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Tigecycline is the Most Effective against Multi-Drug Resistant *Klebsiella pneumoniae* Recovered from Burn Wound Infections in Two Hospitals in Al-Kut City, Iraq

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Abstract

Klebsiella pneumoniae is among the most frequent microorganisms isolated from infections of burn wounds. This cross-sectional study aimed to investigate the distribution of multi-drug resistant (MDR) *K. pneumoniae* in two burn hospitals and the antibiotic resistance profile in different burn regions of the same patient. It was performed in two hospitals (Al-Zahraa and Al-Karama) in Al-Kut, Iraq, between January and May 2022. Totally, 100 burn swabs were collected from 40 patients of both genders suffering from burn wound infections, with ages ranging between 3 and 50 years. *Klebsiella pneumoniae* were isolated and identified using conventional methods followed by VITEK®2 system and confirmed via polymerase chain reaction targeting the *gapA* gene. Then, the antimicrobial susceptibility pattern was studied by the VITEK®2 system. Of the 100 burn wound swabs, 20 isolates were *K. pneumoniae*. Fifty five percent (11 out of 20) of *K. pneumoniae* isolated in the current study were MDR and 35% of the isolates had the extended spectrum beta-lactamase (ESBL) which is the main antibiotic resistance mechanism. Furthermore, the bacteria isolated from different burned areas of the same patient showed variable pattern of antibiotic susceptibility. To conclude, *K. pneumoniae* contaminating the burn wards in the Iraqi hospitals are mostly MDR, against which tigecycline is the most effective antibiotic.

Keywords: Burn infection, *Klebsiella pneumoniae*, Antimicrobial susceptibility, Multi-drug resistance.

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تجيسيكلين هو الدواء الأكثر فاعلية ضد*Klebsiella pneumoniae* المقاومة لعدد من المضادات الحيوية والمعزولة من التهابات جروح الحروق في مستشفيين في مدينة الكوت، العراق

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الخلاصة

تعد *Klebsiella pneumoniae* من بين الكائنات الحية الدقيقة الأكثر شيوعًا المعزولة من التهابات الجروح الحرقية. هدفت هذه الدراسة إلى التحقق من انتشار هذه الجراثيم المقاومة للأدوية المتعددة في مستشفيين للحروق، ومعرفة مظاهر المقاومة للمضادات في مناطق الحروق المختلفة للمريض نفسه. أجريت الدراسة في مستشفيين (الزهراء والكرامة) في الكوت، العراق، في الفترة ما بين كانون الثاني و أيار 2022. تم جمع 100 مسحة حروق من 40 مريضاً من كلا الجنسين وتراوحت أعمارهم بين 3 و 50 سنة يعانون من التهابات جروح الحروق. تم عزل وتحديد هذه الجراثيم باستخدام الطرق التقليدية وتلاها التشخيص بواسطة نظام VITEK®2 وتأكيد العزلات بتفاعل البلمرة المتسلسل الذي استهدف جين *gapA*. جرى دراسة نمط الحساسية لمضادات الميكروبات باستخدام نظام VITEK®2. من بين 100 مسحة من جروح الحروق، كانت 20 عزلة تعود لجراثيم *K. pneumoniae*. كانت معظم العزلات (55٪، 11 من 20 عزلة) في الدراسة الحالية هي متعددة المقاومة للمضادات، و 35٪ من العزلات تمتلك الطيف الواسع لبيتا لاكتاماز (ESBL)، وهي آلية مقاومة المضادات الحيوية الرئيسية. علاوة على ذلك، أظهرت الجراثيم المعزولة من مناطق الحروق المختلفة للمريض نفسه نمطًا مختلفا من الحساسية للمضادات الحيوية. تستنتج هذه الدراسة أن جرثومة *K. pneumoniae* التي تلوث ردهات الحروق في المستشفيات العراقية هي في الغالب متعددة المقاومة للمضادات MDR، و يعد Tigecycline المضاد الحيوي الأكثر فاعلية ضدها.

1. Introduction

Burn wound infections, the most frequent sources of morbidity and mortality in burn victims, still constitute a public health dilemma all over the world [1, 2, 3]. Approximately, 180,000 deaths are expected to take place globally and annually because of burns based on the report of the World Health Organization [2]. Burn wound infections can lead to death in 33 to 80% of the patients, either directly or indirectly [4, 5]. Similarly, infection can cause death in 75% of patients after thermal injuries [6, 7]. Low as well as middle-income nations comprise the majority of these deaths [8].

The susceptibility of burn wound patients to infection is due to breakdown of the skin barrier, altered physiology, along with the acquired immuno-suppression [9, 10]. In the same context, prolonged stay in the hospitals of burn injuries as well as invasive interventions such as the use of catheters, carried out at healthcare facilities can predispose such patients to infection with nosocomial pathogens [11, 12, 13]. Additionally, other factors, such as the patient's age, extent and profundity of burn injuries concomitant with microbial causes, including the number and type of the infecting microorganism, and its ability to produce toxin/enzyme and motility, are considered as determinants of invasive infection [14].

The most common part of the body supporting the growth of microorganisms is eschar because it is avascular, humid and rich in proteins that blocks the arrival of antibiotics and immune cells [6,15]. Therefore, in the absence of effective treatment, the burn injury is regarded as a perfect culture medium for the colonization and proliferation of all types of endogenous (found in gastrointestinal tracts of patients) and exogenous (contaminating external sources, such as fomites' hands, healthcare workers' hands, as well as hospital environments) microorganisms [14, 15]. *K. pneumoniae* is one of the most common nosocomial pathogens that have the capability to cause burn wound infections with severe local and systemic disorders due to colonizing skin and mucosae of the affected patient [16].

In numerous studies, *Klebsiella* spp. have been documented as the mainly frequent bacteria in infected burns [7, 17]. In this context, *Klebsiella* spp. was the most prevalent pathogen of the burn infections constituting a rate of 34.4% in the study of Kehinde *et al.* [17]. Similarly, Perween *et al.* [18] reported *Klebsiella* isolation rate of 22% (195 out of the total 885 isolates) from burn injuries; 153 out of 885 (17.3%) isolates belonged to *K. pneumoniae*, 37/885 (4.2%) were *K. oxytoca* and 5/855 (0.6%) were other species. Another study in Nigeria found that *Klebsiella* spp. among the Gram-negative bacteria was the predominant pathogen causing burn wound infections [19]. Moreover, one of the top ten pathogens identified from burn intensive care units and burn common wards was *K. pneumoniae* [10].

Klebsiella, a member of the family *Enterobacteriaceae*, is known to harbour a horizontally transferable plasmid that mediates extended spectrum β-lactamases (ESBLs) [20]. According to some studies, genes encoding ESBLs are present on plasmids that also contain other resistance genes [21] which make these plasmids harbouring bacteria to become multidrug resistant (MDR) [8]. MDR refers to resistance to at least one antibiotic from three or more distinct antibiotic classes [18], or in another meaning, MDR strains are those that show resistance to three or more antibiotic families [22]. While 54% of the *Klebsiella* spp. isolates were MDR in the research by Perween *et al.* [18], 100% of the *Klebsiella* spp. isolates belonged to MDR in the study by Kabanangi *et al.* [8]. In contrast, extensive drug resistance (XDR) has been reported in 44% of the environmental and 55% of and clinical isolates of *K. pneumoniae* [22]. The emerged XDR strains were defined as those that continue to be vulnerable to only one or two antimicrobial classes, while pandrug resistant (PDR) strains were specified as those not-susceptible to whole antibiotics in every antibacterial category [23]. Recently, *K. pneumoniae* of MDR as well as those with *carbapenem*-resistance have developed as main public health dilemma in the world [24]. The pathogenicity of *K. pneumoniae* is attributed to numerous virulence factors, such as its capability to readily acquire multiple antibiotics resistance [25]. This bacterium has evolved many mechanisms to avoid β-lactam drugs, including cephalosporins, penicillins and carbapenems. Examples of such strategies of drug resistance include the production of extended-spectrum β-lactamase (ESBL), metallo-β-lactamase (MBL) production, AmpC β-lactamase production, and carbapenemase formation along with porin loss [26]. In addition, this bacterium possesses other mechanisms of resistance represented by efflux pumps which augment resistance to β-lactam drugs, chloramphenicol, quinolones and macrolides [27].

The development and selection of MDR bacteria has been further demonstrated to occur due to cleaning practices of hospitals, extreme and extended use of antibiotics, and antibiotic prescription without knowledge of distributing bacteria [28, 29]. Moreover, the ability of these MDR bacterial strains to survive for a long period in the hospital setting can lead to outbreaks [30]. In the recent years, the isolation rates and antimicrobial resistance of *Klebsiella* spp. is increasing over the time. Therefore, the continuous surveillance of microorganisms circulating in the hospitals, along with monitoring their antimicrobial susceptibility, is essential for the control programs of infections, and can also help physicians to choose appropriate antibiotic for empirical treatment [14, 28]. The current study aimed to isolate *K. pneumoniae* from various affected regions of burned patients admitted into two hospitals in Al-Kut city, Iraq, as well as investigating the antimicrobial susceptibility of the isolates.

2. Materials and Methods

2.1 Ethics

The ethics committees at Al-Zahraa Hospital and Al-Karama Hospital in Al-Kut, Iraq, authorized this human-participant study.

2.2 Study Design and Data Collection

This cross-sectional study was conducted at two referral hospitals (Al-Zahraa and Al-Karama) in Al-Kut, Iraq. Between January and May 2022, 40 individuals (24 females and 16 males) with burn injuries were recruited in the research. Demographic information (gender, age) and burn regions were extracted from the patients' medical records (Supplementary Table).

2.3 Samples Collection

The burned region was cleansed with regular saline solution prior to specimen collection. Sterile cotton swabs were then employed to obtain specimens aseptically from the burn wound depth by rotation with adequate pressure [8]. Totally, 100 swabs were taken from different burned areas where the burn degree was the highest. While, 57 specimens were from Al-Zahraa hospital and 43 sfrom Al-Karama hospital. The specimens were then put in transport media and within wo hours delivered to the microbiology laboratory at the College of Science, Wasit University, Iraq. The wound areas that showed presence of pus, discoloration, bad odor, and/or eschar separation were chosen for sample collection. Numerous swabs were obtained from the same patient based on presence of different burned regions in the body of the victim, such as face, neck, shoulder, chest, right/left hands, right/left arms, fingers, right/left flank, abdomen, back, right/left legs, and right/left foot (Supplementary Table).

2.4 Bacteria Identification

For the purpose of isolating and identifying *K. pneumoniae*, 100 wound swabs from 40 patients who were admitted to burn wards were gathered. The swabs were immediately inoculated into MacConkey agar (Oxoid, UK) upon arriving to the laboratory and incubated aerobically for a whole night at 37°C. Later, the lactose fermenter bacterial isolates were selected and purified and their colony characteristics, e.g., morphology, texture, and colour were examined. Subsequently, Gram staining and performing biochemical tests, such as catalase, oxidase, urease and Simons' citrate were done [31]. These isolates were further diagnosed by the VITEK®2 system (bioMerieux, USA).

Partial amplification of the *gapA* gene was used to identify *K. pneumoniae* at the molecular level using specific primers designed by Aziz and Lafta [32] by applying Thermal Cycler. For the molecular diagnosis, the DNA was extracted from all of the 20 isolates using EasyPure® Bacteria Genomic DNA Kit (Cat. no. EE161). Next, 5 µL of each DNA extract was mixed with the other PCR reaction components (12.5 µl of 1x EasyTaq® PCR SuperMix, 1 µl of GapA forward primer: 5'-GTGATGGGCGTTAATGAGAG, 1 µl of GapA reverse primer: 5'-AAGCATTGTTCACCTCTTCG, and nuclease free water at 5.5 µl), in a final reaction volume of 25 µl. The PCR program involved: initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation for 30 sec at 95°C, annealing for 35 sec at 58°C, extension for 30 sec at 72°C, and final extension at 72°C for 10 min. For verification of PCR amplicons, they were loaded into 1.5% agarose gel (1.5 g agarose dissolved into Tris-Borate-EDTA buffer) along with 100bp Plus Opti-DNA Marker (Cat. G193), and the electrophoresis was run at 80 V for 45 min. Finally, using the gel imaging documentation system, the DNA bands stained with ethidium bromide were seen under the UV light, and the gel pictures were then taken.

2.5 Antimicrobial Susceptibility Test

Antibiotic sensitivity tests were carried out by VITEK®2 (bioMerieux, USA), and the ID-GNB and AST-N280 cards (bioMerieux, USA) for Gram negative bacteria were applied according to instructions of the manufacturer. Concisely, a few bacterial colonies were mixed with normal saline to make suspension. The optical density (OD) of the bacterial suspension was amended to 0.5. The Vitek tubes were thoroughly shaken to sustain homogenous suspension before placing them in the Vitek machine. Antimicrobial susceptibility testing card used for Gram-negative bacteria contained 16 antimicrobials involving: amikacin, cefazolin, cefoxitin, ceftazidime ceftriaxone, cefepime, ampicillin, ertapnem, imipenem, gentamicin, ciprofloxacin, levofloxacin, tigecycline, and trimethoprim/sulphamethoxazole, and nitrofurantoin.

3. Results

3.1 Demographic Features of Patients

The ratio of females (60%) was higher than that of males (40%). The patients varied in age from 3 to 50 years. The vast majority of infected burned patients came from Al-Zahraa hospital.

3.2 Bacterial Diagnosis

The suspected *Klebsiella* spp. isolates detected by routine diagnostic methods were further diagnosed to the species level as *K. pneumoniae* by the VITEK®2 system. The DNA was successfully extracted from most of the samples except sample number 11 where the DNA was degraded (Figure 1). All 20 *K. pneumoniae* isolates were successfully confirmed by the PCR reaction followed by agarose gel electrophoresis which showed DNA bands of the expected size of approximately 391 bp of the *gapA* gene partially amplified from *K. pneumoniae* genome (Figure 2). Despite one of the samples (isolate no. 11) that was already diagnosed as *K. pneumoniae* by the VITEK®2 system, revealed a faint band on the gel due to DNA degradation. The molecular data showed that 14 out of 40 (35%) patients were positive to *K. pneumoniae.* While 5 isolates were obtained from Al-Karama hospital, rest 15 isolates were found in Al-Zahraa hospital.

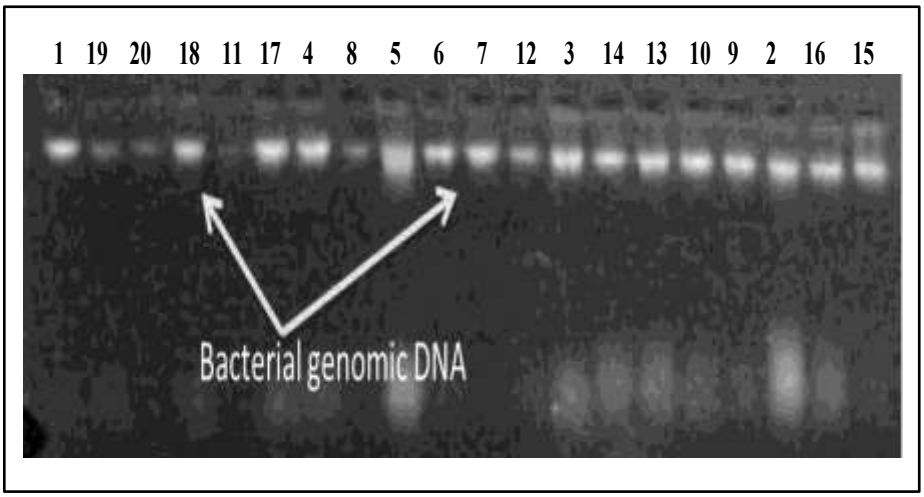


Figure 1: Agarose gel electrophoresis shows bands of the whole genomic DNA isolated from the 20 samples of *K. pneumoniae* isolated from burn wound infections. Lanes 1-20: DNA extracts of the isolated *K. pneumoniae*. Agarose gel of 1.5% was electrophoresed at 80 V for 45 min.

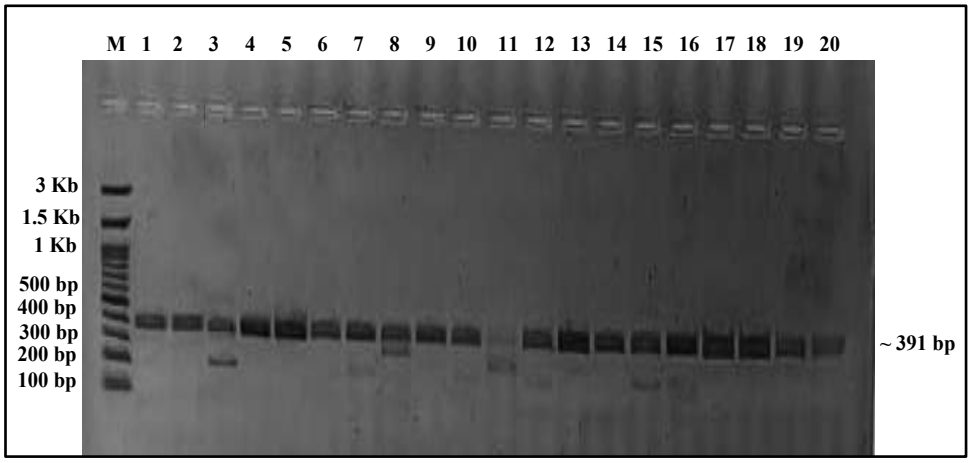


Figure 2: Agarose gel electrophoresis shows DNA bands of roughly 391 bp of the partially amplified *gapA* gene of *K. pneumoniae.* M: 100 DNA molecular size marker, lanes 1-20: burn wound samples positive for *K. pneumoniae.* Agarose gel of 1.5% was electrophoresed at 80 V for 45 min.

3.3 Antimicrobial Resistance

Table 1 illustrates *Klebsiella pneumoniae* isolates and the number of antibiotics they were resistant to and their ESBL status. In this study, 55% (11 out of 20) of *K. pneumoniae* isolates were multidrug resistant. While one isolate was resistant to 5 antibiotics. Some isolates (precisely 3) were resistant to 3 antibiotics and other 4 isolates showed resistance to 4 antimicrobials. Interestingly, 2 isolates were resistant to 11 antimicrobials (Figure 3) and another one resisted 14 antibiotics (Figure 4). The isolate which was resistant to 14 out of 16 antibiotics, known as extensively drug resistant (XDR), was susceptible to ertapenem and tigecycline only (Table 1).

Extended spectrum β-lactamase (ESBL) was found in 35% (7 out of 20) of *K. pneumoniae* isolates. It was noticed that ESBL was absent in the isolates susceptible to most of the antimicrobials. The opposite situation was correct for most of the multidrug resistant (MDR) bacteria which were positive for ESBL. However, 4 MDR bacteria were ESBL negative (Table 1).

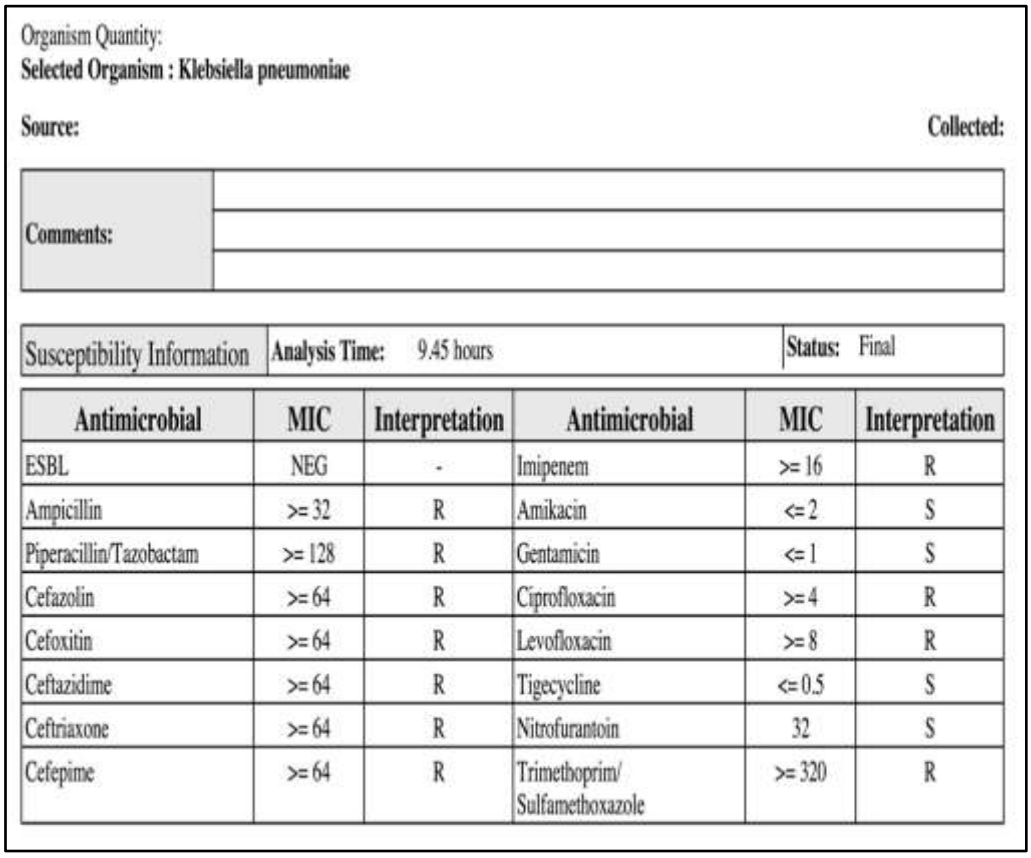
Most of the *K. pneumoniae* isolates (90%) of the present study were resistant to ampicillin. Regarding cefazolin and ceftriaxone, they were effective against only 55% and 45%, respectively of the isolates. Furthermore, 35% of the isolated bacteria were resistant to cefoxitin, whereas the resistance to nitrofurantoin was 30% (Table 2). High intermediate susceptibility (40%) of the isolates also occurred against nitrofurantoin. Ampicillin reported an intermediate susceptibility in 10% of the isolates versus 5% intermediate susceptibility exerted by each amikacin and gentamicin.

With the respect of antimicrobial susceptibility, all of the isolates were 100% sensitive to tigecycline. Ninety five percent of *K. pneumoniae* isolates showed susceptibility to each of amikacin and gentamicin. Each of ciprofloxacin, piperacillin/tazobactam, imipenem, levofloxacin and trimethoprim/sulphamethoxazole was effective against 85% of the isolates. Finally, 80% of the bacterial isolates were sensitive to both ceftazidime and cefepime. Table 2 shows details of the antimicrobial susceptibility profile of *K. pneumoniae* isolated from burn infections.

Table 1: *Klebsiella pneumoniae* isolates and their resistance to antibiotics and ESBL status

Isolate ID	No. of Antibiotics Resistant to	ESBL Status	Isolate ID	No. of Antibiotics Tesistant to	ESBL Status
1	1	-	**11**	1*	-
2	3*	+	**12**	4*	+
3	4*	+	**13**	4*	+
4	5	+	**14**	1*	-
5	4	-	**15**	1*	-
6	3	+	**16**	1*	-
7	11	-	**17**	1	-
8	1*	-	**18**	2	-
9	3*	-	**19**	14	+
10	11*	-	**20**	1*	-

*: means the isolate also shows intermediate susceptibility to another antibiotic.



Organism Quantity:
Selected Organism : Klebsiella pneumoniae

Source: **Collected:**

Comments:	

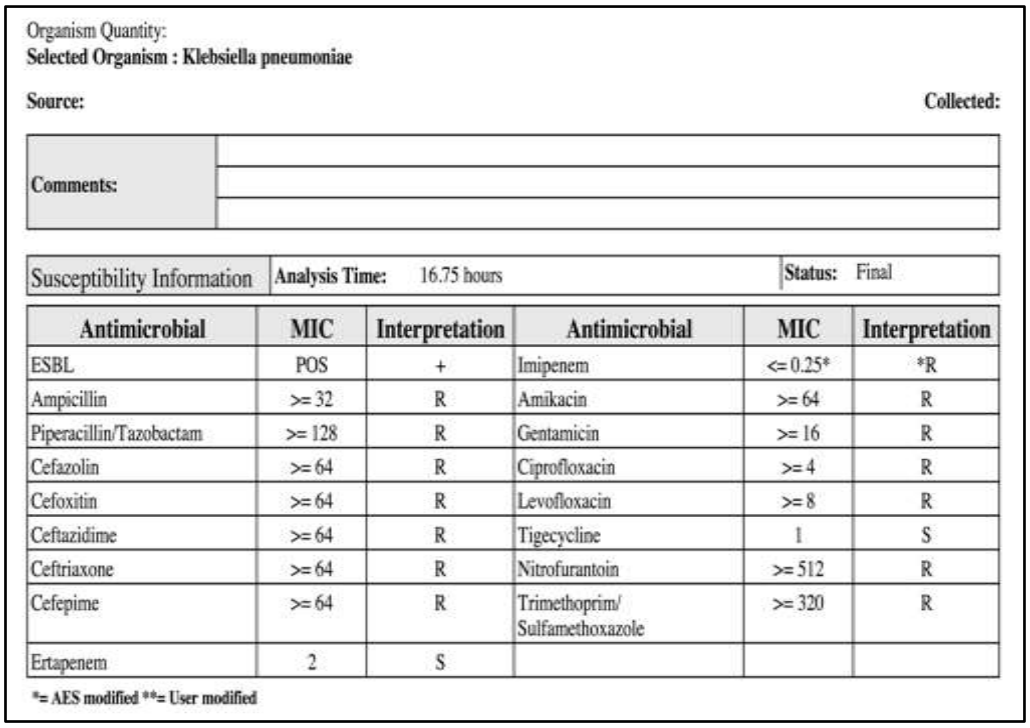
Susceptibility Information	**Analysis Time:** 9.45 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Imipenem	>= 16	R
Ampicillin	>= 32	R	Amikacin	<= 2	S
Piperacillin/Tazobactam	>= 128	R	Gentamicin	<= 1	S
Cefazolin	>= 64	R	Ciprofloxacin	>= 4	R
Cefoxitin	>= 64	R	Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantoin	32	S
Cefepime	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

Figure 3: An isolate of *K. pneumoniae* shows resistance to 11 out of 16 antimicrobial drugs.

Table 2: Antimicrobial susceptibility profile of *K. pneumoniae* isolated from burn wound infections.

Antimicrobial	Antimicrobial Class	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Ampicillin	Penicillins	18 (90%)	2 (10%)	**0**
Piperacillin/ Tazobactam	Penicillins	3 (15%)	0	**17 (85%)**
Cefazolin	1st generation cephalosporin	11 (55%)	0	**9 (45%)**
Cefoxitin	2nd generation Cephalosporin	7 (35%)	0	**13 (65%)**
Ceftazidime	3rd generation cephalosporin	4 (20%)	0	**16 (80%)**
Ceftriaxone	3rd generation cephalosporin	9 (45%)	0	**11 (55%)**
Cefepime	4th generation cephalosporin	4 (20%)	0	**16 (80%)**
Ertapenem	Carbapenem	0	0	**18 (90%)**
Imipenem	Carbapenem	3 (15%)	0	**17 (85%)**
Amikacin	Aminoglycoside	1 (5%)	1 (5%)	**18 (90%)**
Gentamicin	Aminoglycoside	1 (5%)	1 (5%)	**18 (90%)**
Ciprofloxacin	Fluoroquinolones	3 (15%)	0	**17 (85%)**
Levofloxacin	Fluoroquinolones	3 (15%)	0	**17 (85%)**
Tigecycline	Glycylcycline	0	0	**20 (100%)**
Nitrofurantoin	Nitrofuran	6 (30%)	8 (40%)	**6 (30%)**
Trimethoprim/ Sulfamethoxazole	Sulfonamides	3 (15%)	0	**17 (85%)**
ESBL	/	7 + (35%)		**13 – (65%)**



Organism Quantity:
Selected Organism : Klebsiella pneumoniae

Source: **Collected:**

Comments:

Susceptibility Information | **Analysis Time:** 16.75 hours | **Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Imipenem	<= 0.25*	*R
Ampicillin	>= 32	R	Amikacin	>= 64	R
Piperacillin/Tazobactam	>= 128	R	Gentamicin	>= 16	R
Cefazolin	>= 64	R	Ciprofloxacin	>= 4	R
Cefoxitin	>= 64	R	Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	Tigecycline	1	S
Ceftriaxone	>= 64	R	Nitrofurantoin	>= 512	R
Cefepime	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ertapenem	2	S			

*= AES modified **= User modified

Figure 4: An isolate of *K. pneumoniae* shows resistance to 14 out of 16 antimicrobial drugs (extensive drug resistance, XDR).

4. Discussion

Burn injuries occurred in 40 individuals in the current study; 24 females (60%) versus 16 males (40%); of different ages that ranged from 3 to 50 years. Out of 100 wound swabs collected from different burn areas, only 20 samples were positive to *Klebsiella pneumoniae*. If one positive specimen was taken into account from each patient with multiple *K. pneumoniae* isolates, then the incidence rate of this bacterium was 35% (14 out of 40 patients) which was relatively high. In comparison with another study performed in Baghdad, Iraq in 2011, where 70 burned patients were enrolled in the study, only one *Klebsiella* spp. isolate (incidence rate of 1.4%) was obtained out of 72 bacterial isolates grown from 45 patients positive for bacterial culture [33]. Another study conducted in Baghdad during the period from July to October 2018 revealed that the isolation rate of *K. pneumoniae* from burn wounds was 10% [34]. However, many studies agree that *K. pneumoniae* constitutes the second predominant bacterial pathogen associated with burn wound infections [14, 18, 28, 29, 35]. Still other researchers have different observations where *Klebsiella* spp. has ranked number three following *Pseudomonas aeruginosa* and *Acinetobacter* spp. from burn injuries [8, 10].

The use of different treatment practices throughout diverse geographical locations can be the cause behind the variations seen in bacteria isolated from burned patients [28]. In the current research, where 15 *K. pneumoniae* isolates were obtained from Al-Zahraa hospital, only 5 isolates were grown from Al-Karama hospital. The bacterial infections in burned patients have been reported by Mehta *et al.* [36] and Otta *et al.* [37] to differ with place and time as well as infection control measures [8]. In addition, the contaminants associated with wound infections have been suggested to vary with the specimen type [38]. This idea was partially confirmed in the present study where the specimens taken from different regions of the same patient (only 14 out of 40 patients) were not all positive to *K. pneumoniae* (Supplementary Table), despite the fact that research on additional bacterial species was not included in the scope of this study. Positive relationship has been found between burn infection and burn severity, including burn region, depth, severity scores, and presence of inhalation injury [10].

In the current investigation, the VITEK®2 system was used for antimicrobial susceptibility testing to look at the isolates' susceptibility as it provided accurate findings and eliminated the need for human analysis and later preventing erroneous results [39]. The data of this study showed that 55% of the isolated *K. pneumoniae* were multidrug resistance (MDR) which is a term used to refer to a pathogen resistance to more than 3 classes of antibiotics [40]. A higher rate of approximately 83.9% (52 out of 62) of the isolates of *K. pneumoniae* was MDR in Nigeria [41]. In comparison with the study of Kabanangi *et al.* [8] in Tanzania, all *Klebsiella* spp. isolates were found to be MDR.

The isolates of the current study, even from the same patient, differed in their resistance profile, where 4 isolates were found to be resistant to 4 different antimicrobials, whereas other 3 isolates showed resistance to various 3 antibiotics, and one isolate revealed resistance to 5 antibiotics. This is congruent with the findings of Azimi *et al.* [22] who indicated that among 18 burn victims many specimens had distinct patterns of antibiotic resistance. From the author's point of view, the reason behind that could be due to the presence of different strains of *K. pneumoniae* colonizing in different burned regions, or the cause might be linked to the ability of these bacteria to form biofilm as it is well-known that the biofilm production renders the bacteria to be antibiotic resistant. However, Nirwati *et al.* [42] found no significant correlation existing between biofilm formation and MDR in *K. pneumoniae.* Adding to this is the depth of the burned area compared to superficial burn wounds and other factors associated with burn severity mentioned above.

The occurrence and selection of MDR among bacteria circulating in the hospital environment has been attributed to the prolonged use of antibiotics leading to treatment failure and increased complications [24]. Likewise, based on numerous studies, an extended hospital stay was thought to increase the chance of MDR bacterial infections [7, 12, 43]. Importantly, this study also reported presence of an isolate that resisted 11 antimicrobials; and another isolate was found to be resistant to 14 drugs, i.e., extensively drug resistant (XDR) isolate which was merely susceptible to ertapenem and tigecycline. Nevertheless, the present study did not witness the isolation of pandrug resistant (PDR) isolates which are characterized by being resistant to all antibiotics in each antibacterial category [23]. Gupta *et al.* [28] referred to the advent of pandrug resistant as well as extensively drug-resistant bacteria where the isolates were resistant to each of the frequently applied antimicrobials and new generation antibiotics.

Furthermore, 35% (7/20 isolates) of *K. pneumoniae* isolated in the current study had the ESBL which is considered as a main antibiotic resistance mechanism. However, this percentage was lower than that reported by Gong *et al.* [43] who found >90% of these bacteria produced ESBL that inactivated broad spectrum of antibiotics. As this enzyme is encoded by a gene harboured on a plasmid, the antibiotic resistance can be spread horizontally from one bacterial species to another [18]. Moreover, the antibiotic resistance can be conferred by other mechanisms, e.g., the production of aminoglycoside modifying enzymes, target site modification, efflux pumps over-expression and reducing drug penetrance [10]. Thus, the presence of diverse antibiotic resistance mechanisms in bacteria may also explain the different profiles of resistance registered here.

The findings of the current work further denote high resistance (90%) to ampicillin. This is consistent with another study carried out in Baghdad, Iraq, where *K. pneumoniae* isolated from different sources was documented to be unaffected by many antibiotics, including ampicillin and ceftazidime [44]. The isolates of this study also exerted varying degrees of resistance against the four generations of cephalosporins. For instance, resistance of 55%, 45%, 35%, 20% and 20% appeared towards cefazolin, ceftriaxone, cefoxitin, ceftazidime, and cefepime respectively. This is partly because Gram-negative bacteria, including *Klebsiella* spp., produce significant amounts of cephalosporinase type 1 upon exposure to ampicillin, penicillin G and first-generation Cephalosporins, as a result, this enzyme easily hydrolyzes these agents [45]. Compared to the study of Abdulkadir *et al.* [46] the isolates obtained from different samples (urine, stool, wound swab, blood, and sputum) in Baghdad city during February till May 2014, were susceptible to meropenem (90.5%) and imipenem (77.3%), but less susceptible to the third generation cephalosporin. In contrast, a study performed recently in Nigeria found that all *K. pneumoniae* isolates were highly susceptible to all classes of carbapenems, involving ertapenem (91%), meropenem (96%) and imipenem (99%) [47].

While the isolates of this study revealed resistance rate of 30% against nitrofurantoin, a lower resistance of 15% was developed by the isolates against ciprofloxacin, despite it was 100% effective and the most efficient medication for treating most Gram-positive and -negative burn wound isolates in the study by Alwan *et al.* [33]. With respect to imipenem which belongs to carbapenem, this study revealed that 15% of the isolates had resistance to this antimicrobial agent. This result is rather similar to that of Perween *et al.* [18] who observed resistance rate of 16%. By contrast, a lower resistance of 5% was registered by Sanchez *et al.* [48], and no resistance at all was reported by Bayraam *et al.* [49] and Ronat *et al.* [50]. Conversely, all of the isolates of the current study were 100% sensitive to tigecycline, a broad-spectrum semi-synthetic glycylcycline that has recently being commercialized [51]. Nevertheless, 90% of the

isolated bacteria were susceptible to ertapenem, and 95% of the isolates showed susceptibility to each of amikacin and gentamicin.

The limitations of this study include: first, it was performed on only two burn hospitals in Al-Kut city, Iraq. Second, it involved only 40 burned patients, and lastly molecular techniques were not applied to analyse the presence of genes responsible for antimicrobial resistance, though the results of the present research could provide preliminary evidence of antimicrobial resistance and the alternative therapy. Moreover, the appropriate and repeated detection of microorganisms, particularly bacteria, in different burn wound samples is very important to the clinicians.

5. Conclusion

To conclude, most *K. pneumoniae* contaminating the burn wards in the Iraqi hospitals are MDR, and that tigecycline was the most effective antibiotic against all of the isolates. Routine examination of the antimicrobial resistance is a significant demand to choose the drug of choice or combination therapies for complete clearance of these bacteria.

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Author Contribution

Z.E.S. carried out the experiments. I.J.L. conceived the study, supervised and analysed the experiments, and wrote the manuscript. O.A.I. contributed to writing the final form of the manuscript.

Conflict of Interest

No conflicts of interest have been declared by the authors.

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Supplementary Table: Information about burned patients, their sex, age, the samples region and the hospitals from where they were collected.

Patient No.	Sex	Age	Burn region	Sample ID	*Klebsiella pneumoniae*	Hospital 1	Hospital 2
1	F	31	Hand	1	-		/
			Abdomen	2	-		/
			Back	3	+		/
			Foot	4	-		/
2	F	9	Abdomen	5	-		/
			Thoracic	6	-		/
			Back	7	+		/
3	F	11	Hand	8	-		/
			Abdomen	9	-		/
			Back	10	-		/
4	M	6	Hand (R)	11	-		/
			Hand (L)	12	-		/
5	M	35	Hand	13	-	/	
			Leg	14	-	/	
6	F	40	Leg	15	+		/
			Foot	16	-		/
7	F	23	Finger	17	+	/	
8	F	10	Abdomen	18	-	/	
			Back	19	-	/	
			Leg	20	-	/	

9	M	30	Hand	21	-	/	
10	F	5	Hand	22	+		/
11	F	20	Abdomen	23	-		/
			Thoracic	24	+		/
12	F	15	Leg	25	+	/	
13	F	25	Hand	26	-	/	
14	F	19	Hand	27	-	/	
15	F	45	Face	28	+		/
			Leg	29	-		/
			Hand (L)	30	+		/
			Hand (R)	31	-		/
			Back	32	+		/
			Neck	33	+		/
			Thoracic	34	+		/
16	M	10	Hand (R)	35	-	/	
			Hand (L)	36	-	/	
17	M	4	Hand	37	+	/	
18	F	38	Leg (R)	38	+	/	
			Leg (L)	39	-	/	
19	M	20	Hand (L)	40	-		/
			Hand (R)	41	-		/
20	M	7	Hand (L)	42	-		/
			Hand (R)	43	+		/
21	M	13	Finger	44	-		/
22	F	33	Thoracic	45	+		/
			Abdomen	46	-		/
23	F	37	Face	47	+		/
			Back	48	+		/
			Flank (R)	49	-		/
			Flank (L)	50	-		/
24	F	48	Leg (R)	51	+	/	
			Leg (L)	52	-	/	
			Hand (R)	53	-	/	
25	M	3	Hand (R)	54	-	/	
			Hand (L)	55	-	/	
26	F	24	Thoracic	56	-		/
			Abdomen	57	-		/
			Back	58	-		/
			Leg (L)	59	-		/
			Hand (R)	60	-		/
27	F	38	Hand	61	-		/
			Arm (L)	62	-		/
			Arm (R)	63	-		/
			Hand	64	-		/
			Shoulder	65	-		/
			Hand (L)	66	-		/
			Hand (R)	67	-		/
			Leg (L)	68	-		/
			Leg (R)	69	-		/

			Abdomen	70	-		/
			Back	71	-		/
			Thoracic	72	-		/
			Flank (R)	73	-		/
			Flank (L)	74	-		/
			Face	75	+	/	
28	F	50	Hand (R)	76	-	/	
			Hand (L)	77	-	/	
			Foot (R)	78	-	/	
29	M	9	Foot (L)	79	-	/	
			Leg	80	-	/	
30	M	11	Leg	81	-	/	
31	M	30	Hand (L)	82	-	/	
			Hand (R)	83	-	/	
32	F	42	Face	84	-	/	
33	M	6	Hand	85	-	/	
			Hand (R)	86	-	/	
34	F	20	Finger	87	-	/	
			Arm	88	-	/	
35	F	11	Face	89	-	/	
			Hand	90	-	/	
			Face	91	-	/	
36	F	16	Hand	92	-	/	
			Back	93	-	/	
			Leg	94	-	/	
37	M	31	Hand	95	-	/	
38	M	27	Hand	96	-	/	
39	M	7	Hand (L)	97	-	/	
			Hand (R)	98	-		/
40	F	16	Thoracic	99	-		/
			Back	100	-		/
Total				**100**	**20**	**43**	**57**

F: Female, M: Male, L: Left, R: Right. Note/ due to ethical issues, the hospitals names are referred to as by numbers 1 and 2 instead of their real names.