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Microbiological and Immunological Investigations of Postoperative Wound Infections in Mosul Hospitals

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Abstract

Hospital acquired infections or nosocomial infections are the major cause of morbidity and mortality around the world. These are major public health problems worldwide, but particularly in the developing countries. The amies of this study was to analyses the frequency of the microorganisms taken from the surgical wounds and to examine the susceptibility of antimicrobial agents for some isolates and to study the relation between some of immunological parameters of the patient and wound infection. Wound swab was examined by different conventional method using MacConkey and blood agar, antimicrobial susceptibility was done using Kirby-Bauer disc diffusion method. Minividas used to demonstrate the concentrations of IgM through different period of the infection for the study the relation between the immunological factors and wound infections. A total of 146 wound samples were collected from patients and examined. The study showed male 89(60.96) and female 57(39.04), wound infection was highest among the age group (40-49) years followed by the age (30-39) years. The lowest wound infection was in the age of (0-9) years. The study also showed the highest incidence of the infection was in Staphylococcus aureus constitute (38.16%) from the infections followed by pseudomonas aeruginosa (25%), while the lowest incidence of the infection was in klebsiella (6.58 %). The study also showed between the kind of surgery the rate was higher in plastic surgery (8.90%) followed by gynecology surgery (7.53%), the lowest incidence of infection rate was seen after urinary tract surgery (2.05%). The study showed resistant to almost of the antibiotics, levofloxacin was the 100 % resistant to almost all the microbial isolates. The immunological changes in the samples also observed in this study, the majority of the sample showed clinically significant at the 10 day of the infection, while preoperative there was no any significantly different in the level of IgA.

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التحريات الميكروبيولوجية والمناعية لالتهابات الجروح بعد العمليات الجراحية في مستشفيات الموصل

حماد شهاب أحمد ويثرب ما شاء الله حامد

الخلاصة

تُعد حالات العدوى المكتسبة من المستشفيات (أو العدوى المشفوية) سببًا رئيسيًا للمراضة والوفيات في جميع أنحاء العالم. وتمثل هذه الحالات مشكلات صحية عامة كبرى على مستوى العالم، ولا سيما في الدول النامية. هدفت هذه الدراسة إلى تحليل تواتر (تكرار) الكائنات الحية الدقيقة المأخوذة من الجروح الجراحية، وفحص مدى حساسية بعض العزلات للمضادات الميكروبية، فضلاً عن دراسة العلاقة بين بعض المعايير المناعية للمريض وعدوى الجروح.

تم فحص مسحات الجروح بطرق تقليدية مختلفة باستخدام آغار ماكونكي والآغار المغذي بالدم(Blood agar) ، كما تم إجراء اختبار الحساسية للمضادات الميكروبية باستخدام طريقة انتشار القرص لكيربي-باور .(Kirby-Bauer) واستُخدم جهاز (Minividas) لإيضاح تركيزات الأجسام المضادة من نوع (IgM) خلال فترات مختلفة من العدوى، وذلك لدراسة العلاقة بين العوامل المناعية والتهابات الجروح. تضمنت الدراسة جمع وفحص ما مجموعه 146 عينة جرح من المرضى؛ حيث أظهرت النتائج إصابة 89 من الذكور بنسبة (60.96%) و57 من الإناث بنسبة (39.04%). وكانت عدوى الجروح في أعلى مستوياتها بين الفئة العمرية (40-49) عامًا، تليها الفئة العمرية (30-39) عامًا، في حين سُجلت أدنى نسبة لعدوى الجروح في الفئة العمرية (0-9) سنوات.كما أظهرت الدراسة أن أعلى معدل للإصابة بالعدوى كان لبكتيريا المكورات العنقودية الذهبية (*Staphylococcus aureus*) حيث شكلت نسبة (38.16%) من الإصابات، تليها بكتيريا الزوائف الزنجارية (*Pseudomonas aeruginosa*) بنسبة (25%)، في حين سجلت بكتيريا الكلبسيلة (*Klebsiella*) أدنى معدل إصابة بنسبة (6.58.(%وفيما يتعلق بنوع الجراحة، أظهرت الدراسة أن المعدل كان أعلى في جراحات التجميل بنسبة (8.90%)، تليها جراحات أمراض النساء بنسبة (7.53%)، بينما سُجلت أدنى نسبة للإصابة بالعدوى بعد جراحات المسالك البولية بنسبة (2.05%). وأظهرت الدراسة مقاومة لمعظم المضادات الحيوية، حيث بلغت نسبة مقاومة مادة الليفوفلوكساسين (Levofloxacin) نسبة 100% تقريبًا لجميع العزلات الميكروبية.

كما لُوحظت التغيرات المناعية في العينات خلال هذه الدراسة، حيث أظهرت غالبية العينات دلالة سريرية واضحة في اليوم العاشر من الإصابة، في حين لم يكن هناك أي اختلاف معنوي (ملحوظ) في مستوى الأجسام المضادة من نوع (IgA) قبل إجراء العمليات الجراحية.

1. Introduction

wound infections are considered one of the major problems facing hospital workers and causing morbidity and mortality for patients undergoing a surgical operations and result in prolongation of hospitalization increment the cost of treatment and the loss of working days. wound infections can be defined as invasion of microorganisms to the tissues of local and internal organs host defense. The prevalence of wound infections following laparotomies may be as high as 20-30% globally and up to 50% in low-and middle-income nations because of the inconsistency of perioperative practices and infection control. Reducing surgical site infections in low-income and middle-income countries (Ademuyiwa et al., 2021). Post-operative wound infections can be caused by Different types of microorganisms can cause inflammation in the site of the surgical operations , it can come from the patient body (endogenous flora) or it can come from the hospital staff ,contaminated needles and instruments(exogenous) .in minor wound infections there is pus discharge without associated excessive discomfort or systemic signs(Ban et al., 2017) .its very important and useful to explore the source of these infections in order to detecting time and removing the causative agents .In order to minimize the postoperative wound infections its very important to safe the environment by controlling the main four sources which are personal ,equipment ,the environment and patients risk factors(Sebola, 2023).

wound infections considered the commonest and the most disruptive condition of wound healing, its reported that surgical site infections(ssi) is increase the risk factors and associated with 75 % of mortality(Awad, 2012). Though all the individuals risk factors of ssi are well indexed, there is little comparative study clarifying the interaction effect between the urgency of the surgical operation and the risk factors on the rate of the hospital-based infection. Among the factors increasing the risk of wound infections are also those associated with the patient (radiation therapy, immunosuppressive therapy, nutritional disorders and disease resulting in immunosuppression), the duration of the operation, blood transfusions during the postoperative period, overuse of electrocauterization, deviations from the surgical technique and the use of irrelevant prosthetic materials. In addition, supportive treatment administered to the patient in the presence of wound infection (nutritional support, immunotherapy, administration of antibiotics) and the actual immunological parameters of the patient have an effect it has been studied that emergency cases have more lesions during contamination, emphasizing the necessity of rigorous wound care and aseptic measures. Complete eradication of wound infection is not feasible, a reduction in the infection rate to minimal could have significant benefits in terms of both patient comfort and medical resources used. A successful surveillance program includes the use of epidemiologically-sound infection definitions and effective surveillance methods, stratification of SSI rates according to risk factors associated with SSI development, and data feedback(Haley et al., 1985). This study carried out in different hospitals in Mosul city .146 wound and pus samples were collected from patients underwent surgical operations and diagnosed using different methods to identify the types of microorganism and the susceptibility of antibiotics as well the effect of immunological factors on the wound infection. Immunoglobulin A (IgA) plays an important protective role in wound infections, especially in preventing microbial colonization and supporting the early immune defense at exposed tissues. Its mainly protects mucosal and epithelial surfaces. When a wound occurs, microorganisms can enter the tissue, and IgA helps limit their ability to infect that largely dominates mucosal humoral immunity. It is generally accepted that SIgA Abs in mucosal and glandular secretions protect mucosal surfaces by directly cross-linking environmental microorganisms or macromolecules, thus preventing their contact with the surface of epithelial cells and hence facilitating their elimination by peristalsis or mucociliary movement, a mechanism called immune exclusion (Cerutti & Rescigno, 2008).

2. Materials and Methods

This study conducted at different hospitals in Mosul city. A total of 146 samples were collected and examined using different methods at the laboratory. Swabs are collected form suspected cases by using sterile cotton swabs. Two swabs were taken, one for gram stain and the second for inoculation on plates of MacConkey agar and blood agar. The blood agar (containing blood agar base and 5% human blood agar) was used for the growth of fastidious microorganisms especially gram-positive bacteria. MacConkey agar was used for the selective isolation of gram-negative bacteria. The plates were incubated 24 to 48 hrs at 37 c in bacteriological incubators. Antibacterial susceptibility testing was prepared using Kirby-Bauer disc diffusion method(Lalitha, 2004). With the aid of a pair of forceps sterilized by flaming and cooled, (7) antibiotic discs were placed on the surface of each of the inoculated plates. The discs were placed equidistant from each other and firmly pressed on to the surface of the plate to ensure adequate contact with the agar. The plates were placed in the in the incubator at 370C overnight. IgA immunoglobulin that serves as a fist line of defense to repel pathogenic microorganisms and determined as immunological factor in the first day and the 10 th day of the infection. Minividas was used as a technique for the determination of immunoglobulin. Blood samples were collected from patients and centrifuged to obtain the serum of the patients and the concentration is gained for IgA in the first and 10 day of the infection. In this study, 24 wound samples were analyzed to investigate the role of Immunoglobulin A (IgA) in wound infections, and the methodology and results were demonstrated through minivoids.

3. Results

This study conducted in Mosul city to evaluate the incidence and the effectiveness of microorganisms on the patients underwent different surgical operations, as well to study the influence of immunological factors on wound infections. A total of 146 different samples were collected from different lab centers and hospitals in Mosul city. The subject included 89 (60.96%) males and 57 (39.04%) females. Patients age ranged from 2 weeks to 78 years, as shown in Table1 and Fig.1.

Table1: Represents the Total Number of The Infected Cases Based on Age and Sex

Age	Number of infected cases	Number of males	%	Number of females	%
0-9	3	2	2.25	1	1.75
10-19	11	7	7.87	4	7.02
20-29	18	13	14.61	5	8.77
30-39	33	14	15.73	19	33.33
40-49	42	27	30.34	14	24.56
50-59	16	12	13.48	4	7.02
60-69	15	8	8.99	7	12.28
70-79	8	6	6.74	3	5.26
Total	146	89		57	

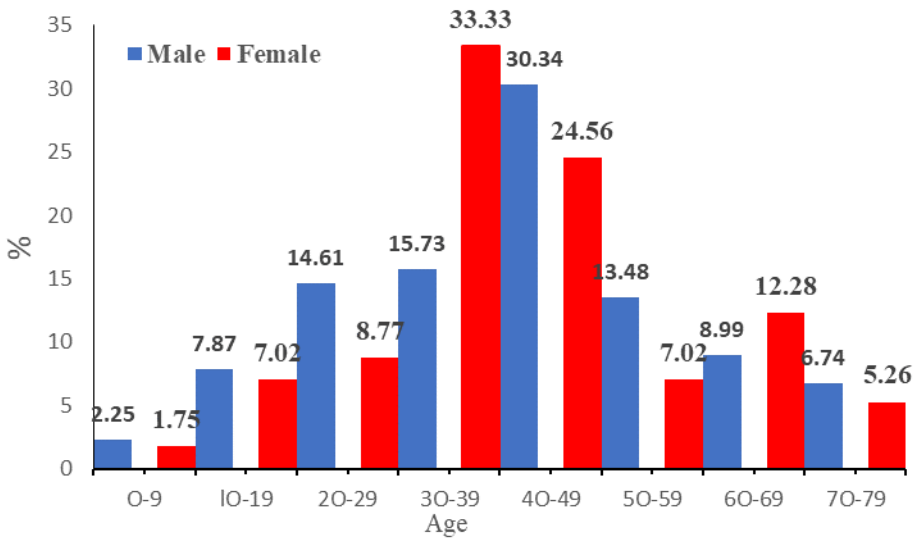

Figure1: Represents the Distribution of The Infected Cases by Age and Sexes, Where Blue Color Refers to Males and Red Color Refers to Males

In this study various bacterial isolates obtained and almost of them as a normal flora. Pseudomonas aeruginosa (19 cases) 25%, Escherichia coli (12 cases) 16%, Streptococcus spp. (8 cases)10%, and proteus Proteus mirabilis (3 cases)4 %, staphylococcus (29 cases)38%, klebsiella (5 cases)7%, as indicated in Table2 and Fig.2.

Table2: Frequency Distribution of Bacterial Species Isolated from Clinical Specimens

Type of organism	Number isolated	Percentage %
Staphylococcus aureus	29	38%
Escherichia coli	12	16%
Pseudomonas aeruginosa	19	25%
Proteus mirabilis	3	4%
Klebsiella spp.	5	7%
Streptococcus spp.	8	10%

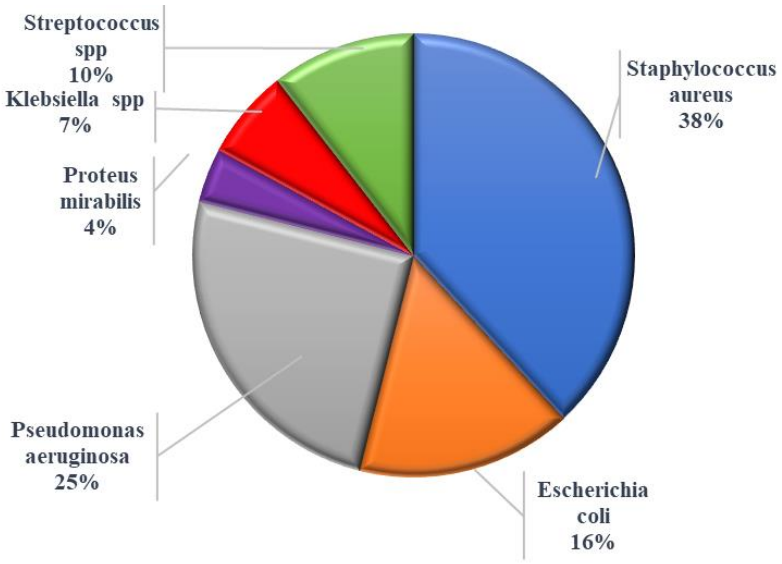

Figure2: Percentage Distribution of Bacterial Isolates Identified in Clinical Samples

In this study 39 cases (26. 71) were diagnosed and distributed in various types of surgery including plastic surgery (8.90%) and gynecology (.7.53). The postoperative wound infection rate was very low in orthopedic surgery (2.74%), see Table3 and Fig.3.

Table3: Percentage of Total Infected Cases (n = 39)

Type of Surgery	Total Number of Cases	Number of Infected Cases	Percentage of Infected Cases (%)
Gynecology surgery	43	11	28.21
Plastic surgery	22	13	33.33
Orthopedic surgery	39	4	10.26
Digestive tract surgery	22	8	20.51
Urinary tract surgery	20	3	7.69
Total	**146**	**39**	**100.00**

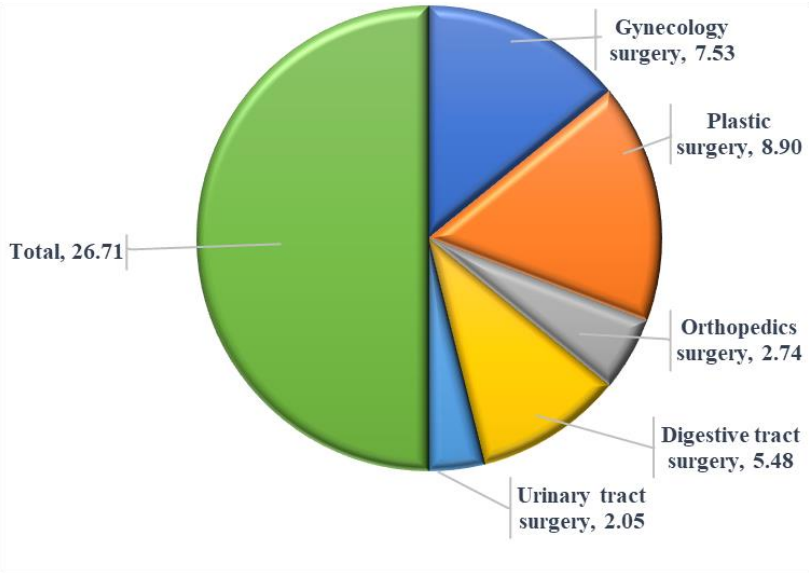

Figure3: Distribution of Surgical Site Infection Cases According to Type of Surgery

In this table the result varied in the susceptibility to all the antibiotics and the majority showed multiple resistance to antibiotics. Staphylococcus and streptococcus were 100% resistant to levofloxacin. Klebsiella and proteus also showed 100% resistant to levofloxacin, as shown in Table4.

Table4: Antimicrobial susceptibility profiles of bacterial isolates against the tested antibiotics

Bacterial isolate	n	Amoxicillin	Gentamicin	Nalidixic acid	Augmentin	Chloramphenicol	Levofloxacin	Tetracycline
Staphylococcus aureus	29	3 (10.3) / 23 (79.3)	12 (41.4) / 17 (58.6)	0 (0.0) / 29 (100)	0 (0.0) / 29 (100)	10 (34.5) / 19 (65.5)	28 (96.6) / 0 (0.0)	8 (27.6) / 21 (72.4)
Escherichia coli	12	3 (25.0) / 9 (75.0)	5 (41.7) / 7 (58.3)	2 (16.7) / 10 (83.3)	0 (0.0) / 12 (100)	0 (0.0) / 12 (100)	10 (83.3) / 2 (16.7)	4 (33.3) / 8 (66.7)
Pseudomonas aeruginosa	19	2 (10.5) / 17 (89.5)	9 (47.4) / 10 (52.6)	3 (15.8) / 16 (84.2)	5 (26.3) / 14 (73.7)	2 (10.5) / 17 (89.5)	16 (84.2) / 3 (15.8)	3 (15.8) / 16 (84.2)
Proteus mirabilis	3	0 (0.0) / 3 (100)	3 (100) / 0 (0.0)	1 (33.3) / 2 (66.7)	2 (66.7) / 1 (33.3)	0 (0.0) / 3 (100)	3 (100) / 0 (0.0)	0 (0.0) / 3 (100)
***Klebsiella* spp.**	5	1 (20.0) / 4 (80.0)	2 (40.0) / 3 (60.0)	5 (100) / 0 (0.0)	1 (20.0) / 4 (80.0)	1 (20.0) / 4 (80.0)	5 (100) / 0 (0.0)	0 (0.0) / 5 (100)
***Streptococcus* spp.**	8	0 (0.0) / 8 (100)	7 (87.5) / 1 (12.5)	5 (62.5) / 3 (37.5)	0 (0.0) / 8 (100)	2 (25.0) / 6 (75.0)	8 (100) / 0 (0.0)	3 (37.5) / 5 (62.5)

Data are presented as n (%). Values represent the number and percentage of susceptible/resistant isolates for each bacterial species. Percentages were calculated using the total number of isolates for each species.
Abbreviations: Amx, amoxicillin; Gen, gentamicin; Nal, nalidixic acid; Aug, amoxicillin/clavulanic acid; Chl, chloramphenicol; Lvx, levofloxacin; Tet, tetracycline.

In this table, 10 samples have been demonstrated for the level of IgA, the majority of the sample showed clinically significant at the 10 day of the infection, while preoperative there was no any significantly different in the level of IgA, as shown in Table5.

Table5: Changes in Serum Immunoglobulin A (IgA) Levels Between Day 1 and Day 10 Following

Subject	**Serum IgA at Day 1 (mg/dL)**	**Serum IgA at Day 10 (mg/dL)**
1	78	81
2	123	125
3	96	112
4	55	57
5	222	196
6	180	192
7	33	41
8	367	412
9	116	190
10	290	302
Comparison	**Mean ± SD (mg/dL)**	**P-value**
Day 1	156.0 ± 108.1	0.1183
Day 10	170.8 ± 115.6	
Reference range: Serum IgA = 70–400 mg/dL.		

4. Discussion

Despite the advance techniques in the surgical operations , and better understanding to the sources of the pathogens, postoperative wound infections continue to be the major source of morbidity and mortality of the patients undergoing surgical procedures , and also view its financial resources and increased the requirements of effective management of the health system(Bowler et al., 2001). In the present study 148 infected samples were processed and diagnosed in different hospitals in Mosul city. The result showed that the incidence of wound infection is higher in male 89(60.96) than female 57(39.04). Staphylococcus aureus has been described as the most common pathogen isolated from postoperative wound infection. In our study a total of 29(38.16%) isolated cases from different site infections followed by pseudomonas aeruginosa 19(25%) , while the less incidence of infection was klebsiella spp. 5 (6.58 %) . Staphylococci's aureus strains accounted for 38.16% of the total bacterial isolates recovered. This value is similar to the 25% value reported by(Zahradnický, 1985). This result is similar to that of Serra et al., (2015) who reported that S. aureus was the most common organism isolated from wounds. MONTOFIORE & OKUBADOJO, (1970) reported comparable value of 25.3% in a large study done by Bandy et al., (2022) over a period of 6 years, S. aureus isolates constituted (28.2%) of total bacterial isolates in 676 surgery patients with symptoms indicative of wound infections. The recovery of 25.0% Pseudomonas aeruginosa in this study is not unusual. This organism remains a serious cause of infection and septic mortality in burn patients, particularly when nosocomial acquired(Tredget et al., 2004). The incidence of wound infection was highest among the age group (40-49) years with 41 cases followed by the age (30-39) years with 33 cases. The lowest wound infection was in the age of (0-9) years with 3 cases, as compared to 7.4% in patients having age less than 26 years. An odd ratio for surgical wound infection is 1.2 for every 10 years of age(Lizán-García et al., 1997) . It can be due to multiple factors like low healing rate, malnutrition, malabsorption, increased catabolic processes and low immunity(Mainous & Deitch, 1994). Amongst different kinds of surgery ,higher infection rate

were noted after plastic surgery 13(8.90%) followed by gynecology surgery 11(7.53%) ,the lowest incidence of infection rate were seen after urinary tract surgery 3(2.05%). Obesity is known to be a well-established risk factor for postoperative wound infection. In this study a body mass index of more than 40kg/m2 was associated with a higher rate of postoperative wound infection. Obesity contributed as strongly as the surgical procedure category to a patient's likelihood of acquiring a surgical site infection(Barber et al., 1995). Similarly, there was statistically high infection rate in emergency surgeries as compared to the elective ones. High rate of infection was observed in plastic surgery (8.90%) and gynecology (.7.53), while very low rate was observed in orthopedic surgery(2.74%).Similar observations have been made by others (Agarwal et al., 1984) . In emergency surgeries, a combination of various factors like physical condition of the patient, operations on the potentially contaminated sites i.e., intestinal perforations, obstructions, strangulated hernia, short time interval for preparing the case for operation and lacking of rigorous aseptic measures due to urgency may predispose the individual to the infection. The rate of postoperative wound infection in diabetic patients was 9.23% in this study, which is significantly higher, though sugar levels were controlled before operation by giving insulin. The increased susceptibility to infection in diabetics is an established risk factor(Johnston, 1994). Anemia itself is not an established factor for postoperative wound infection. However, a higher incidence of postoperative wound infection was noted with initial low hemoglobin levels. It can be due to the effect of blood transfusions, which were given preoperatively to bring the hemoglobin level up to 10 gm/dl. Many people in the state may be practicing self-medication, thereby leading to inappropriate use of antibiotics. This would encourage resistance of microbes to the drugs(Mulu et al., 2006). stated that the resistance might be a reflection of inappropriate use of antimicrobials, lack of diagnostic laboratory services or unavailability of guideline regarding the selection of drugs. attributed it to the abuse and overuse of the antibiotics in the environment. In this study 29 of staphylococcus isolates were resistant to Nalidixic acid (48.33%), Augmentin (42.65%) , Tetracycline (36.21%), Chloramphenicol (31.15%), Amoxicillin (35.94%), Gentamicin (44.74%).These result suggest that Staphylococcus aureus are less susceptible to traditionally employed antimicrobials, such as tetracycline, erythromycin and chloramphenicol which may be epidemiologically important in treating infections caused by this organism. This is comparable to the work of (Mulu et al., 2006). Paul which reported that Staphylococcus aureus showed high level drug resistance against penicillin (59%). Tetracycline (57%). Ampicillin (55%) and cotrimoxazole (35%). Gedikoglu (1986), evaluating the bacteriology of wound infections, observed that most of the Staphylococcus aureus strains isolated from wounds often demonstrate a high rate of resistance to most of the antimicrobials tested in Kenya. Other investigation corroborates these findings(Montefiore et al., 1989). The gram negative organisms, like gram positive organisms, displayed high resistance to the different antimicrobial agents used in this study. Pseudomonas aeruginosa isolates were the most predominant gram negative isolates were resistant to Amoxicillin (26.56%), Gentamicin (26.32%), Naldixic acid (26.27%), Augmentin (20.59%), choropgenicol (27.87%),Tetracycline (27.59%) . Such observation has also been reported by other researchers (Ambreetha et al., 2024). However, this is contrary to the reports by , who reported no resistance to gentamycin in their study of puncture wounds of the foot in children(Russell & Gould, 1988). The mechanism of resistance of Pseudomonas aeruginosa to aminoglycosides and β-lactams is antibiotic inactivation by certain enzymes. Richmond & Sykes, (1973) reported the possession of these enzymes by Pseudomonas aeruginosa. The outer membrane of this organism may also act as a barrier limiting the entry of antimicrobial agents (Shittu et al., 2003).

Conclusion

Although advanced sterilization techniques and modern surgical procedures and prophylactic use of good antibiotics, postoperative wound infections remain a major contributory factor of patient's morbidity. This study showed that a variety of bacteria, including opportunistic bacteria are found in different wounds and more than one type of bacteria species infected the wound. The majority of bacterial isolates were resistant to almost all the antibiotics used. The study showed a high incidence of multiple antibiotic resistance among both gram positive and gram-negative bacterial species. This, of course, may cause prolong wound healing and it may increase the cost of therapy to the patients, this can be achieved by taking proper measures to improve our operation theatres and wards environments, and methods of sterilization. This study suggests better clinical evaluation of antimicrobial therapy which would lead to the practice of rational use of drugs. Further investigation is needed to understand the level of self-medication by the people and to use the proper way to understand the method of antibiotics.

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