

Detection the Antibiotyping Patterns of the Most That Common Bacterial Pathogens Susceptible to Different Antimicrobial Agents That Cause Wound Infections, in Duhok, Iraq



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ABSTRACT

Background: Wound infections are considered a major problem in the field of surgery for a long time, with growing bacterial resistance to drugs that were once considered the first line of treatment for post-operative wound infections, according to data spanning two years. The objectives of this research were to evaluate the prevalence of bacteriological profiles that cause surgical wound infections and the antimicrobial susceptibility patterns between outpatient clinics at Azadi teaching hospital in Duhok, Iraq. **Methods:** 165 wound swab samples were collected from surgical wound infections and tested on Blood agar, MacConkey agar, and Nutrient agar, then identified using different cultures and standard biochemical tests. On Muller Hinton Agar, all of the isolates were tested for antimicrobial susceptibility using the disk diffusion technique, as defined by the Clinical and Laboratory Standards Institute (CLSI). **Results:** 90 (54.5%) of the 165 surgical wound swab samples cultured had a positive bacterial culture, with *Klebsiella pneumoniae* 25 samples (27.8%) being the most common pathogen, followed by *Staphylococcus aureus* (20 samples). Some gram-negative bacteria such as *Pseudomonas aeruginosa* (14 samples), *Escherichia coli* (13 samples), *Proteus mirabilis* (7 samples), and *Enterobacter cloacae* were also isolated (2 samples). Except for amikacin, imipenem, and ciprofloxacin, the majority of pathogens isolated demonstrated greater resistance to most other antibiotics. **Conclusion:** In this research, the incidence of surgical wound infection was found to be higher in the study region. *Klebsiella pneumoniae* was isolated as the most common causative agent, with multi-drug resistance and antibiotic patterns that varied.

1. INTRODUCTION

Wound infections are considered as a difficulty ordeal of many patients because it causes many distresses related with morbidity and mortality, boost hospital entering, weak wounds recovering and excessive pain, and need long costs to receive healthcare. However, healthcare specializes are implicated in the barring, and protection from wound infections [1]. In addition, wound infections caused many severe problems of surgery and can extend hospitalization from 5 for 20 days and raise the medical costs significantly [2]. Wound infections primarily based on the host defenses of patients, wound conditions and microbial population. Exogenous and endogenous bacterial flora play an important role in contamination of surgical wounds. Also, use of antimicrobial has a significant part of prevalence microorganisms in hospital [3, 4].

The goal of the study is to identify the most common bacteria that cause wound infections, as well as the patterns of antibiotic resistance of the most important isolates. Antibiotyping patterns were also shown, to be necessary to differentiate between strains and detecting relatedness among isolates in order to track the source of infections in our environment.

2. MATERIAL AND METHODS

2.1. Study Participants

Between June 2013 to November 2015, a total of 165 samples were collected from outpatients at Azadi teaching hospital in Duhok City, Iraq, who had evidence of surgical wound infections.

2.2. Identification of Bacteria

From postoperative wound infections, the clinical suspected specimens were collected with sterile precautions of pus specimens and wound swabs and then directly transported to the laboratory. After that, the samples were routinely treated in the bacteriology department in Central Public Health Laboratory. Many culture media and biochemical tests were used for isolating, identifying and

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testing the sensitivity of the clinical isolates to the most common used antibiotics. The following culture media and biochemical tests were used in this study: Blood agar (with 5-7% defibrinized blood), MacConkey agar, Nutrient agar, Mannitol salt agar, Simmons citrate agar, kligler Iron Agar (KIA), Mueller-Hinton agar, Sulfide formation indole production, Motility Test (SIM), Methyl Red-Voges Proskauer broth, Coagulase, Catalase, Urease, and Oxidase tests. All of the above media and reagents were obtained from (Difco. USA). All of these culture media were prepared based on the manufacturer's instructions in 500 mL bottle and sterilized by autoclaving at 121°C for 20 min. The bacterial isolate plates were incubated at 37°C for 18-24 hr in an incubator. The culture media plates were visualized the next day and the time extended for 48 hr if there wasn't microbial growth during 24 hrs. Isolated bacterial colonies were undergoing to Gram staining procedure and biochemical tests for identification [5, 6, 7].

2.3. Antibiotic Sensitivity Test

Antibiotic sensitivity testing was performed using commercially prepared antibiotic sensitivity disks (Oxoid, England). Using adapted Kirby-Bauer method was used as a standard method according to (CLSI) guidelines using MuellerHinton agar as a medium. The antimicrobial susceptibility inhibition zone criteria were considered from Clinical and Laboratory Standards Institute (CLSI) tables on interpretative zone diameters [8, 9].

3. RESULTS

Among (165) suspicious surgical wound samples approx. (54.5%) (90) had a positive bacterial culture as shown in Table 1. In table 2, displays the most significant bacteria that cause wound infections disease. *Klebsiella pneumoniae* (25) isolate (27.8%), *Staphylococcus aureus* (20) isolate (22.2%), *Streptococcus pyogenes* with a lowest causative agent (3.3%), and *Enterobacter cloacae* (2) (2.2%). In the table 3, it shows the different types of wound infection, bacterial isolates had different rates of sensitivity to antibiotics use in this study. A larger value of the sensitivity rate to antibiotics was the antibiotic amikacin, imipenem & ciprofloxacin, respectively. The majority of other commonly used antibiotics, on the other hand, were found to be ineffective. The antibiotic trends of *Klebsiella pneumoniae* isolates are shown in table 4 for widely used antibiotics. All the strains were found to be multi-resistant, i.e., resistant to more than one antibiotic. The antibiotyping patterns results detected that out of (25) strains, (10), multidrug resistant isolates belong to (4) different antibiotyping patterns; (3) was typical antibiotics.

Table 1: Total and percentage of isolated samples from surgical wounds.

Total isolated samples	Positive cultures (%)	Negative cultures (%)
165	90 (54.5%)	75 (45.5%)

Table 2: The prevalence of bacterial species in wound infections.

Bacteriological agents	No. of Organisms %
<i>Klebsiella pneumoniae</i>	25 (27.8%)
<i>Staphylococcus aureus</i>	20 (22.2%)
<i>Pseudomonas aeruginosa</i>	14 (15.6%)
<i>Escherichia coli</i>	13 (14.4%)
<i>Proteus mirabilis</i>	7 (7.8%)
<i>Staphylococcus epidermidis</i>	6 (6.7%)
<i>Streptococcus pyogenes</i>	3 (3.3%)
<i>Enterobacter cloacae</i>	2 (2.2%)
Total	90 (100%)

Table 3: Antimicrobial Susceptibility test of various bacterial species causing wound infections.

Bacteriological profile	Antibiotics										
	AMC %	CL %	CN %	CIP %	CRO %	IPM %	DO %	F %	E %	AK %	** %
<i>Klebsiella pneumoniae</i>	8	3	7	25	2	18	11	3	1	27	80
<i>Staphylococcus aureus</i>	14	24	12	28	4	22	15	7	17	35	90
<i>Pseudomonas aeruginosa</i>	4	3	20	23	2	13	5	0	5	28	90
<i>Escherichia coli</i>	10	2	21	31	0	31	11	14	0	33	70
<i>Proteus mirabilis</i>	0	4	12	23	12	19	0	11	0	42	90
<i>Staphylococcus epidermidis</i>	32	9	9	32	0	32	18	13	0	14	70
<i>Streptococcus pyogenes</i>	25	42	25	41	25	43	33	0	34	25	10
<i>Enterobacter cloacae</i>	0	0	20	20	0	41	0	10	0	50	100

Table 4: Antibiotyping patterns of *Klebsiella pneumoniae* isolates.

Antibiotyping patterns	Resistance spectrum phenotypic	No. of isolates (%)
Antibiotype 1	E, CRO, AK, DO, CL, CN, AMC, F, CIP	2 (20)
Antibiotype 2	E, CRO, AK, CL, CN, AMC, F, CIP	2 (20)
Antibiotype 3	E, CRO, CL, CN, AMC, F	4 (40)
Antibiotype 4	E, CRO, AK, DO, CL, CN, AMC, F	2 (20)
Total		10(100)

4. DISCUSSION

More learning into the bacterial infections and the laboratory sensitivity testing involved many types of microorganisms may make medicine chosen more reasonable in antimicrobial chemotherapy. This would further minimize or remove flaws in the scientific collection of both unsuccessful and costly medications. The outcome of the current project to exhibit that surgical wound

infections had an average infection rate of (54.5%). The prevalent isolates were *Klebsiella pneumoniae* with an incidence of (27.8%) followed by *Staphylococcus aureus* (22.2%), *Pseudomonas aeruginosa* (15.6%), *Escherichia coli* (14.4%), *Proteus mirabilis* (7.8%), *Staphylococcus epidermidis*, *Streptococcus pyogenes* (7.8%) and (6.7%) respectively. *Enterobacter cloacae* (2.2%).

According to another study, the infection rate of hospital - acquired infections is 7.5 %. *Staphylococcus aureus* (44%) was the most common, followed by *Bacteroides fragilis* (11%), *Escherichia coli* (11%), *Proteus spp.* (11%), *Pseudomonas spp.*, and *Klebsiella spp.* (8.3 % of each) [10].

Other research showed that *Staphylococcus aureus* isolates were the most common (51.6%), accompanied by *Klebsiella spp.* (22.6 %), *E. coli* (6.45%), *Pseudomonas aeruginosa*, and *Proteus spp.* (9.7) [11]. In Bosnia and Herzegovina, a similar discovery was made [12]. However, in Jos, Oguachuba discovered that *Proteus spp.* was the most prevalent surgical wound infection isolate with a rate of 41.9 %, followed by *Staphylococcus aureus* with a rate of 25.6 % [13]. In addition, a study conducted in Ghana found that *Proteus spp.* were the most common wound isolates [14]. In an Iranian research, the average infection rate of surgical wounds was 8.29%. It should be noted that anaerobic species need extremely strict isolation conditions, so there are challenges associated with anaerobic cultures in this country; this may explain why anaerobes were not included in our research.

The antibiogram outcome of pathogenic isolates revealed that the amikacin had significant activity against most leading species, with the exception of *Staphylococcus epidermidis*, as follows: *Klebsiella pneumoniae* (27%), *Staphylococcus aureus* (35%), *Pseudomonas aeruginosa* (28%), *Escherichia coli* (33%), *Proteus mirabilis* (42%), *Staphylococcus epidermidis* (14%), *Streptococcus pyogenes* (25%) and *Enterobacter cloacae* (50%). Other studies have also confirmed this in [15, 16, 17]. As a result, this drug is still successful and may be seen as an additional treatment choice for surgical wound infections in our region. A research conducted in Pakistan, on the other hand, discovered that amoxiclav and imipenem were the most effective drugs against wound isolates [18]. Only three pathogens were less or moderately susceptible to ciprofloxacin among the total pathogens recovered in our study: *Pseudomonas aeruginosa* (23%), *Proteus mirabilis* (23%) and *Enterobacter cloacae* (20%). Similar findings were made by [19]. According to the findings, the majority of the isolated species were resistant to the antibiotics studied, which included erythromycin, amoxiclav, cephalixin, and cefuroxime. However, this revealed that these isolate types were all multi resistant strains. Data similar to this was recorded by [10, 11, 16]. The latter finding is especially significant because it means that these antibiotics are no longer effective as standard treatment for surgical wound infections in the study field. Therefore,

updating the antibiogram on a regular basis would significantly decrease the occurrence of postoperative wound infections.

Antibiotyping is a phenotypic approach that involves measuring bacterial strains against a collection of randomly selected antibiotics, resulting in the generation of a resistance pattern that is specific to a strain and is thought to characterize isolates for epidemiological purposes. The aim of this study was to see if antibiotyping patterns of *Klebsiella pneumoniae* isolates could be useful.

As previously mentioned, we determined the sensitivity of *Klebsiella pneumoniae* to a variety of antibiotics, and the majority of the isolates were multi resistant. The study's findings showed that out of 25 *Klebsiella pneumoniae* isolates, 10 of them were multi-drug resistant (with the same antibiotic sensitivity pattern) and belonged to four distinct antibiotic patterns. Antibiotypes 3 was the most prevalent, accounting for 40% of all isolates. Furthermore, the disk susceptibility patterns of those isolates from different outpatients were similar, suggesting that they were related. In general, this simple typing system is capable of distinguishing between strains and determining relatedness among *Klebsiella pneumoniae* isolates in order to trace the source of infections in our environment.

Since many of the causative species have likely begun to develop some sort of drug resistance to the commonly used antibiotics, the overall outcome of this study clearly emphasizes that the severity of the surgical wound infection issue could be increasing. The average surgical wound infection rate was (54.5%), which is relatively high as compared to the socially appropriate surgical wound infection rate. As a result, continuous monitoring in the form of wound surveillance will certainly allow each institute in our setting to identify and correct unique problem areas. Furthermore, this research contributes to the collection of national data on the prevalence of antimicrobial-resistant pathogens that cause surgical wound infections in Duhok.

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الكشف عن الممرضات البكتيرية الأكثر شيوعا المسببة لالتهابات الجروح والحساسية لمختلف المضادات الحيوية في محافظة دهوك/ العراق

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الخلاصة:

اعتبرت التهابات الجروح مشكلة كبيرة في حقل الجراحة لفترة طويلة، ولوحظ تزايد مقاومة البكتيريا للأدوية التي كانت تعتبر في يوم من الأيام الخط الأول في علاج التهابات الجروح بعد الجراحة، وفقاً لبيانات تمتد لعامين. هدف البحث الى تقييم مدى انتشار الأنماط البكتيرية التي تسبب التهابات الجروح وتقييم أنماط الحساسية للمضادات الحيوية بين المرضى الوافدين الى مستشفى آزادي التعليمي في دهوك/ العراق. تم جمع 165 عينة من مسحات عدوى التهابات الجروح وتشخيصها بالاعتماد على التقنيات المايكروبيولوجية القياسية. كما أجري اختبار فحص الحساسية للمضادات الحيوية للعينات المجموعة بالاعتماد على معهد المعايير السريرية والمختبرية (CLSI). كانت النتائج 90 عينة (54.5%) من مجموع 165 عينة إيجابية النمو على الأوساط الزراعية الخاصة لنمو البكتيريا. حيث وجد 25 عينة من *Klebsiella pneumoniae* (27.8%) أكثر مسببات الأمراض شيوعاً، تليها *Staphylococcus aureus* (20 عينة) بنسبة (22.2%). كما وتم عزل بعض البكتيريا سالبة الكرام مثل *Pseudomonas aeruginosa* (14 عينة) و *Escherichia coli* (13 عينة) و *Proteus mirabilis* (7 عينات) و *Enterobacter cloacae* (عينتان). وبعد اجراء فحص الحساسية للعزلات البكتيرية لوحظ انه باستثناء المضادات الحيوية (Amikacin, Imipenem, and Ciprofloxacin)، أن غالبية المسببات المرضية المعزولة مقاومة بشكل أكبر لمعظم المضادات الحيوية الأخرى المستعملة. الخلاصة: في هذا البحث، تبين أن معدل الإصابة بعدوى التهابات الجروح ذات نسبة عالية، خاصة في المرضى الوافدين الى المستشفى. كما وتبين أن *Klebsiella pneumoniae* هي العامل المسبب الأكثر شيوعاً، مع وجود مقاومة متعددة للأدوية وغيرها من أنماط المضادات الحيوية المتنوعة المستعملة