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Isolation of Positive and Negative bacteria, and determination of antibiotic resistance of pathogenic bacteria isolated from wounds Infection

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Abstract :

Wound infection is a major global problem in surgery resulting in numerous complications and increased morbidity and mortality. It may occur as a primary wound infection acquired in hospital (nosocomial infection) and varies from hospital to hospital or as a secondary wound infection due to some other complication. One of the reasons for the prevalence of wound infection is the lack of standardized criteria for diagnosis. The present study aimed to isolation and identification of bacteria and evaluating pro inflammatory cytokines as Interleukin ٦. ١٢٠ samples (swabs and blood samples) were collected from patients visiting and inpatients in Baghdad hospitals (Baghdad Teaching Hospital and Ghazi Al-Hariri Hospital) from the October, 19, 2023 until the end of May, 2024. The samples included swabs collected from wounds (from the consulting department, emergency wards, men's and women's surgical wards, internal medicine wards and intensive care) with a sample from both sexes and of different ages. Sterile cotton swabs were used to take wound samples. The results of the study showed variation in the resistance of bacterial isolates to antibiotics. The negative bacterial isolates showed the highest resistance to the antibiotic Chloramphenicol, and the positive bacterial isolates showed the highest resistance to the antibiotics penicillin, trimethoprim, methicillin, doxycycline, and clindamycin, at a rate of 100%. A blood sample was also taken from healthy people with a total of 30 samples The concentration of IL-6 was estimated using the analysis kit produced by Sunlong, China, according to the steps attached to it .The results of the current study showed significant differences ($P<0.05$) between patient groups (7.84±11.4) and healthy groups (0.59±0.35)in the IL-6 rateood prognosis of wound infection.

Key words: (Wound infection, IL-6 , MRSA, Antibiotics).

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عزل البكتيريا الموجبة والسالبة وتحديد مقاومة البكتيريا المسببة للأمراض المعزولة من عدوى الجروح للمضادات الحيوية

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الملخص

تعد عدوى الجروح مشكلة عالمية كبرى في الجراحة مما يؤدي إلى العديد من المضاعفات وزيادة معدلات الإصابة بالأمراض والوفيات. قد تحدث كعدوى جرح أولية مكتسبة في المستشفى (عدوى المستشفيات) وتختلف من مستشفى إلى آخر أو كعدوى جرح ثانوية بسبب بعض المضاعفات الأخرى. أحد أسباب انتشار التهابات الجروح هو عدم وجود معايير موحدة للتشخيص. هدفت الدراسة الحالية إلى عزل وتشخيص البكتيريا وتقييم السيتوكينات المؤيدة للالتهابات مثل إنترلوكين ٦. تم جمع ١٢٠ عينة (مسحات وعينات دم) من المرضى الزائرين والمرضى المنومين في مستشفيات بغداد (مستشفى بغداد التعليمي ومستشفى غازي الحريري) للفترة من ١٩ تشرين الأول ٢٠٢٣ حتى نهاية أيار ٢٠٢٤. وتضمنت العينات مسحات تم جمعها من الجروح (من القسم الاستشاري، أقسام الطوارئ، أقسام الجراحة للرجال والنساء، أقسام الباطنة والعناية المركزة) بعينة من الجنسين ومن مختلف الاعمار. تم استخدام مسحات القطن المعقمة لأخذ عينات من الجروح. أظهرت نتائج الدراسة تبايناً في مقاومة العزلات البكتيرية للمضادات الحيوية .وأظهرت العزلات البكتيرية السلبية أعلى مقاومة للمضادات الحيوية الكلورامفينيكول، وأظهرت العزلات البكتيرية الإيجابية أعلى مقاومة للمضادات الحيوية البنسلين والتريميثوبريم والميثيسيلين والدوكسيسيكلين والكليندامايسين بنسبة ١٠٠%. كما تم أخذ عينة دم من أشخاص أصحاء بإجمالي ٣٠ عينة وتم تقدير تركيز IL-6 باستخدام عدة التحليل التي تنتجها شركة Sunlong الصينية حسب الخطوات المرفقة بها. أظهرت نتائج الدراسة الحالية فروق ذات دلالة إحصائية ($P<0.05$) بين مجموعات المرضى (٧.٨٤±١١.٤) والمجموعات السليمة (٠.٥٩±٠.٣٥) في معدل الإنترلوكين-٦ لعدوى الجرح.

الكلمات المفتاحية: (عدوى الجرح، انترلوكين ٦، MRSA، مضادات الحيوية)

INTRODUCTION

The skin is the body's biggest organ, encompassing its whole external surface. The skin comprises three layers: the epidermis, dermis, and subcutaneous layer, each with distinct anatomical features and functions. The skin structure comprises a complex network that

serves as the body's principal barrier against viruses, UV radiation, chemicals, and mechanical harm. This organ regulates temperature and the volume of water expelled into the environment [1]. Human skin constitutes a protective barrier against the external environment and serves as our primary protection against poisonous, UV, and pathogenic threats. Our skin delineates our exterior appearance, safeguards our inside structures and organs, functions as a sensory interface, and inhibits dehydration. Colonizing microorganisms are essential to the skin's barrier function, offering protection against infections, modulating immunological responses, and fortifying the epithelium [2]. The skin's microbiota serves as a barrier against the invasion, colonization, and infection by foreign and harmful microorganisms. Skin microorganisms inhabit polymicrobial communities, vie for resources, and have developed strategies to directly oppose their rivals. Various species of CoNS, including Staphylococcus hominis, synthesize antibiotics characterized by distinctive chemistry and significant inhibitory efficacy against the predominant skin pathogen *Staphylococcus aureus* [3]. Risk factors that contribute to the aggravation of hospital infections, especially in developing countries, include frequent use of antimicrobial prescriptions, physiological condition of tissues in wounds, immune status of the host, malnutrition, low socioeconomic status, in addition to the level of microbial contamination, age of patients, duration of surgical operations, and the presence of chronic diseases [4]. The majority of bacteria causing wound infections are *S. aureus*, and among the Gram-negative bacilli are *Escherichia coli, Klebsiella, Pseudomonas aeruginosa*, and *Proteus mirbila.* There is an increase in the incidence of post-operative wound infections caused by bacteria resistant to antimicrobials such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA) [5]. The local immune response in a surgical wound is mainly associated with the activation of innate immune mechanisms, where neutrophils and monocytes flock to the wound and produce cytokines and chemokines. Local production of inflammatory factors increases during surgery and wound infections, which may enhance the systemic immune response, known as the systemic inflammatory immune response (SIRS) [6] [7]. IL-6 plays an important role in the wound healing process, which in turn plays a role in protecting tissues from infection and promoting healing. IL-6 also stimulates cell proliferation (such as keratinocytes and fibroblasts) that contribute to the construction of new tissue and wound closure . The postoperative inflammatory reaction is characterized by the production of large amounts of pro-inflammatory cytokines - Interleukins (IL-1β, IL-6, and tumor necrosis factor TNF-α)[8]. Cytokines are small secreted proteins (<40 kDa), which are produced by almost every cell to regulate and influence the immune response [9]. They are proteins,

glycoproteins, or peptides that are soluble in water, and are produced by many cells, including T & Blymphocytes, macrophages, dendritic cells, epithelial cells, monocytes, and natural killer cells. They are the language of communication between the innate and acquired immune systems [9, 10].

MATERIALS AND METHODS

Sample Distribution

In this study, 120 swabs and blood samples were collected from patients admitted to Baghdad Teaching Hospital, Ghazi Al-Hariri Hospital, and from outpatient clinics with wound infections, for both sexes, and of different ages (15-75 years)**.**

Preparation of culture media

The culture media were produced following the manufacturer's instructions, sterilized in an autoclave, placed onto dishes, and incubated at 37°C for 24 hours to confirm the absence of contamination. The items were subsequently stored in the refrigerator at 4°C until utilized. The media were categorized based on their preparation methods into:

Prepared media: A- Liquid media: made following the manufacturer's specifications on the packaging, wherein the medium is dissolved in distilled water and subsequently sterilized in an autoclave at 121°C for 15 minutes under a pressure of 15 pounds. A multitude of these media comprises:

1-Nutrient broth

2-Pepton water

3-MR-VP broth

4-Brain- heart infusion broth

B- Solid media: which were prepared according to the manufacturer's instructions on the box, where the medium is dissolved in distilled water and then sterilized in an autoclave at 121°C for 15 minutes and at a pressure of 15 pounds. Ang, and left to cool at 45 °C, and poured into dishes and left to solidify, then incubated for 24 hours at 37 °C to ensure that no contamination occurs during preparation and kept at 4 °C until used, from these solid media: Nutrient agar ,EMB agar ,MacConkey agar ,Mannitol salt agar ,Muller Hinton agar

C Solid slant media: Prepared according to the manufacturer's instructions and placed in test tubes, then placed in the oven for 15 minutes at 121 °C and under a pressure of 15 pounds Ang, then left slanted until solidified from these media Simmon citrate agar and Kligler iron agar.

Bacterial isolates

From October 19, 2023, to May 22, 2024, 120 samples (swabs and blood samples) were collected from patients attending and hospitalized in Baghdad hospitals (Baghdad Teaching Hospital and Ghazi Al-Hariri Hospital). The samples comprised swabs obtained from wounds across various departments, including the consultation department, emergency wards, male and female surgery wards, internal medicine wards, and critical care, encompassing both sexes and diverse age groups. Sterile cotton swabs were utilized to collect wound specimens. A total of 30 blood samples were collected from healthy individuals.

Identification of bacterial isolates

The bacterial isolates under investigation were examined under a microscope by transferring a portion of the colony to a sterile glass slide and staining it with Gram stain. This allowed researchers to evaluate the bacteria's response to the stain, as well as the shape and aggregation of the cells.

Cultural Properties

The first diagnosis of bacterial colonies on citramide blood agar, blood agar, and MacConkey agar was used to study the cultural properties. The morphological features of the colonies, including their size, height, color, shape, and texture, as well as their ability to break down blood by producing hemolysin on the blood agar medium, were observed, and biochemical tests were conducted to identify bacterial isolates.

Antibiotic Susceptibility of Bacterial isolates

The Kirby-Bauer method [٨]. On Muller-Hinton agar was used to assess susceptibility to Ten antibiotics, as follows: Chloramphenicol 10mg, Ciprofloxacin 10mg, Ampicillin 25mg, Trimethoprim 10mg, Doxycycline 10mg, Rifampicin 5mg, Clindamycin 2mg, Azithromycin 15mg, Penicillin 10mg, Vancomycin 30mg were used for Gram-positive bacterial isolates, Ten antibiotics, as follows: Chloramphenicol 10mg, Ciprofloxacin 10

mg, Ampicillin 25 mg, Trimethoprim 10mg, Piperacillin 100mg, Amikacin 10 mg, Gentamycin 10 mg, Meropenem 10 mg, Imipenem 10 mg, Cefotaxime 30 mg for Gram-negative isolates. The results were recorded, and the resistance and susceptible isolates were determined by measuring the diameter of the inhibition zone according to [11].

Estimation of the concentration of interleukin IL-6

The Chinese-made Sunlong Company analysis kit and the accompanying instructions were used to quantify the concentration of IL-6 using ELASA kits.

Statistical analysis

All results of the current study were subjected to statistical analysis. SPSS version [12] was used for this purpose. The Chi-square test and the one-way and multiple analysis of variance test were applied according to the least significant difference (LSD). The confidence interval was adopted equal to 95% and the probability level value was less than 0.05.

RESULTS AND DISCUSSION

Distribution of samples by age group

The samples taken from patients with wound infections were distributed according to age groups, where the age group (15-25) years was (16.7%), while the age group (26-35) years was (11.6%), while the age group (36-45) years was (15.8%) and the age group (46-55) was (25.9%), while the percentage was (17.5%) for the age group (56-65) and the age group (66-75) was (12.5%) as shown in Table (1**).**

Table (1): Distribution of isolated samples according to age groups

Number and percentage	Age groups
(%١٦.٧)٢٠	٢٥-١٥
(%١١.٦)١٤	٣٥-٢٦
(%١٥.٨)١٩	٤٥-٣٦
(%٢٥.٩)٣١	٥٥-٤٦
(%١٧.٥)٢١	٦٥-٥٦
(%١٢.٥)١٥	٧٥-٦٦
(%١٠٠)١٢٠	total

The distribution of samples according to age groups showed different percentages, as the age group (46-55 years) had the highest percentage among patients with wound infections, 25.9%. This is attributed to the physical, sensory, and cognitive changes associated with aging and association with environments that are not prepared according to the needs of the elderly, and the lowest percentage was for the age group (26-35 years) by 11.6%. The results of our study agreed with the findings of the researcher [13]**,** as the rates of wound infection were highest in the age group of 40-55 years, at a rate of 30%, and the lowest in the age group of 15-25 years, at a rate of 20%. Our study dis-agree with the study of [14]. The highest percentage was 43.7% for the group (26-40 years) and the lowest percentage was for the age group (55-80 years) at 12.5%.

Distribution of samples by gender

The collected samples were distributed by gender, where the results showed that 65 (54.2%) of the collected samples were males, while 55 samples were females 45.8%). The reason for the higher levels of males may be due to behaviors associated with risk-taking and occupational risks, such as working in high places or using alcohol and drugs at a higher rate compared to females. **As shown in Figure 1.**

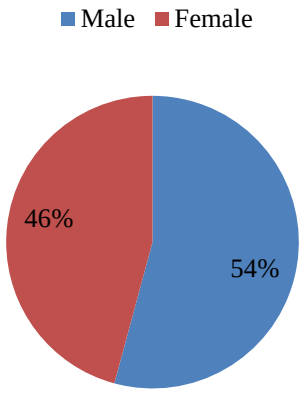

Figure (١): Distribution of samples by gender

This is consistent with a study conducted in northern Iraq/Erbil Governorate, where the number of samples taken from males with wound infections (65.5%) was higher than that of females (35.5%) [14]. The present study disagreement with a study conducted in Baghdad, where the number of samples taken from females was 52.4%, 47.6% more than

from males [15], and it also does not agree with the study (Al-Mousawi et al., 2022)[16], where the percentage of samples taken from females was 72%. Much higher than males, 28%. Males are generally considered more susceptible to infection than females due to differences in immune responses as well as variation in genes linked to the sex chromosome [17]**.**

Diagnosis of samples

The samples taken from the patients were cultured on different culture media. In order to diagnose the bacterial isolates, a set of tests were performed, where similarities between the culture characteristics, microscopic examination and biochemical tests helped in identifying the bacterial isolates that matched those described previously. The diagnosis was confirmed using the VITEK 2 device. The results showed that 96 samples showed positive growth on the media used, at a rate of 80% of the total samples taken, while the samples that did not show growth on MacConkey medium and blood agar were 24 samples at a rate of 20% of the total smears.

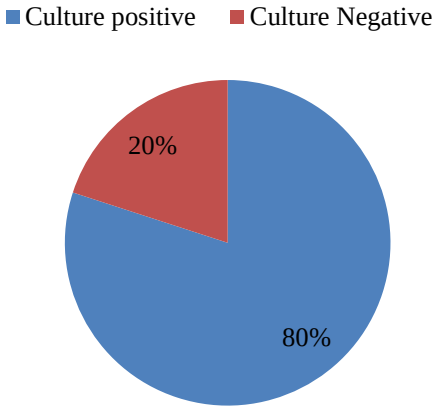

Figure 2. Culture results of swabs taken from wound infections

Our results are consistent with a study conducted in Karbala, where the percentage of positive isolates was 75.8% and the percentage of negative isolates was 24.2% [18]. It is also consistent with a study conducted in Egypt, where the percentage of positive culture isolates was (83%) from wound infections [19]**.** The results of the current study were close to the results of (Abdulqader and Saadi 2019)[14]**,** where the percentage of positive samples was 64.5% of the total samples taken from wound infections, and also consistent

with (Ahmad et al., 2021)[13]**,** where the percentage of positive samples was 54.5% of the total samples.

The reason may be due to a difference between researchers and the taking of antibiotics by the patient, which leads to negative culture results. The difference may be attributed to the causes of bacterial infection and the source of infection in the wounds from which the samples were obtained. Another reason that may explain this difference is the adopted protocol for infection control and antibiotic prophylaxis which may play a crucial role in bacterial growth. Also, the growth conditions of some types of bacteria may be responsible for their inability to grow [20]

Bacterial identification

A total of 96 bacterial isolates were obtained from wound infections. After collecting the samples, all samples were cultured on blood agar, MacConkey agar, mannitol salt agar and other used culture media and incubated aerobically at 37°C for 24 hours. Different types of Gram-positive and Gram-negative bacterial isolates were obtained, the highest percentage of Gram-negative bacteria was *P. aeruginosa* (33.3%), followed by *K. pneumonia* (26%), *E. coli* (15.6%) and *P. Mirabilis* (9.3%), while Gram-positive bacteria were less isolated, as *S. aureus* (8.6%) was isolated, followed by *S. saprophyticus* (5.2%) and *S. epidermidis* (2%) as shown in the Table (2**).**

Table (2): Types of bacteria isolated from wound infections

Bacterial isolates	**No.**	**Percentage**
Staphylococcus aureus	8	%٨.٦
Staphylococcus epidermidis	2	%٢
Staphylococcus saprophyticus	5	%٥.٢
Escherichia coli	15	%١٥.٦
Pseudomonas aeruginosa	32	%٣٣.٣
Klebsiella pneumonia	25	%٢٦
Proteus mirabilis	9	%٩.٣
total	96	%١٠٠

The results of the current study, shown in **Figure (),** showed the resistance of the isolated bacteria to the antibiotics used in the study, The *S. aureus* were resistant to Penicillin by 100%. The bacteria were also resistant to Methicillin and Trimethoprim by

(75%) to each of them and by (62.5%) to Doxycycline. The *S. aureus* showed sensitivity to Azithromycin by 87.5% and to Vancomycin. Vancomycin and Clindamycin 75% For each of them and for Ampicillin and Chloramphenicol at 62.5% each. The *Staphylococcus epidermidis* also showed high resistance to chloramphenicol methicillin, clindamycin, doxycycline, and trimethoprim by 100%, moderate resistance by 50% to ciprofloxacin, penicillin, and ampicillin, and showed high sensitivity of 100% to vancomycin and azithromycin. While the bacteria *S. Saprophyticus* showed high resistance by 100% to penicillin and azithromycin, by 80% to methicillin, doxycycline, and Trimethoprim, and by 60% to Clindamycin and Chloramphenicol. It also showed sensitivity by 60% to ciprofloxacin, ampicillin, and vancomycin.

The study found that the bacteria *Staphylococcus aureus*, was 100% resistant to penicillin, and this was confirmed by many studies, including the study of the researcher [21] and the researcher [22] of the pathogenic isolates, and the study of the researcher [23]. The rate of resistance to penicillin in all studies was 100%. The resistance of Staphylococcus aureus bacteria to penicillin antibiotics results from several mechanisms, including their ability to produce lactamase enzymes that degrade the β-lactam ring, in addition to the mechanism of changing the penicillin binding proteins present within the group of penicillins, thus leading to the inactivation of the antibiotic [24].

Microlide antibiotics work by inhibiting protein synthesis in the bacterial cell by binding to the ribosomal subunit 50. This binding process prevents the activity of the Peptidyl transferase enzyme, and interferes with the transfer of amino acids during the translation and assembly of proteins. The semi-synthetic macrolide Azithromycin is also used clinically to treat infections caused by other bacterial species Staphylococci commensal in the body will be exposed to macrolides, and this may contribute to resistance to Erythromycin and azithromycin from the group of macrolides that commonly occurs in clinical isolates [25].

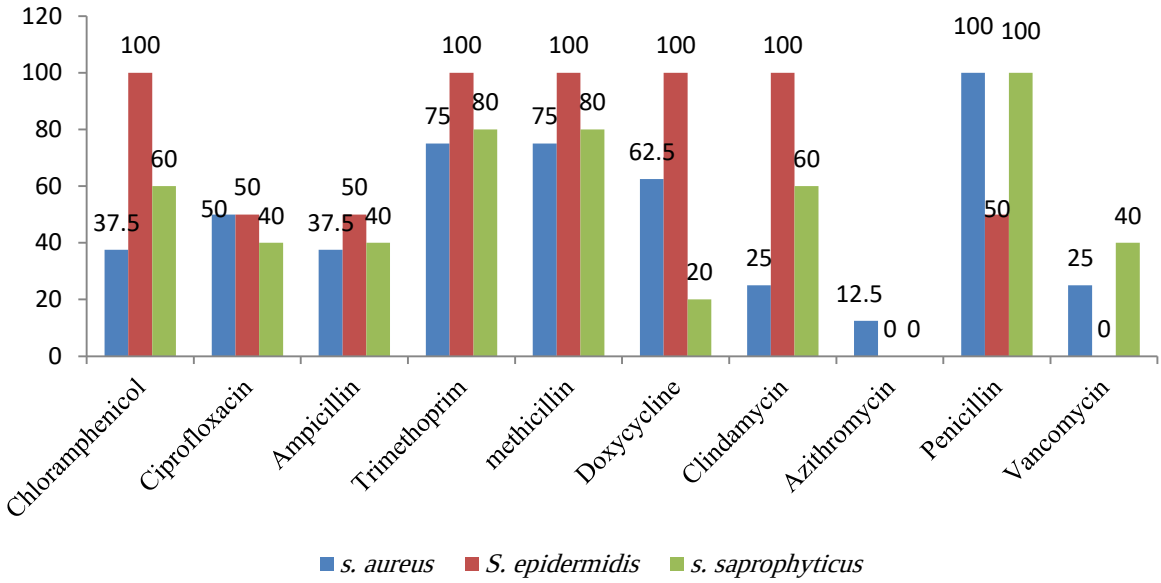

Figure : Antibiotic resistance of gram-positive bacteria

The results of the current study, as shown in Figure (), showed that the bacteria ***Pseudomonas aeruginosa*** was sensitive to the antibiotic meropenem at a rate of 84.4%, imipenem and ampicillin at a rate of 75%, amikacin at a rate of 71.9%, and cephotaxime, piperacillin, ciprofloxacin, and trimethoprim at a rate of (69%, 62.5%, 59%, 53.2%), respectively.

Escherichia coli bacteria, were resistant to chloramphenicol at a rate of 73.3%, piperacillin at a rate of 66.6%, and both amikacin and cephotaxime at a rate of 60%, while they showed high sensitivity at a rate of 86.6% to imipenem, meropenem at a rate of 80%, ampicillin at a rate of 73.4%, and a rate of 66.6% to each of ciprofloxacin and gentamycin. and 53.4% for Trimethoprim.

The bacteria were ***Klebsiella pneumoniae***. pneumonia was resistant to chloramphenicol at a rate of 72%, and to amikacin and gentamycin at rates of 64% and 56%, respectively, while it showed sensitivity to the antibiotics imipenem at a rate of 88%, meropenem at a rate of 75%, and cephotaxime, ciprofloxacin, piperacillin, and ampicillin at rates of 68%, 64%, 60%, and 56%, respectively. ***P. mirabilis*** showed high resistance to gentamycin at a rate of 88.8%, trimethoprim at a rate of 77.8%, and chloramphenicol at a rate of 66.6%, while it showed sensitivity to cephotaxime, meropenem, and amikacin at a rate of 77.8%. It

was also sensitive to the antibiotics ciprofloxacin and imipenem at a rate of 66.6% each, and was sensitive to the antibiotics ampicillin and amikacin at a rate of 77.8%. 55.6%.

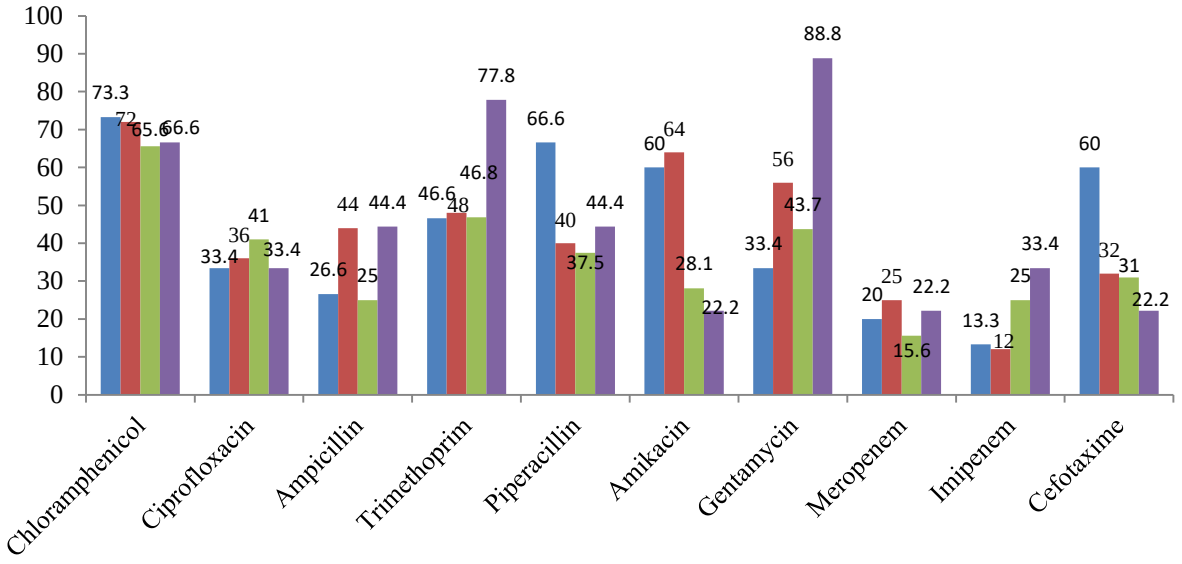

Figure : Antibiotic resistance of gram-Negative bacteria

The reason for the resistance of some isolates may be due to their possession of modified enzymes, anti-aminoglycosides (AMES), and sometimes bacterial resistance is due to a mutation in ribosomes or their possession of a permeability barrier [26] .

Immunological study results

Study of Interleukin-6values in patient and healthy groups

The results of the current study showed significant differences ($P<0.05$) between patient groups (7.84±11.4) and healthy groups (0.59±0.35) in the IL-6 rate as shown in Figure (3).

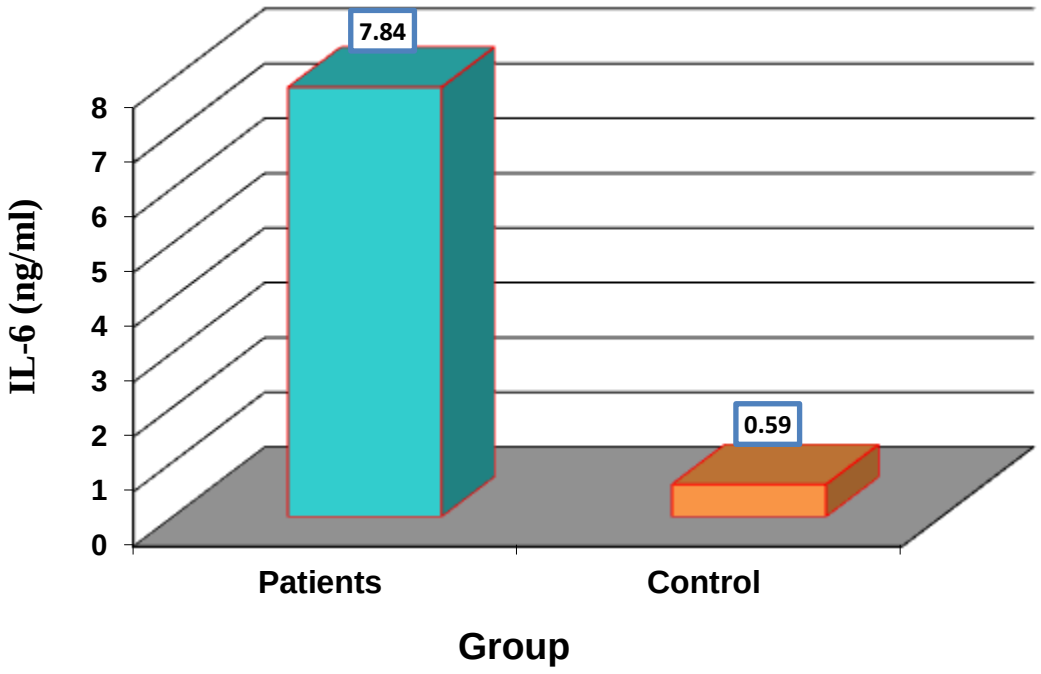

Figure 3. Levels of IL-6 (ng/ml) between patient and healthy groups

Wound healing depends on three stages: inflammation, proliferation, and remodeling, and IL-6 plays a major role in all of these stages by promoting collagen deposition and blood vessel generation. Since 64.58% of patients had a significant increase in IL-6 levels, this may be due to severe tissue damage with a high rate of bacterial contamination, which showed an increase in the numbers of white blood cells and neutrophils, causing an increase in IL-6 levels. Seven microbial [27]. The study by [28]showed that after surgery, macrophages produced more IL-6 in wound infection, a result that aligns with [29]. A study showed that the level of IL-6 was elevated in infected patients compared with control. This suggests that elevated interleukin-6 level is caused by inflammation, infection, and autoimmune disorders [30]. Farhan *et al*., 2021 revealed that the level of IL-6 in the serum of patients with chronic wound infection increased significantly in the first days of hospitalization and decreased after standard treatment, but did not reach healthy levels [31]. These cytokines govern the mechanisms of the immune system, as they are involved in all facets of innate and adaptive immune responses, encompassing cell growth, differentiation, and inflammation, along with their extensive role in other biological processes such as activating specific cells and inducing particular functional alterations.

Cytokines are synthesized by several cell types upon antigenic stimulation and serve multiple roles in the human immune system, functioning as communicative mediators among all immune cells.

CONCLUSION

This study concluded increase pro-inflammatory cytokines IL-6 that suggested good prognosis of wound infection. In addition bacterial infection is more prevalence in wound infection.

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