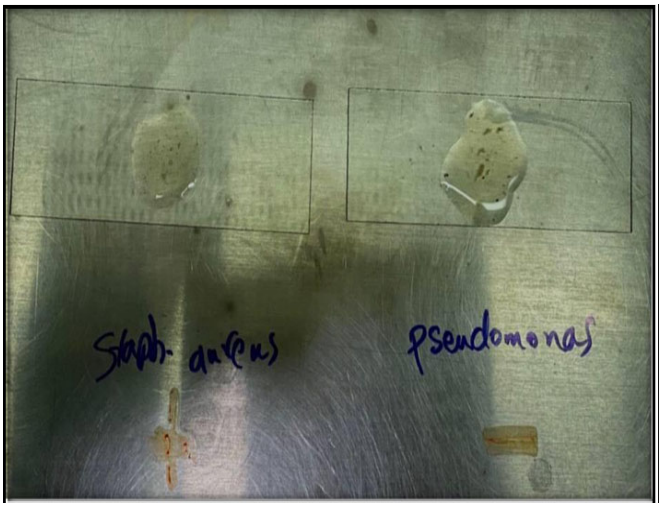

Figure 6. Coagulase test

Biochemical test for some pathogenic bacteria

Bacteria	Indol	Ureaes	Coagulase
Klebsiella	-	+	-
Staph.aureus	+	+	+
Pseudomonas aeruginosa	-	+	-

In addition, this study assessed the susceptibility of bacteria to antibiotics by employing a total of seven different types of antibacterials. Pseudomonas aeruginosa bacteria were employed and, during a 24-hour incubation period, these bacteria were discovered to exhibit resistance against all categories of antibacterials, including Florfenicol, Tobramycin, Vancomycin, Clarithromycin, Tetracycline, Amikacin, and Clindamycin. As shown in figure

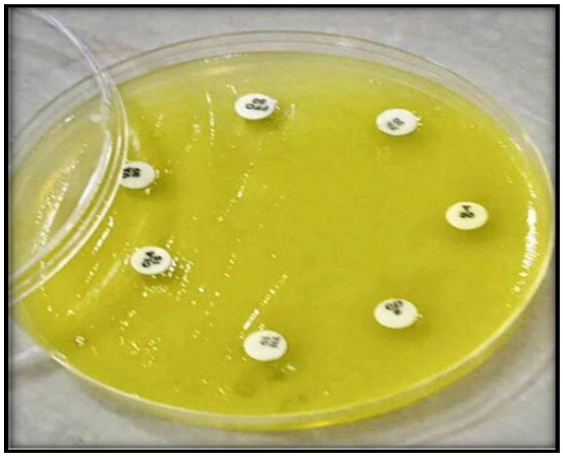

Figure 9. Pseudomonas aeruginosa on Muller-Hintonagar

Conclusion

Our findings indicate that some bacterial species, which are both contaminated and pathogenic, are beneficial for treating burns and wounds, particularly those that have been exposed to contamination in a hospital setting.

REFERENCES

1. Angel, D. E.; Lloyd, P.; Carville, K. and Santamaria, N. (2011). The clinical efficacy of two semi-quantitative wound swabbing techniques in identifying the causative organism(s). Int Wound J; 8:176–185.
2. Al-Muhammadi, M. O. and Azeez, H. A. (2011). Some physiological changes in burn patients. Medical Journal of Babylon., 8(3): 303 – 319.
3. Abston, S.; Blakeney, P. and Desai, M. (2000). Acute Burn Management. Resident Orientation Manual. Galveston Shriners Burn Hospital and University of Texas Medical Branch Blocker Burn Unit.
4. Agnihotri, N.; Gupta, V. and Joshi, M. (2004). Aerobic bacterial isolate from burn wound infections and their antibiotics: a five-year study. J. Burns., 30: 241-3.
5. Bjarnsholt, T. (2013). The role of bacterial biofilms in chronic infections. APMIS Suppl. 136:1-51.
6. Bessa, L. J.; Fazii, P.; Di Giulio, M. and Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. Int Wound J 2015; 12:47–52.
7. Barret, J. P. and Herndon, D. N. (2003). Effects of burn wound excision on bacterial colonization and invasion. Plast. Reconstr. Surg., 111:744 - 750
8. Benbow, M. (2010). Wound swabs and chronic wounds. Practice Nurse 39(9):27– 30.
9. Bowler, P. G.; Duerden, I. and Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. Clin Microbiol Rev. 14(2): 244– 269
10. Baron, C., and B. Coombes. (2007). Targeting bacterial secretion systems: benefits of disarmament in the microcosm. Infect. Disord. Drug Targets 7:19-27.
11. Cutting, K. F. (2010). Addressing the challenge of wound cleansing in the modern era. Br J Nurs (Tissue Viability Supplement). 19(11): S24–S29.
12. Church, D.; Elsayed, S.; Reid, O.; Winston, B. and Lindsay, R. (2006). Burn wound infections. Clin. Microbiol. Rev., 19 (2), 403 – 434.
13. Clark, N. M.; Patterson, J. and Lynch III, J. P. (2003). Antimicrobial resistance among gram-negative organisms in the intensive care unit. Curr. Opin. Crit. Care .9:413 - 423.
14. Church, D.; Elsayed, S.; Reid, O.; Winston, B. and Lindsay, R. (2006). Burn wound infections. Clin. Microbiol. Rev., 19 (2), 403 – 434
15. Cooper, R. and Lawrence, J. C. (1996). The isolation and identification of bacteria from wounds. J Wound Care. 5:335–40.
16. Collee, J.G., Fraser, A. G., Marmion, B. P. and Simmon, A. (1996). Makic and McCartncy medical microbiology. 14th. the Churchill living stone. Inc. USA.11:e221-e238.
17. Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1975): "Staining methods", "Tests for identification of bacteria" In: "The practice of medical
18. microbiology", Cruickshank, R., Duguid, J.P.; Marmion, B. P. and Swain, R.H.A. (Eds) Twelfth edition. Volume 2. Churchill Livingestone, Edinburgh. London. New York pp31-157, 170-189 and 444- 448.
19. Dow, G.; Browne, A. and Sibbaild, R. G. (1999). Infection in chronic wounds: controversies in diagnosis and treatment. Ostomy/ Wound Manage. 45: 23-40
20. Edwards, R. and Harding, K. G. (2004). Bacteria and wound healing. Curr. Opin. Infect. Dis., 17:91–96.
21. Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. (1998). Bailley and Scott Diagnostic Microbiology. 10th ed., Mosby.
22. Gupta, M.; Gupta, O. K.; Yaduvansh , R. K. and Upadhyahy , J. (1993). Burn epidemiology: The pink city scene. Burns. 19: 47-51.
23. Greenhalgh, D.G.; Saffle, J.R.; Holmes 4th, J. H.; Gamelli, R.L.; Palmieri, T. L. and Horton, J.W. (2007). American Burn Association, Consensus Conference to Define Sepsis and Infection in Burns. J. Burn Care Res., 28(7):776 - 90.
24. Kaplan, O. and Bakir, U. (1998). The effect of chemical crosslinking of invertase with dimethyl suberimidate on its pH stability. World J. Microbiol., and Biotechnol., 14: 277-280.
25. Kasten, K. R.; Makley, A. T. and Kagan, R. J. (2011). Update on the critical care management of severe burns. J Intensive Care Med. 26(4):223-36
26. Liwimbi, O. M. and Komolafe, I. O. O. (2007).
27. Epidemiology and bacterial colonization of burn injuries in Blantyre. Malawi Medical Journal. 19(1): 25-27.
28. Luterman, A.; Dasco, C. C. and Curreri, P. W. (1986). Infection in Burn Patients. Am. J. Med., 81: 45-52.
29. Luthi-Peng, Q., Dileme, F. B. & Puhan, Z. (2002). Effect of glucose on glycerol bioconversion by Lactobacillus reuteri. Appl Microbiol Biotechnol 59, 289–296.

30. Mayhall, C. G. (2003). The epidemiology of burn wound infections: then and now. Clin Infect Dis 37: 543–550.
31. Martineau, L. and Dosch, H. M. (2007). Biofilm reduction by a new burn gel that targets nociception. J Appl Microbiol. 103(2):297-304.
32. Mah, T.-F.C. and O'Toole, G.A. (2001). Mechanism of biofilm resistance to antimicrobial agents. Trends Microbiol 9: 34–39.
33. Marvaki, C.; Joannovich, I.; Kiritsi, E.; Iordanou, P. and Iconomo, T. (2001).The effecttiveeness of early enteral nutrition in burn patients. Annals of Burns and Fire Disasters. 16 (4).
34. Magnet, M. H.; Arongozeb; Khan, G. M. and Ahmed, Z. (2013). Isolation and identification of different bacteria from different types of burn wound infections and study their antimicrobial sensitivity pattern. International Journal of Research in Applied Natural and Social Sciences. 1(3): 125-132.
35. Manson, W. L.; Pernot, P. C.; Fidler, V. and Sauer, E. W. (1992). Colonization of burns and duration of hospital stay of severely burned patients. J. Hosp. Infect., 22: 55-63.
36. Monafo, W. W. and Freedman, B. (1987). Topical Therapy for Burns. Surg Clin North Am. 67: 133-145.
37. Mooney, D. P. and Gamelli, R. L. (1989). Sepsis Following Thermal Injury. Comp. Ther. 15: 22 29.
38. Mooney, D. P. and Gamelli, R. L. (1989). Sepsis following thermal injury. Comp Ther. 15:22 – 29.
39. Manus, A.; Mason, A.; Manus, W. and Pruitt, B. (1994). A decade of reduced gram negative infections and mortality improved isolation of burned patients. Arch. Surg., 129: 1306 - 9.
40. MacFaddin, J.F. (2000) Biochemical Tests for Identification of Medical Bacteria. 3rd Edition, Lippincott Williams & Wilkins, Philadelphia
41. National Committee for Clinical Laboratory Standards. (1993). Methods for determining bactericidal activity of antimicrobial agents. Tentative Guidelines, M26-T NCCLS. Villanova, PA.
42. Ogunsola, F. T.; Oduyebo, O.; Iregbu, K. C.; Coker, A. O. and Adetunji, A. (1998). A review of nosocomial infections at LUTH: Problems and strategies for improvement. J. Nigerian infection Control Association., 1: 14-20.
43. Pruitt, B. A.; Colonel, M. C. and McManus, A. D. (1984). Opportunistic infections in severely burnt patients. Am. J. Med., 76: 164-154.
44. Rashid, M. H.; Rumbaugh, K.; Passador, L.; Davies, D.G.; Hamood, A.N.; Iglewski, B.H. and Kornberg, A. (2000) Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of Pseudomonas aeruginosa. Proc Natl Acad Sci USA 97: 9636–9641.
45. Roth, J. J. and Hughes. W. B. (2004). The essential burn unit handbook. Quality Medical Publishing, St. Louis, Mo.
46. Ramzy, P. I.; Wolf, S. E.; Irtun, O.; Hart, D. W.; Thompson, J. C. and Herndon. D. N. (2000). Gut epithelial apoptosis after severe burn: Effects of gut hypoperfusion. J. Am. Coll. Surg., 190(3): 281 - 287.
47. Sambrook, J. and Rusell, D.W. (2001). Molecular cloning a laboratory manual. Cold spring Harbor, NY: Cold spring Harbor laboratory press.32:154-165.
48. Weinberg, E. D. (1997). The Lactobacillus anomaly: total iron abstinence. Perspect. Biol. Med., 40: 578 – 583
49. White, R. J.; Cooper, R. and Kingsley, A. (2001). Wound colonization and infection: the role of topical antimicrobials. Br J Nurs. 1 0:563–78.
50. Wysocki, A. B. (2002). Evaluating and managing open skin wounds: colonization versus infection. AACN Clin. Issues. 13:382 - 397.u
51. Young, L. (2012). Identifying infection in chronic wounds. Wound Practice and Research: Journal of the Australian Wound Management Association. 20(1): 38 – 44.
52. Yang, E.; Maguire, T.; Yarmush, M. L.; Berthiaume, F. and Androulakis, I. P. (2007). Bioinformatics analysis of the early inflammatory response in a rat thermal injury model. BMC Bioinformatics. 8(10).
53. Yrios, J. W. 1977 Comparison of rabbit and pig plasma in the tube coagulase test. J. Clin. Microbiol. 5:221-224.

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