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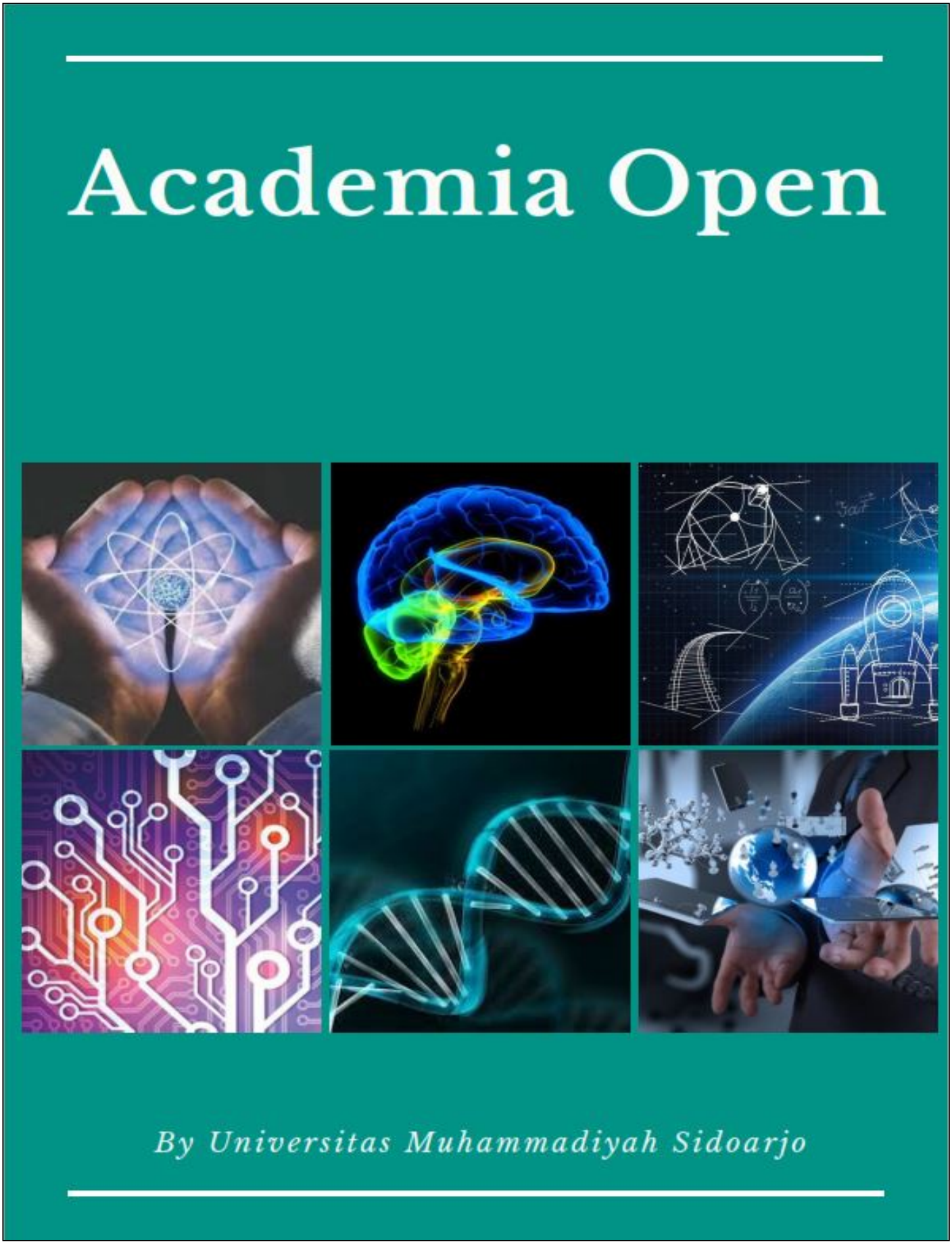

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Resistance pattern of some bacterial isolates from burns and wound infection

Pola resistensi beberapa isolat bakteri dari luka bakar dan infeksi luka

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Abstract

Wound and burn infections present ongoing challenges in healthcare due to multidrug-resistant bacteria. This study aimed to identify bacteria from these infections, assess antibiotic resistance, and evaluate treatment efficacy. Samples from 86 patients were cultured and tested for antibiotic susceptibility. Imipenem showed effectiveness against Gram-negative bacteria, while ciprofloxacin was more effective against Gram-positive bacteria. Rifampicin demonstrated reduced sensitivity. Staphylococcus aureus and Enterobacteriaceae were prevalent, indicating increasing multidrug resistance. These findings emphasize the need for tailored treatment strategies to combat antibiotic resistance effectively in wound and burn infections.

Highlight:

1. **Resistance Challenge:** Addressing antibiotic resistance in wound and burn infections.
2. **Tailored Treatment:** Importance of customized approaches for effective antibiotic management.
3. **Predominant Pathogens:** Identifying prevalent bacteria to guide targeted treatment strategies.

Keyword: Wound Infections, Burn Infections, Antibiotic Resistance, Bacterial Identification, Treatment Efficacy

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INTRODUCTION

Wounds pose medical risks because they often develop as a result of burns ,surgery, trauma and chronic diseases such as diabetes . The healing process is Delays because infection microbes lead to persistent infections, especially if they are in the form of biofilmsanThe biofilms are antibiotics resistances. Therefore, new chemical dressing methods and material with antibacteria, antibiofilms and wound healing proportion need to bedeveloped Nanomaterials (NM) have (unique property) that lead to a wide range of applications due to their large surface areaa and size . Many NMs have antimicrobial activity related to wound repair mechanisms and therefore show promise for various types of wounds. .[1]

There are several factors that increase the risk of infection and sepsis in burn patients; these include underlying disease factors, percentage of total body surface area with inflammation, delayed wound healing, and number of viruses/bacteria. Organisms that cause wound infections vary depending on the time and location of the wound; and evaluation has many aspects. Bacterial infections still include Staphylococcus and Pseudomonas spp.[2]Burns can seriously damage skin , compromise one of the most important barriers to infectious. The cause of death in trauma patients is infection. Even in patients who survive, the disease can be difficult to treat and can cause long-term damage, including delayed recovery and extended hospital stays. Biofilm formation at the wound site is a significant contributor to wound healing failure and wound death. Bacteria form a biofilm, or community of bacteria, surrounded by a polysaccharide matrix that is highly resistant to infection, thus enhancing the host's immune system and is particularly resistant to antibiotics. Inflammatory wounds associated with biofilms are also believed to serve as bacterial vectors for deep, systemic infections and ultimately bacteremia and sepsis. In this review, we will discuss some pathogens that cause wound inflammation and show how they regulate biofilm formation in thewound environment. We also discuss new and emerging models available to study biofilm burns in vivo.

METHOD

Sterilization method

Wet hot sterilization:-

Placed the media In an autoclaves and sterilize It at 121 ° to 15 min. After sterilization, take out the media by using asbestos gloves and keep at appropriate condition

Preparation reagents and solution

Reagent:-

A-Catalase reagent: storedHydrogen peroxide 3% in a (brown bottle) under refrigerate. An 18 - 24-hour cultures of the organisms to be tests

B-Oxidase reagent:- oxidative test uses 0.5% or 1% aqueous solution of TPD

C- Methyl red reagent:-Prepare this reagent from dissolve 0.1g of stain of methyl red in 300.0ml of ethanol Alcohol in concentration 100% and then fill the volume to

500.0ml by use distal water

3.2.2.2 Solution

A- Urea solution: Prepare urea from dissolve 4g from urea powders in 10ml of distalled water [4]

Stain

A-Gramstain:-

Analysis the Bacteria can be differentiated as Gram-positive and Gram-negative based on their morphology cell wall structure and chemical composition.

Prepare culture media:-

Prepare all media that use in study depend on information of factory company that

Fix on the package and sterilize this media in autoclave device for 15 minute, 121C And 1atm then poured in Petri dish and put in incubate for 24h in 37C and put in Refrigerator.

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Collection of specimens:-

86 samples (swab) were collected from clinical sources that included 74 wounds and 12 burns from Rafidian patients who suffer from burn and wound infections and under medical supervision in hospitals.

identification of isolated bacteria -:

Bacterial isolates grown on blood agar solid medium and macConkey solid medium and Brain heart infusion agar solid medium Were diagnosed according to One of the following grounds:

1-Cultural characteristics Cultural features. The phenotypic characteristics of bacterial colonies growing on blood agar and MacConkey agar were investigated and culture characteristics were determined in terms of colony color, size ,shape, odor. [5]

2- Microscopic characteristics: A tiny quantity of the developed bacterial colonies were removed and spread out on microscopic slides together with a drop of distilled water. The smear was then stained with Gram stain and examined under a light microscope to see the color of the bacteria and The form and arrangement of the cells were examined under an oil immersion.3-Biochemical test: - 3.1 Coagulase Test:- Place two drops of fresh water or salt water solution in two circles drawn on the glass surface with a wax pencil. Carefully remove colony material from the organism to identify it in the water each round. Add a drop of plasma coagulase Into the suspension in one of the circles and stir with a wooden stick. To check, add another drop of water or saline to the other wheel, continue sliding back and forth and watch for agglutination to stop the test and show a positive result.[6]**(Appendix 3)**

3.2 indole test: - **Kovacs reagent containing PDAB can be used. Approximately five drops of Kovac's reagent were added to the salt culture. The tube is shaken and if indole is present, a red color appears.** [7] .(Appendix 4)

3.3 Catalase:- **Using a pin or safety pin, move the growth from the center of the column to the top of the glass. Add a drop of 3% hydrogen peroxide and observe the formation of the (positive) tube**. [6]

3.4 Citrate Utilization:-**A well-isolated colony is selected from the dominant isolation medium and plated in a single layer on the surface of citrate agar. The tube is placed at 35°C for 24-48 hours. ResultsDescriptionA positive test is indicated by the development of a dark blue color within 24-48 hours; This indicates that the test organism is able to utilize citrate in the medium.**[6](Appendix 7)

3.5- Methyl Red Test :- **Prepare the MR VP tube according to the required volume and put 10 ml into the tube. Sterilize by autoclaving. Insert the MR VP tube containing the culture from the feeding plate. Incubate at 37°C for 24 hours. After incubation, add drops of methyl red solution to all tubes. Writing the answers. Based on the color change of the MR-VP salt to cherry red, bacteria are classified as methyl red (MR) positive (appears red, indicating acid production) or negative (appears yellow).**[8] (Appendix 5)

3.6 - Voges-Proskauer's Test-:

noculate MR. VP broth with loopful of culture from nutrient agar

plates. Incubate at 37°C for 24 hours. After incubation, add 0.5 ml (8-10 drops) of unftol solution and 0.5 ml of 40% KOH solution containing 0.5 ml of 40% KOH solution. 3% creatine. Mix well and let sit for 5 to 30 minutes. Save the results. Color change from yellow to red compared to the red color change of MR-VP was a sign of good results. This test was used to measure the ability of bacteria to produce acetoin as a result of sugar fermentation. [8] **(Appendix 6**)

3.7-Oxidase test: - **Various methods are available to perform oxidation testing. A drop of reagent is added to the filter paper and a wooden stick is used to leave colonies on the filter paper. The development of a lavender color within 10 to 15 seconds is a positive reaction.**[7]

3.8- The urease test **In a test performed by injecting light ureaProcesses are sequenced and stored at 37°C for 24 hours. An average color change from yellow to light brown indicates a positive result; If any color change is positive** [9] (Appendix 8)

3.9-Triple Sugar Iron Agar (TSI) Test-:

TSI Agar is employed to distinguish between different types of gram-negative enteric bacteria by looking at how they digest carbohydrates and produce hydrogen sulfide. This test is pe rformed by fermenting the glucose, lactose, and sucrose contained in the baseline growth medium and measuring t he hydrogen sulfide content to ascertain the organism's capacity to attack a particular carbohydrate. (HS) pro-duction. A pH When fermentation occurs, the indicator will cause the media's color to change. Which sugar is fermented is indicated by a color shift in the medium during fermentation. The existence of black signifies the production of HS.

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In this modia, ferrous sulphate and HS combine to form ferrous sulphide, which is black in culture. **(Appendix 9)**

3.10 Motility test:

Bacterial growth was added to test media for motility in tubes. stabbing until three centimeters deep, being careful not to puncture the tubes' bottom. After that, the tubes were incubated at 37 °C for 24 hours. A good outcome is indicated by the hazy forms that surround the stabbing site.

preservation bacteria isolation

Tubes containing slant brain heart infusion agar were inoculated by streaking then they were incubated at 37 °C for 24 hours and then preservation in refrigerator with packaging Para film

Antibiotic susceptibility test

The Kirby-Bauer method was utilized to evaluate the isolates' sensitivity to specific antibiotics in accordance with C LSI (2020) guidelines. The results showed as follows. Up to five colonies cultured on nutritional agar were transferr ed to tubes filled with regular saline until they reached MacFarland's standard turbidity of 1.5x108 cell/mL. In orde r to remove extra feed, a sterile cotton swab was placed inside a tube holding bacterial suspension, twisted, and pr essed up against the tube's inner walls. The swab was then distributed in various directions over laboratory dishes holding Muller-Hinton agar to guarantee a uniform growth. Using sterile forceps, antibiotic disks used in the study listed in table 3.6 were pressed into the surface of the cultured media at an identical distance from one another. disks then comparing them to the standards mentioned in CLSI, 2023.((Appendix10)

RESULT

This study aims to discover the bacterial isolates that cause different skin complications in burns and wound infecti ons. circumstances and these bacterial isolates' resistance to antibiotics. A total of (86) specimens, consisting of wo und (74), burn (12), and wound swabs (74), were taken from various patients; the specimens were for (47) female a nd (39) male patients, with an average age ranging from 2 to 70 years.

And for the period from September to December 2022 from Fallujah Teaching Hospital and Al-Ramadi Teaching Hospital, and after conducting the final diagnosis of the samples, then obtaining 55 isolates of bacteria, with a rate of 63.95% . this percentage is high of previous study conducted by Edrees and Banafa, (2021) in government hospital sana city,yemen with 175/278 (62.95%)

Type of specimen	No.sample	No.of isolate	percentage
Burn	12	9	16.36%
Wound	74	46	83.63%

Table 1.

They investigate the antimicrobial susceptibility of isolated bacteria from patients with wound infections that visit certain government hospitals in Sana'a City, Yemen and it is lower than that found in Baghdad city by khudhair,alaudyi (2023) which prevalence of 93/103 (90.29%) of wound and burn infection in Baghdad.

Table (1).number specimen wound and burn and isolate percentage

Positive culture			Negative culture		
Age	Male		Female	Total	Male
No.patient	24		31	55	16
2-10	2 (8.3%)		2) 6.45 %(	4(7.27)	3 (18.75%)
11-20		1)4.2%)	0) %(	1(1.82)	2(12.5%)

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21-30		7)29.1%)	6) 19.4 %(	1(23.64%)	2(125%)
31-40		4(16.7%)	7)22.58%)	11(20 %)	6(37.5%)
41-50		4) 16.7% (	5) 16.12%(	9(16.36%)	1(6.25%)
51-60		2) 8.3%(	6) 19.35%(	8(14.55%)	1(6.25%)
61-70		4)16.7%(	5)16.1%)	9(16.36%)	1(6.25%)
percentage		43.63%	56.36%	63.95%	51.61%

Table 2.

Our analysis shows that The majority of the participants in the study were females with 31/55 (56.36%) incidence,the Prevalence of bacterial wound and burn infection were higher in the Age group 31-40 with 7\31(22.58%) followed by patients who are 21-30 and between 51-60 have save result 6/31 (19.35%) and followed by patient who are 41-50and between 61-70 have same result 5\31(16.12%) have the same result 6/24(15.79%) the lowest result seen in age group of 2-10 2\31 (6.45%) and the age group of 11-20 that have negative culture result compared to these results, males have lower prevalence rates 24/55 (43.63%) the prevalence of bacteria in Male is the highest in age group of 21-30 7/24 (29.16%) followed by the age group of 3140 and the group of 41-50 and61-70 have same result 4\24(16.66%)the lower result seen in the age group of 51-60 2/24 8.33%) and the lowest result was in the age

group of 11-20 1/24(4.16% All of isolated bacteria subjected to number of identification tests including cultural and microscopical with number of biochemical tests. After that the bacteria were detected to al level of genus and species by the collaborative identification of all aspects of diagnostic tests,Table (4-3) results of biochemical test

Table (2) percentage of the isolated bacteria from wound and burn samples

Bacteria genus	No.bacterial isolate	percentage
Staphylocccus auerus	14	25.45%
Proteus mriabilis	13	21.81%
Escherichia coli	12	18.18%
Pseudomonas aergunosa	3	5.45%
Staphylococcus epidermides	3	5.45%
Klebsiella pneumonia	1	1.81%
Streptococcus pyogenes	2	3.63%
Proteus vulgarus	1	1.81%
Klebsiella oxytoco	1	1.81%
Enterobactercloacae	1	1.81%
Citrobacter frenudii	4	7.27%
Total isolate	55	63.95%

Table 3.

found were *staphylococcus.aureus* the most common microorganism isolated of wound and burn infection in

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present study accounting 14/55 (28.81%) followed by *proteue.mriabilis* 13\55 (23.63%)then *Escherichia.coli* 10\55(18.18%) *Citrobacter. frenudii*4\55(7.27%) then *Pseudomonas. aergunosa* and *Staphylococcus.epidermides* and 3\55(5.45%) followed by *Streptococcus .pyogenes* 2\55(3.63%) then *Klebsiella. pneumonia* and *Proteus .vulgarus* and *Klebsiella. oxytoco* and *enterobactercloacae*

1\55(1.81% In our study, we used more than one type of antibiotic with different concentrations to determine which one is the most effective against the isolated bacteria we found imipenem, 89.2% as the most effective antibiotic followed by ciprofloxacin 67.6%, levofloxacin51.3%, gentamicin 35.1% ,cefotaxime 29.7% ceftriaxone 16.2% ,amikacin 10.8%, and finally rifampicin 8.1against gram negative bacteria isolated. As shown above imipenem has the highest sensitivity, and this is due to newly and conservative using of this antibiotic in Iraq, The percentage of antibiotic sensitivity test to gram

negative bacteria was show in Table(4-5)

Table (3). The percentage of antibiotics sensitivity and resistance to gram negative bacteria

Antibiotics	No.sensitive gram negative bacteria	No. resistance gram negative bacteria	
CIP	25(67.6%)	9(24.3%)	
AK	4(10.8%)	30(81%)	
CTX	11(29.7%)	22(59.4%)	
CN	13(35.1%(	18(48.6%)	
RA	3(8.1%)	33(89.2%)	
CRO	6(16.2%)	30(81%)	
LEV	19(51.3%(	12(32.4%)	
IMP	33(89.2%)	1(2.7%)	

Table 4.

While most effective antibiotic against gram positive bacteria isolated found ciprofloxacin 84.2%followed vancomycin 73.7%, imipenem 63.2% and amikacin 57.9%, gentamicin 47.4%levofloxacin 31.6%,,ceftriaxone 26.3% rifampicin 21.1%

,cefotaxime 21.1%.As shown above ciprofloxacin has the highest sensitivity,

Table (4). The percentage of antibiotics sensitivity and resistance to gram positive bacteria

Antibiotics	No.sensitive gram positive bacteria	No. resistance gram positive bacteria isolated
CIP	16(84.2%(	3(15.7%)
AK	11(57.9%)	6(31.6%)
CTX	4(21.1%)	12(63.2%)
CN	9(47.4%)	8(42.1%)
RA	4(21.1%)	15(78.9%)
CRO	5(26.3%)	12(63.2%)
LEV	6(31.6%)	5(26.3%)
IMP	12(63.2%)	5(26.3%)
VA	14(73.7%)	5(26.3)

Table 5.

Table (5) Pattern Resistant to most common isolate

Name bacteria	No.isolate	Pattern the resistance
E.Coli	3	CN,CRO,CTX,AK,CIP,LEV,RA
E.Coli	3	CN,CRO,CTX,AK ,LEV,RA
E.Coli	1	CN,CRO,CTX ,CIP,LEV,RA
E.Coli	1	CTX,AK ,RA
E.Coli	1	CN, ,AK ,CRO
E.Coli	1	CN,CRO, ,AK ,LEV,RA

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E.Coli	1	CTX,AK ,LEV,RA
E.Coli	1	CN, ,CTX,AK ,LEV,RA
P.mirabilis	5	CN,CRO,CTX,AK ,LEV,RA
P.mirabilis	2	CRO,CTX,AK, ,LEV,RA
P.mirabilis	2	CRO,CTX,AK ,LEV
P.mirabilis	1	CN,CRO,CTX,AK,CIP,LEV,RA
P.mirabilis	1	CN,CRO,CTX,AK,CIP,LEV,RA
P.mirabilis	1	CN,CRO,CTX,AK,CIP,LEV,RA,IMP
P.mirabilis	1	CN,CRO,CTX ,LEV,RA
P.mirabilis	1	CRO,CTX,AK, ,LEV,RA
S.aureus	2	CN,CRO,CTX,AK,CIP,LEV,RA,VA
S.aureus	1	CN,CRO,CTX,VA ,LEV,RA
S.aureus	1	CN,CRO,CTX, ,VA ,RA
S.aureus	1	CRO,CTX
S.aureus	1	CTX,AK, ,RA
S.aureus	1	CN,CRO,LEV
S.aureus	1	CN,CRO,CTX , IMP ,RA
S.aureus	1	CN,CTX,CRO
S.aureus	1	CN,CRO,CTX,IMP,LEV ,RA
S.aureus	1	CN,LEV ,CTX, ,RA
S.aureus	1	CRO,CTX,AK, ,RA,CN,VA
S.aureus	1	CN,CTX,RA,AK
S.aureus	1	CRO,LEV,CTX,RA,AK

Table 6.

DISCUSSION

After being exposed to heat, burns are initially sterile but later get colonized by both gram-positive and gram-negative bacteria. Certain populations are more vulnerable to resistant organisms colonizing them early or late. A rise in fungal colonization has been observed due to excessive use of antibiotics. Male gender, older age, lower back pain, inflammation throughout the body, delayed treatment, and pre-existing diabetesput patients at higher risk of infection. These infections require systemic antibiotics, and wound infections require immediate antibiotic treatment and excision. Fungal infections cause rare and serious disease. Infection leading to the septic system in burn patients is difficult to explain due to the hypermetabolic immune response of the injured body. Possible sources of sepsisinclude wound infections and disseminated infections. The American Burn Association Sepsis Criteria were defined in 2007 and are specifically defined for the diagnosis of sepsis and septicemia. The best way to reduce wound infections is to avoid them. Effective practices include isolation rooms, hand washing, good wound care, early vaccination and dressing, antibiotic stewardship, and nutritionalsupport[10].Inflammatory wounds inhibit healing, increase pain, scarring, risk of sepsis, and quality of life. Clinical decision making needs to be supported by evidence. The value of evidence obtained from a physical examination may be diminished if details of the infection are damaged between tests. The purpose of this review was to determine whether wound infection has been identified and whether there are differences in the criteria used to define wound infection amongclinical studies with febrile patients. [11] . Evaluation 11(2), 268, 2021Burns impose a huge economic burden on healthcare infrastructure worldwide. They are often associated with increased deaths from serious complications. The fact that infection is a common complication highlights the importance of rapid and accurate diagnosis to prevent adverse outcomes and improve patient outcomes. Here we review current trends in the treatment of burns and chronic wound infections and then reviewstudies based on fluorescence imaging, a novel bacterial detection technique. Through our five years of published research on bacterial fluorescence imaging (MolecuLight i:X device), we summarized the value of effectiveness, analyzed it, and compared it to modern medicine; clinical examination and microbiological analysis. We highlight the potential benefits of this technology, as well as's challenges and opportunities in integrating this new approach into healthcare.[12]

CONCLUSION

1- Considering the findings of the present investigation, the ensuing deductions may be made: Infection of burns and wounds with germs resistant to drugs is on the rise.

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2 This study has demonstrated that staphylococcus .aureus andEnterobacteriaceae family were the most common microorganism isolated of wound and burn infection

3- In this study the most sensitive for most gram negative bacteria is imipenem and most resistance antibiotic for most bacteria is rifampicin and sensitive for most gram positive bacteria to ciprofloxacin and resistance to rifampicin

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