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PREVALENCE OF MULTIDRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATES FROM INFECTED WOUNDS AND BURNS AT A TERTIARY CARE HOSPITAL IN SYRIA

Razan AL Debs* and Mohammad Maarouf

Department of Biochemistry and Microbiology, Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic

Klebsiella pneumoniae is a critical opportunistic pathogen and a major concern for public health, associated with a broad spectrum of hospital-acquired infections that are often resistant to conventional therapies. This study aimed to evaluate the prevalence and antimicrobial resistance patterns of Klebsiella pneumoniae isolated from patients with wound and burn infections.

A total of 90 clinical specimens were collected from patients admitted to the General Surgery and Burn & Plastic Surgery Departments. Identification of Klebsiella pneumoniae was conducted based on colony morphology, Gram staining, and biochemical tests following cultivation on selective media. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and multidrug-resistant (MDR) isolates were characterized.

Klebsiella pneumoniae was isolated in 30 out of 90 samples, representing an isolation rate of 33.33%. The majority of isolates (65.6%) were from male patients. The isolates exhibited high susceptibility to colistin (63.3%) but were highly resistant to most other antibiotics tested. All isolates displayed 100% resistance to Aztreonam, Ampicillin/Sulbactam, and Erythromycin. Moreover, all isolates were classified as MDR, with resistance phenotypes ranging from four groups of antibiotics (3.33%) to eleven groups (13.33%).

The study reveals the high prevalence and extensive antimicrobial resistance of Klebsiella pneumoniae in wound and burn infections, and its significant role in nosocomial infections that are challenging to manage due to multidrug resistance.

Consequently, the research emphasizes the necessity for focused prevention and monitoring strategies, along with the judicious and targeted use of antibiotics, to help mitigate the spread of these resistant strains.

Keywords: *Klebsiella pneumoniae, multidrug resistant (MDR), wound infections, burn infections*

INTRODUCTION

Any disruption in the integrity of the skin increases the risk of infections that can adversely affect the healing process[1]. Various types of wounds can be distinguished, including post-surgical wounds, trauma-related wounds, and burn wounds[2]. Burns and wounds create a moist, nutrient-rich environment that facilitates microbial colonization and proliferation at the site of injury[3]. The Centers for Disease Control and Prevention (CDC) has reported a global rise in infections caused by MDR pathogens, posing a significant threat to healthcare systems and contributing to the failure of antibiotic therapies[4,5]. Wound and burn infections are major public health issues, being leading causes of morbidity and mortality, particularly in developing countries[6,7]. These infections are often polymicrobial, with *Klebsiella pneumoniae* being one of the predominant Gram-negative

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*Corresponding author: Razan AL Debs, E-mail: pharmarazma@gmail.com

bacilli isolated from such infections[8]. *Klebsiella pneumoniae* is a Gram-negative, encapsulated bacillus from the family *Enterobacteriaceae*. It is facultatively anaerobic, ferments sugars, and produces mucoid colonies on culture media[9]. As an opportunistic pathogen, *Klebsiella pneumoniae* primarily affects immunocompromised individuals, and is a major cause of nosocomial infections, rapidly spreading among hospitalized patients and leading to outbreaks. It has been reported as the second most significant threat to public health, following *Escherichia coli* according to the World Health Organization (WHO) in 2014[10,11,12].

Klebsiella pneumoniae is frequently isolated from surgical site infections and is notorious for its high level of antibiotic resistance[13]. Studies have demonstrated the extensive resistance of *Klebsiella pneumoniae* isolates from hospitalized patients[14]. This bacterium is responsible for a variety of infections, including urinary tract infections, wound infections, otitis media, meningitis, neonatal sepsis, and bacteremia[15]. *Klebsiella pneumoniae* exhibits intrinsic resistance to penicillin due to its production of the penicillinase enzyme. It can also easily acquire resistance genes for other classes of antibiotics, contributing to its resistance to β-lactam antibiotics through the production of extended-spectrum β-lactamases (ESBLs), which has facilitated its widespread presence in hospitals and contributed to increased mortality rates[16].

In recent years, *Klebsiella pneumoniae* has shown rising resistance to several classes of antibiotics, including third-generation cephalosporins, carbapenems, and aminoglycosides, making it a multidrug resistant pathogen[17]. A bacterial strain is classified as MDR when it demonstrates resistance to at least one agent in three or more different antibiotic classes[18].

Variations in the bacterial species associated with wound and burn infections, as well as their antibiotic resistance patterns, can significantly influence treatment strategies and available therapeutic options[19].

In recent decades, antimicrobial resistance (AMR) has significantly increased among bacteria in the *Enterobacteriaceae* family, which includes *Klebsiella spp.*, *Escherichia coli*, and others. *Enterobacteriaceae* are normal inhabitants of the gut flora and are important infectious agents in both hospital and community settings. These bacteria are easily transmitted between humans through hand contact, contaminated food or drink, and environmental sources. They can acquire antimicrobial resistance via plasmids, transposons, or other mobile resistance elements[20].

AMR is a significant issue in the Middle East, particularly due to the high prevalence of MDR infections, especially among Gram-negative bacteria. There has been a concerning rise in antibiotic resistance across Middle Eastern Arab countries, creating serious challenges for healthcare providers treating infectious diseases. Additionally, antibiotic-resistant nosocomial infections (NIs) are becoming increasingly common in the region, with surgical site infections (SSIs) and bloodstream infections (BSIs) being the most frequently reported types[21].

The studies indicated that resistance rates in low-income countries are higher than those in high-income countries. Additionally, there are significant data gaps in many low-income settings, where the resistance situation may be worse than what the collected data shows[22].

In a study of Carbapenem-resistant *Enterobacterales* from clinical samples collected in Qatar between April 2014 and November 2017, it was found that 81 isolates, or 54.4%, were *K. pneumoniae*[23]. Another study conducted on samples from hospitals in Saudi Arabia revealed that *K. pneumoniae* was present in 87.86% of the isolates, with 89.9% of these isolates being Carbapenem-resistant[24].

Recently, the war in the Middle East has created significant economic challenges that are severely impacting the health care system. As a result, many individuals are suffering from serious and life-threatening infections due to a shortage of essential medications, such as antibiotics, in various regions. Additionally, the inability to implement newer and more advanced diagnostic methods has exacerbated this widespread issue. Moreover, the improper use of antibiotics has led to increased antibiotic resistance, particularly in Syria, a war zone known for a high incidence of hospital-acquired infections.

In Syrian hospitals, physicians seldom assess antibiotic susceptibility. Instead, they

often resort to high doses of new-generation broad-spectrum antibiotics to manage infections. This practice overlooks the potential consequences of developing bacterial resistance, which can arise from inappropriate antibiotic use and inadequate methods for identifying and isolating resistant strains.

In Damascus, 87% of pharmacists sell antibiotics without requiring a prescription, while only 3% refuse to dispense them without a doctor's approval. This practice has contributed to an increase in antibiotic resistance in Syria and has led to a reduced effectiveness of antibiotics compared to the period before the conflict. Antibiotic resistance is known to spread to other countries, particularly through travel or migration from conflict zones to Europe. This situation could potentially create new strains of MDR bacteria in European nations and elsewhere. Therefore, the issue of MDR in Syria is no longer a purely national problem; it has become an international concern with significant financial implications[25].

Given the widespread prevalence of *Klebsiella pneumoniae* in hospitals and its involvement in hard-to-treat infections due to its multidrug resistance, this study aims to assess the prevalence of this pathogen in patients with wounds and burns. Additionally, the study seeks to characterize its antibiotic susceptibility profiles to improve therapeutic options and develop effective treatment plans for MDR strains.

MATERIALS AND METHODS

A total of 90 clinical samples were collected from patients admitted to the General Surgery and Burn & Plastic Surgery Departments at Al-Mouwasat University Hospital in Damascus after obtaining informed consent. The trial was approved by the Research Ethics Committee (UDDS-528-18062019/SRC-1810).

The collection took place between November 2023 and February 2024. Samples were taken using sterile swabs from infection sites in patients whose wounds exhibited signs of infection. Each sample was labeled with the patient's name and gender. The swabs were immediately placed into sterile transport media to preserve bacterial viability until they were transported to the laboratory[26].

Isolation and Identification of Bacteria

Isolation

Bacterial identification was made by culturing the samples on selective media, followed by Gram staining and biochemical testing to confirm the bacterial species. Samples were cultured on Eosin Methylene Blue (EMB) agar sourced from Himedia Laboratories, India. The medium was prepared according to the manufacturer’s instructions. Streak plate technique was employed, and plates were incubated at 37°C for 24 hours.

Identification

Once pure bacterial colonies were obtained, morphological characteristics were studied. Colonies of *Klebsiella pneumoniae* on EMB agar appeared pink with a highly mucoid with a dark center. Microscopic examination revealed the characteristic Gram-negative, non-motile bacilli of *Klebsiella pneumoniae.*

Fig. 1: *Klebsiella pneumoniae* colonies on EMB agar.

Further biochemical tests were conducted on pure cultures from isolated colonies using media from Himedia Laboratories, India. The identification was confirmed by performing tests according to Cowan and Steel's Manual for the Identification of Medical Bacteria[27]. Tests included citrate utilization, urease hydrolysis, glucose and lactose fermentation, and negative indole production. **Table (1)** lists the biochemical tests used for identifying *Klebsiella pneumoniae*.

Table 1: Identification tests for *Klebsiella pneumoniae.*

Characteristics	***Klebsiella pneumoniae***
Shape	Bacillus
Gram Staining	Negative
Motility	Negative
Oxidase	Negative
Catalase	Positive
Indole Production	Negative
Methyl Red	Negative
Voges Proskauer	Positive
Citrate	Positive
Glucose	Positive
Lactose	Positive
H2S	Negative

Antibiotic Susceptibility Testing of *Klebsiella pneumoniae*

The Kirby-Bauer disk diffusion method was employed to assess antibiotic susceptibility. Bacterial suspensions were prepared from each strain of *Klebsiella pneumoniae* colonies with a turbidity of 0.5 McFarland standard, which corresponds to a bacterial load of approximately 1.5×10^8 CFU/ml. The suspension was evenly spread on the surface of Mueller-Hinton agar plates and with no gaps. Antibiotic discs were then placed on the agar surface using sterile forceps, and the plates were incubated at 37°C for 24 hours. **Table (2)** shows used antibiotic discs belonging to different antibiotic classes.

After incubation, inhibition zones around each disc were measured to determine whether the isolates were susceptible (S), intermediate (I), or resistant (R) to the antibiotic. The inhibition zone diameters were compared with standard values according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[28]. Isolates were considered MDR if they exhibited resistance to at least one agent in three different antibiotic classes[18].

Table 2: Names and classes of antibiotics used in the study.

Antibiotic	**Class**
Ampicillin /Sulbactam	Penicillin's
Cefepime	Cephalosporin's
Cefoperazone	
Ceftazidime	
Aztreonam	Monobactams
Amikacin	Aminoglycosides
Gentamycin	
Ciprofloxacin	Flouroquinolones
Colistin	Polymyxins
Cotrimoxazole	Sulfonamides
Doxycycline	Tetracyclines
Tigecycline	
Chloramphenicol	Chloramphenicol
Imipenem	Carbapenems
Meropenem	
Erythromycin	Macrolides

Statistical analysis

Microsoft Excel was used to illustrate the results obtained with appropriate charts.

SPSS® Statistics v24 was used to Conduct statistical analysis. Independent Student's t-test was used to evaluate the statistical differences in resistance rates between departments, considering the significance level value to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Distribution of Samples

The 90 samples collected from patients exhibiting signs of infection at the wound site were distributed as follows: 41 from the General Surgery Department and 49 from the Burn & Plastic Surgery Department, as shown in **Fig 2**.

Of the 90 samples collected, 59 (65.6%) were from male patients, while the remaining 33 (34.4%) were female patients.

Of the 90 samples collected, the mean age was 47.3 ± 11.9 years and the age ranges from 15-71 years.

Prevalence of *Klebsiella pneumoniae* Isolates

Klebsiella pneumoniae was isolated from 33.33% of the samples (30 out of 90). Of these, twenty isolates were from the Burn & Plastic Surgery Department, and ten isolates were from the General Surgery Department, as shown in **Fig 2.**

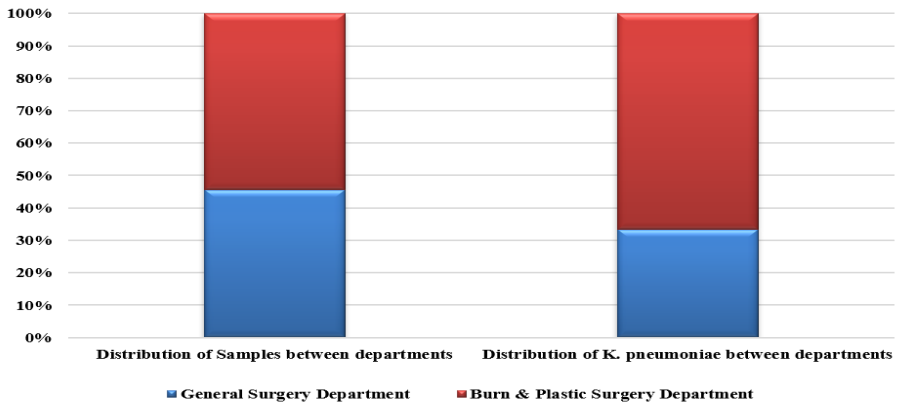

Fig. 2: The distribution rate of samples and prevalence of *k. pneumonia* between departments.

Antibiotic Susceptibility and Resistance of *Klebsiella pneumoniae* Isolates

All *Klebsiella pneumoniae* isolates exhibited 100% resistance to Ampicillin/Sulbactam, Aztreonam, and Erythromycin. High levels of resistance were also observed to third- and fourth-generation Cephalosporins, with resistance rates ranging from 86.7% to 96.7%.

Additionally, 93.3% of the isolates were resistant to Cotrimoxazole. Moderate resistance, ranging from 60% to 66%, was noted for Aminoglycosides. However, 63.3% of isolates were susceptible to Colistin, as shown in **Table (3)**.

Table 3: Susceptibility and Resistance of *Klebsiella pneumoniae* Isolates to antibiotics.

Antibiotic	Resistant R (%)		Intermediate I (%)		Susceptible S (%)	
Amikacin	18	(60)	3	(10)	9	(30)
Ampicillin /Sulbactam	30	(100)	0	(0)	0	(0)
Aztreonam	30	(100)	0	(0)	0	(0)
Cefepime	26	(86.7)	1	(3.3)	3	(10)
Cefoperazone	29	(96.7)	0	(0)	1	(3.3)
Ceftazidime	29	(96.7)	0	(0)	1	(3.3)
Chloramphenicol	22	(73.3)	3	(10)	5	(16.7)
Ciprofloxacin	22	(73.3)	3	(10)	5	(16.7)
Colistin	5	(16.7)	6	(20)	19	(63.3)
Cotrimoxazole	28	(93.3)	0	(0)	2	(6.7)
Doxycycline	25	(83.4)	1	(3.3)	4	(13.3)
Erythromycin	30	(100)	0	(0)	0	(0)
Gentamicin	20	(66.7)	3	(10)	7	(23.3)
Imipenem	22	(73.4)	1	(3.3)	7	(23.3)
Meropenem	21	(70)	1	(3.3)	8	(26.7)
Tigecycline	12	(40)	2	(6.7)	16	(53.3)

Resistance Profiles of *Klebsiella pneumoniae* Isolates

All *Klebsiella pneumoniae* isolates studied were classified as MDR. A total of 17 distinct antibiotic resistance phenotypes (Antibiotypes) were identified among the MDR *Klebsiella pneumoniae* isolates. The prevalence of these Antibiotypes ranged from 3.33% for isolates resistant to four classes of antibiotics, to 33.33% for isolates resistant to ten classes, as summarized in **Table (4)**.

Comparison of Antibiotic Resistance between Departments

The number of antibiotic classes to which the isolates were resistant ranged from 6 to 11 classes in Burn & Plastic Surgery Department, and from 4 to 10 antibiotic classes in General Surgery Department.

The significance level value is greater than 0.05 between the departments in the research sample, meaning that at the 95% confidence level there are no statistically significant differences in the resistance rates between the Burn & Plastic Surgery and General Surgery departments as shown in **Table (5)**.

Table 4: Antibiotypes of MDR *Klebsiella pneumoniae* isolates.

Antibiotype	Isolate No.	Aminoglycsides	Penicillins	Monobactams	Cephalosporins	Chloramphenicols	Flouroquinolones	Polymyxins	Sulfonamides	Macrolides	Carbapenems	Tetracyclines	Number of Antibiotic Classes	%
A1	*Kp* 5		R	R	R					R			4	3.33
A2	*Kp* 2		R	R	R				R	R		R	6	3.33
A3	*Kp* 10		R	R	R	R			R	R		R	7	16.66
A4	*Kp* 16	R	R	R	R				R	R	R		7	
A5	*Kp* 17		R	R	R	R	R		R	R			7	
A6	*Kp* (29, 30)		R	R	R		R		R	R		R	7	
A7	*Kp* 1		R	R	R	R			R	R	R	R	8	16.66
A8	*Kp* 3	R	R	R	R		R		R	R	R		8	
A9	*Kp* 4		R	R		R	R	R	R	R		R	8	
A10	*Kp* 15	R	R	R	R				R	R	R	R	8	
A11	*Kp* 27		R	R	R	R	R			R	R	R	8	
A12	*Kp* 12		R	R	R	R	R		R	R	R	R	9	13.33
A13	*Kp* 23	R	R	R	R		R		R	R	R	R	9	
A14	*Kp* (20, 28)	R	R	R	R	R			R	R	R	R	9	
A15	*Kp* (6, 8, 9, 14, 19, 21, 24, 25, 26)	R	R	R	R	R	R		R	R	R	R	10	33.33
A16	*Kp* 11	R	R	R	R	R	R	R	R	R		R	10	
A17	*Kp* (7, 13, 18, 22)	R	R	R	R	R	R	R	R	R	R	R	11	13.33

Table 5: Results of Student's t-test to study the differences in resistance rates between departments

	Department	Number of antibiotics classes	Mean	Standard Deviation	T- value	P- value	Decision
Antibiotic Resistance	Burn & Plastic Surgery	11	9.25	1.44	1.98867	0.056591	The difference is not statistically significant
	General Surgery	11	8	1.789			

Discussion

The isolation rate of *Klebsiella pneumoniae* in this study was 33.33%, aligning with the results of Nakamura *et al.* (2012), where the isolation rate was 28.58%[29]. However, this rate was notably higher than that reported by Gharrah *et al.* (2017), where *Klebsiella pneumoniae* was isolated in only 15% of cases[30].

The predominance of male patients in this study could be attributed to the higher exposure of males to injuries, likely due to the nature of the professional activities they engage in, which is consistent with findings from Michael Pack *et al*. (2011)[31].

The resistance rate to Ceftazidime in this study was 96.7%, significantly higher than the 65.5% reported by Alam *et al.* (2021)[8]. Similarly, the resistance to Cefepime in our isolates was also higher than that reported by Kaapu *et al.* (2022), whereas the resistance rates to Cotrimoxazole in both studies were similar, with the isolates in Kaapu's study showing a resistance rate of 95.7%[32].

As for Chloramphenicol, the resistance rate in our study was higher than in both the Aljanabi study (2016) and the Woldu study (2015)[33,34]. Furthermore, resistance to Ciprofloxacin was also higher in our isolates compared to those in the study by Hashemi *et al.* (2014)[35].

Regarding Gentamicin resistance, the isolates in our study exhibited a lower resistance rate than those reported by Kaapu *et al.* (2022), but a higher rate than that found in the study by Wadekar e*t al.*[32,36]. Resistance to Colistin in our study was 16.7%, which was lower than the 40.9% reported by Nobel *et al.* (2022)[37]. However, our isolates showed higher resistance to Meropenem compared to the resistance rates reported in the studies by Nobel, Alam, and Aljanabi[8,33,37].

The high resistance observed in our *Klebsiella pneumoniae* isolates, as well as the variability in antibiotic susceptibility across studies, may be attributed to differences in the used treatment protocols. Additionally, the widespread and inappropriate use of antibiotics can contribute to the development of resistance mechanisms in bacteria, depending on the antibiotics used, leading to the emergence of MDR strains.

The antibiotic resistance phenotypes (Antibiotypes) observed in our *Klebsiella pneumoniae* isolates underscore the increasing prevalence of MDR strains in the studied hospital. This finding is in agreement with the study by Loper *et al.*, which identified 14 distinct Antibiotypes[38].

The diversity in resistance phenotypes can be explained by *Klebsiella pneumoniae* employing various mechanisms to resist antibiotics used. This is further reinforced by the horizontal transfer of resistance genes between strains.

Conclusions

This study emphasizes the significant presence of *Klebsiella pneumoniae* infections among patients admitted to the wound and burn units of the hospital. The high infection rate contributes to prolonged hospital stays and increases the risk of cross-infection among other patients. The isolated strains demonstrated multidrug resistance, with resistance spanning four to eleven classes of antibiotics. This highlights the ability of Klebsiella pneumoniae to acquire and develop multiple resistance mechanisms, severely limiting available treatment options.

Therefore, to improve patient survival rates, it is crucial to reduce the transmission of Klebsiella pneumoniae. This can be achieved by implementing effective infection control methods in healthcare settings.

Key strategies include maintaining a sterile environment through cleaning and

disinfection of surfaces to ensure the eradication of pathogens. Additionally, medical equipment should be properly sterilized, and the integrity of air filters in healthcare facilities must be ensured, as Klebsiella pneumoniae is often associated with healthcare environments such as sinks and medical facilities. In addition, the direct transmission of Klebsiella pneumonia between patients requires early identification of infections caused by these strains.

Early identification of infections caused by these strains is necessary because of direct transmission between patients. This can be accomplished using accurate modern diagnostic methods, such as molecular techniques. Following diagnosis, isolating carriers and infected patients can help limit the spread within healthcare settings.

Antimicrobial stewardship is also essential in reducing the risk of infections associated with multidrug resistance that can arise from the selective pressure of antibiotic use. This can be achieved by conducting susceptibility tests to prescribe appropriate antibiotics, monitoring their usage, and evaluating treatment outcomes to assess efficacy. Additionally, restrictions should be imposed on the improper dispensing of antibiotics.

Furthermore, molecular methods should be employed to detect resistance genes, as these resistance elements can be carried on transferable genetic elements, facilitating their transmission within hospital environments. Phenotypic resistance tests cannot distinguish between chromosomal and plasmid-encoded genes; therefore, genotyping should be integrated to adopt protocols for prescribing antibiotics that enhance appropriate use and ensure accuracy.

These efforts are vital to curbing the development of resistance and the spread of these multidrug-resistant strains.

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انتشار سلالات الكليبسيلا الرئوية متعددة المقاومة المعزولة من إنتانات الجروح والحروق في مستشفى للرعاية الثالثية في سورية

رزان الدبس* – محمد معروف

قسم الكيمياء الحيوية والأحياء الدقيقة، كلية الصيدلة، جامعة دمشق، دمشق، الجمهورية العربية السورية

تعد الكليبسيلا الرئوية من أهم العوامل الممرضة الانتهازية التي تهدد الصحة العامة، وتتسبب بطيف واسع من الإنتانات المستشفوية المعندة على العلاج. هدفت هذه الدراسة إلى تقصّي انتشار جراثيم الكليبسيلا الرئوية وتقييم أنماط مقاومتها لدى المرضى الذين يعانون من إنتانات الجروح والحروق. تم جمع ٩٠ عينة وتحديد وجود الكليبسيلا الرئوية من خلال تلوين غرام وشكل المستعمرات والاختبارات الكيميائية الحيوية. تم تحديد حساسية السلالات المعزولة تجاه المضادات الحيوية باستخدام طريقة الانتشار من الأقراص الورقية (Kirby-Bauer disk diffusion method)، وتم تحديد العزلات ذات المقاومة المتعددة MDR. أظهرت النتائج وجود الكليبسيلا الرئوية في ٣٠ عينة (٣٣.٣٣%)، وكانت غالبية العينات من المرضى الذكور (٦٥.٦%). أظهرت معظم العزلات حساسية تجاه الكوليستين (٦٣.٣%)، إلا أنها أبدت مقاومة عالية تجاه أغلب المضادات الحيوية، ومقاومة بنسبة ١٠٠% تجاه كل من الأزتريونام (Aztreonam)، والأمبيسيلين/سولباكتام (Ampicillin/Sulbactam)، والإريثرومايسين (Erythromycin). كانت جميع العزلات متعددة المقاومة (MDR)، وتراوحت أنماط مقاومتها بين ٤ إلى ١١ زمرة من المضادات الحيوية.

تظهر الدراسة الانتشار الواسع والمقاومة العالية للمضادات الحيوية لجراثيم الكليبسيلا الرئوية في عداوى الجروح والحروق، ودورها المهم في العداوى المستشفوية والتي يصعب علاجها بسبب مقاومتها المتعددة للأدوية. وبالتالي، يؤكد البحث على ضرورة وضع استراتيجيات الوقاية والمراقبة المركزة، إلى جانب الاستخدام الحكيم والمستهدف للمضادات الحيوية، للمساعدة في الحد من انتشار هذه السلالات المقاومة.