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Identification of pathogenic bacteria isolated from wound infections, and effect of some antibiotics

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***Abstract*---**In clinical practice, where empiric treatment of infections is common, wound infections are still a problem. Purpose: In this study, it was aimed to identification of pathogenic bacteria isolated from wound infections, and Effect of some antibiotics at Wasit, Iraq. Methods: Records of 102 patients' wound swabs taken with a suspicion of wound infections. Swabs from the wounds were used to inoculate and culture smears. Gram-stained bacterial colonies were studied under a microscope. To determine the types of pathogens, biochemical tests were conducted. For evaluating antibiotics, the Kirby-Bauer disk diffusion method was employed. Results: Infections in wounds were prevalent 81.4% (CI: 60.869). 83 infected wounds yielded a total of 10 distinct species. *Staphylococcus aureus* (26.5%) was the most prevalent bacterial species found, followed by *Pseudomonas aeruginosa* (14.4%), *Proteus mirabilis* (12%), *Escherichia coli* (10.8%), and *Enterobacter cloacae* (8.4%). Two species made up the majority of the 25 (30.1%) polymicrobial infections that were discovered in the samples. The *P. aeruginosa* and *P. mirabilis* relationship was the most frequent. The studied antibiotic resistance levels among the bacterial isolates varied (8.3% to 100%) resistance. Amoxicillin-clavulanate resistance was present in all isolates. Age and sex had no bearing on the prevalence, type of agent, and pattern of antimicrobial resistance. Conclusion: Clinical isolates were shown to have a higher incidence of resistance to the most popular antibiotics in general, and *E. cloacae* being the most resistant.

***Keywords*---**bacterial isolation, skin wound infections, antimicrobial resistance.

Introduction

A wound is defined as an interruption of the integrity or dysfunction of the skin layers [1, 2]. Wounds are classified as acute or chronic in the most cases. The first type includes burns, scratches, abrasions, and bug bites are examples of acute ulcers that are expected to heal in an expected time. Patients' quality of life is drastically reduced when their skin is damaged by trauma or burn injury [3]. However, depending on the degree of the injury, therapeutic There are ways to quicken the healing process [4]. Moreover, in the majority of cases, serious burn or gunshot wounds necessitate surgery, debridement, and antibiotic therapy. Processes related accompanied by an underlying risk factor that is diabetes mellitus (D.M) or immunological weakness, on other hand, are the most common cause of chronic wounds. Leg and artery ulcers, non-healing surgical site, and Diabetic foot ulcers are examples of chronic wounds [5]. Another risk agents for chronic wounds include foreign objects, advanced age, chronic conditions, such as diabetic mellitus, obesity, immune-deficient diseases, and infection with microbes [6, 7]. Wound infections, one of the most common types of nosocomial pathogens, infects millions of individuals each year all over the world and has a high fatality rate [8, 9]. Healthy and immunocompetent people are naturally protected against pathogens by their immune systems. However, in cases of immunodeficiency, a variety of microbial illnesses will damage the underlying tissues throughout the body, harming or even killing organs[10]. Wound infections are frequently induced by pathogenic bacteria entering the skin gaps from other regions of the body or the outside, and according to the pathogen, additional tissues could be implicated [11].

Common pathogenic microbes that induce wound infections include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Acinetobacter baumannii* [8, 12]. By employing numerous virulence factors, these bacteria can cause harm and diseases [13]. Wounds, particularly burn injuries, are inhabited by Gram-positive Micro-organism (M.O), including *S. aureus* in first week, and Gram-negative bacteria penetrate the wound in the second week [14]. As bacteria multiply, they begin to invade deeper tissues, and sepsis develops when pathogenic bacteria penetrate the lymphatic system and capillaries. One of the most dangerous clinical forms of infection in patients with inflamed wounds is sepsis, which requires prompt treatment, especially in patients in burn units, Due to the presence of the extremely poisonous lipopolysaccharides (LPS) in their cell walls, Dangerous and harmful bacteria include *P. aeruginosa* and *A. baumannii* [15]. The United Nations recently declared this a worldwide threat, estimating that ten million deaths will occur by 2050 as a result of these diseases [16, 17]. Antibiotics are unable to invade the injured tissue due to these causes. Because of enzymatic activity, dilution by effusion fluids, or perhaps other processes involving inflammatory mediators, antibiotics used topically for wound infections may not be effective [18]. In this study, it was aimed to appraise activity of some antibiotic against some pathogenic bacteria in the skin wound infections at Wasit, Iraq, by examining the common M.O implicated in wound infections, related variables, and antibiotic resistance patterns.

Material and Method

Study design

This research was cross-sectional study during the period from 9st November, 2021 to 15th February, 2022. A wound swab samples taken from patients with suspected skin wound infections, including burn, surgery, diabetic foot, and trauma, between the ages of 17 and 70. Admitted to Al-Zahra Teaching Hospital, Al Karma Hospital, Fairuz hospital and private clinics in Wasit province. Samples were taken by sterile disposable cotton swabs and transport swab. They were, then, cultured onto (MacConkey agar, Blood agar, and Chocolate agar/ Liofilchem) plates before incubating aerobically and (anaerobically with Co_2) at 37°C for (24h to 48h). After incubation, identified based on colony morphology, microscopic Gram stain investigation, and standard biochemical tests [19].

Exclusion and inclusion criteria

In order to include all patients in the study who had suspected wound infections, the records were carefully examined. Patients were considered to have a wound infections if they displayed any of the following symptoms: fever, erythema, localized warmth, foul smell, and darkening of granulation tissue. Patients who were really unwell and those who had had antibiotic treatment one to two weeks before to the research were excluded. The patients' age, sex, and wound type were recorded. After superficial pre-cleansing of wounds with normal saline, Excess saline was removed carefully by using sterile gauze, each specimen was collected by rotating a sterile swab during the wound surface of a $1cm^2$ area in a zig-zag motion, from the center of the wound to outside.

Antibiotic Susceptibility

Susceptibility to antibiotic chemotherapy was determined by the Kirby-Bauer disc diffusion method, and results interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2022). The antibiotics tested were amikacin 30 µg, amoxicillin-clavulanate 30 µg, azithromycin 15 µg, ceftazidime 30 µg, ciprofloxacin 5 µg, gentamicin 10 µg, ceftriaxone 30 µg, nitrofurantoin 30 µg, imipenem 10 µg, levofloxacin 5 µg, meropenem 10 µg, nalidixic acid 30 µg, piperacillin 100 µg, trimethoprim sulfamethoxazole 25 µg, and tobomycin 30 µg.

Statistical analyses

Statistical-Package-for-Social-Science (SPSS, version 25.0) for Windows was used to do statistical analysis on all data. All findings with a significant level ($P \leq 0.05$) were analyzed using Chi Square.

Result

Prevalence of wound infections

The current study was conducted on 102 specimens from skin wound infections of suspected patients, 57 (55.9%) male and 45 (44.1%) female subject. The results

were distributed according to the patient's age between 17-70 years old. The lowest incidence was among 17-20 age group (5.9%), while the highest incidence was among 41-50 age group (27.4%), as showed in the (Table 1).

Table (1): The distribution of patients according to age groups and gender

Variable		Frequency	%	*P*-value
Gender	Male	57.0	55.9	0.013
	Female	45.0	44.1	
Age (years)	17-20	6	5.9	
	21-30	22	21.5	
	31-40	19	18.6	
	41-50	28	27.4	
	51-60	15	14.7	
	61-70	12	11.8	

P<0.05, Chi-square= 14.443

Type of wound

One hundred and two swab specimens from skin wound infections were collected in this study which include burn (n=30, 29.4%), diabetic foot (n=27, 26.4%), surgery (n=25, 24.5%), and trauma (n=20, 19.6%). about 83 swab (81.4%) show positive culture of bacterial growth from different skin wound sites versus 19 swab (18.6%) show negative results for culturing. The majority of swabs taken from patients with trauma wounds resulted in bacterial growth (85.0%), as showed in the (Table 2).

Table 2: The distribution of sample collection according to site of wound and gender

Type of wound	No. of swab	Patient's with infected wounds		No. of infected swabs	Patient with infected wounds		*P*-value
		Males	Females		Males	Females	
Burn	30(29.4%)	17	13	25(83.3%)	14	11	0.285
Diabetic foot	27(26.4%)	17	10	21(77.8%)	15	6	
Surgery	25(24.5%)	10	15	20(80.0%)	7	13	
Trauma	20(19.6%)	13	7	17(85.0%)	11	6	
Total	102	57	45	83(81.3%)	47	36	

P<0.05, Chi-square= 3.788

Age and sex

a higher incidence of wound infections in the 17 to 70 year age group, but age did not significantly correlate with the incidence of wound infections. Both the type of wound and the type of organism that was isolated did not significantly correlate with the subject's sex (p = 0.28 and p = 0.57, respectively).

Isolation of bacteria

A culture study based on morphological and biochemical tests revealed a high incidence of Gram negative bacteria 46(55.5%), that includes *Pseudomonas aeruginosa* showed that a high percent 12(14.4%), followed *Proteus mirabilis* 10(12.0%). Moreover, *Escherichia coli* 9(10.8%), *Enterobacter cloacae* 7(8.4%), *Klebsiella Pneumoniae* 7(8.4%), , while *Enterococcus facieum* was the least detected isolate 1(1.2%). Whereas Gram-positive bacteria recorded 37(44.5%), that includes *S. aureus* had the highest percentage of isolated M.O in this investigation 22(26.5%), followed by *S. epidermidis* 7(8.4%), *S. hominis* 4(4.8%) and *S. haemolyticus* 4(4.8%). *S. aureus* was the predominant Gram positive M.O isolated, accounting for almost quarter of the isolates. *P. aeruginosa* was the predominant Gram negative M.O isolated from the wound swabs. No significant differences between type of M.O and gender (p = 0.57), (Table 3). However, there was significant associated between organism and wound types (P = 0.0002), (Figure 1).

Table 3: Frequency of isolated microorganisms

Isolated microorganism	Total	Male	Female	*P*-value
S. aureus	22	10	12	0.579
S. epidermidis	7	2	5	
S. hominis	4	3	1	
S. haemolyticus	4	3	1	
P. aeruginosa	12	8	4	
P. mirabilis	10	6	4	
E. coli	9	4	5	
E. cloacae	7	5	2	
K. Pneumoniae	7	5	2	
E. facieum	1	1	0	

P<0.05, Chi-square= 7.558

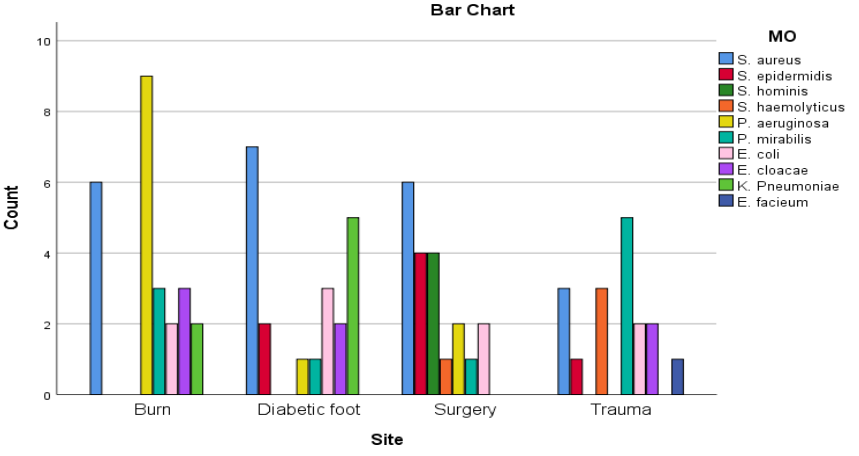

Figure 1: Frequency of organisms based on wound types

Antibiotic resistance

In current study, examined the sensitivity of antibiotics to a group of bacteria isolated from wound infections, which include *P. aeruginosa*, *P. mirabilis*, *E. coli*, and *E. cloacae*. The evaluated antibiotic resistance in the bacterial isolates varied, with most isolates having values between 8.3% and 100%. The M.O. isolate was completely resistant to amoxicillin-clavulanic acid, with the exception of *P. mirabilis* was 90%. Approximately 50% of M.O were resistant to nalidixic acid as well. The lowest observed resistance level for *E. cloacae* was 42.9%. *E. cloacae* displayed a very high resistance to the tested antibiotics. *P. aeruginosa* was relatively susceptible to Meropenem and Piperacillin (Table 4).

Table 4: Antibiotic susceptibility of several isolated microorganisms expressed as a percentage of resistance

Antibacterial agent	*E. coli*	*P. mirabilis*	*E. cloacae*	*P. aeruginosa*
AK	22.2	20	71.4	41.7
AUG	100	90	100	100
AZM	55.6	20	100	-
CAZ	44.4	50	85.7	58.3
CIP	44.4	50	71.4	50
CN	56.6	90	100	41.7
CRO	77.8	70	71.4	-
F	22.2	60	71.4	-
IMI	77.8	20	71.4	33.3
LEV	22.2	60	42.9	50
MRO	22.2	20	71.4	8.3
NA	66.7	70	100	100
PRL	77.8	20	100	8.3
SXT	44.4	80	100	-
TOB	55.6	50	100	66.7

AK = Amikacin, AUG = Amoxicillin-clavulanate, AZM=Azithromycin, CAZ= Ceftazidime, CIP = Ciprofloxacin, CN = Gentamicin, CRO = Ceftriaxone, F = Nitrofurantoin, IMI = Imipenem, LEV = Levofloxacin, MRP = Meropenem, NA = Nalidixic acid, PRL = Piperacillin, SXT = Trimethoprim sulfamethoxazole, TOB = Tobomycin.

Discussion

Hospital wound bacterial contamination is a significant issue, especially with surgical operations where the site of a sterile surgery may become contaminated [20, 21]. These studies showed that harmful bacteria were present in wounds at significant rates (81.4%). This high number is consistent with results from other studies conducted in Nigeria [22, 23, 24, 25], although it differs from one conducted in East Africa that reported a prevalence of 70.5% [26]. These variations may be a result of the study's design. The average might be just as high a strong suspicion of infection are looked into rather than all wounds. The majority of swabs from traumatic wounds generated significant bacterial growth

in 85.0 percent of cases, which was considered to indicate infection even though there was a correlation between the type of wound and the type of M.O identified. The patient's age should ideally have some influence on wound infections and healing, with persons in the extremes of life being more prone to wound infections. However, in these study found no relationship between age and wound infections, which is in contrast to the findings of a study conducted in the Niger Delta [27]. As in current studies, *S. aureus* and *P. aeruginosa* as the most common pathogens isolated from wound infections. These result agreed with other study [28]. Anaerobic cultures were not performed in the majority of investigations, including this one, for a number of reasons, the primary one being a lack of resources (equipment and money). So it was unable to separate anaerobic bacteria, which are also crucial for wound infections. Antibiotic abuse and misuse by the public in general and health-care professionals are among the causes that have contributed to the rise in antimicrobial - resistant rates [29]. In this investigation, it is clear that the maximal rates of resistance in *E. coli* have been towards amoxicillin/clavulanate was 100%. Similar results (100%, 72%, 59.7% and 72%, respectively) have been reported in many other studies around the world [30, 31, 32, 33]. Moreover, resistance to 3rd generation cephalosporins, ceftazidime, and ceftriaxone was shown to be substantial 44.4% and 77.8%, respectively. *P. mirabilis* represents one of the most concerning pathogens colonized with multiple antibiotic resistant [34]. antibiotic sensitivity test revealed maximum resistant to gentamicin 90%, amoxicillin/clavulanate 90% and nitrofurantoin was 70%. These data are in accordance with the research done in Nigeria was 90% [28]. Resistance percentage to ceftriaxone was 70%, these result are agreed with study reported in Bangladesh was 72.7% [35]. Resistance percentage to ciprofloxacin was 50%, these result are disagreed with other study reported was 70.4% [35]. In *E. cloacae*, all isolates displayed high level of resistance to Gentamicin, amoxicillin/clavulanate, trimethoprim/sulfamethoxazole, ceftriaxone, tobomycin, and piperacillin was 100%. These result disagreed to that reported was 3.6%, 15.8%, 26.1% respectively in Saudi Arabia [36].

In *P. aeruginosa*, the percentage 100% of the isolates were resistance to amoxicillin/clavulanate and nalidixic acid, respectively. In addition, 66.7% of the isolates exhibited resistance to tobomycin, these result are in agreement with the study performed in Wasit 74.6% [37]. The percentage of resistance of ceftazidime in this study was 58.3%, these result similar to that reported was 57% [38]. While these result disagreed with the study in Iraq that revealed was 26.8% [39]. It has been established that inadequate access to antibiotics and their unauthorized or inappropriate usage are factors in the rise of antimicrobial resistance in developing nations [40].

Conclusions

According to the results of the current study, *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *E. coli*, *E. cloacae*, *K. pneumonia*, *S. epidermidis*, S. *haemolyticus*, *S. hominis*, and *E. facieum* were the most common microorganism infections in wound infections. Additionally, mixed cultures, particularly P. aeruginosa and P. mirabilis, have been discovered. Clinical isolates generally shown a high level of

resistance to the most widely used antibiotics, especially *E. cloacae* was the most resistant to most antibiotics.

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