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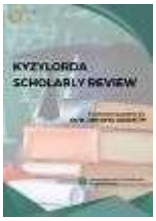

Investigation of Antibiotic Resistance and some Virulence Genes in Klebsiella Pneumoniae Isolated from Post-Plastic Surgery Patients

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Abstract. Post-operative wound infections after plastic surgery and reconstructive surgery are prevalent and can be serious, especially if the infection is due to multi drug resistant bacteria. Of these pathogens, Klebsiella pneumoniae is an important surgical site infection pathogen. The present study was designed to study the prevalence of K. pneumoniae in surgical wound infections, its antimicrobial susceptibility and selected phenotypic and genotypic virulence factors. A descriptive cross-sectional study was conducted on 150 patients who underwent plastic surgery in Azadi hospital in the period from March to October 2026. Conventional and differential media were used to culture wound swab specimens. Biochemical tests and DL system were used to identify the bacteria and the Kirby–Bauer disk diffusion method was used to determine antimicrobial resistance. Phenotypic determination of biofilm, capsule, proteinase, hemolysin, gelatinase and hyaluronidase activities were carried out. Also, the virulence genes magA, mrkD, hly and t1fim were studied. Of the 150 samples, 85 (56.7%) were found to contain bacteria. Out of 85 bacterial isolates, 17 (20.0%) were found as K. pneumoniae which is 11.3% of total bacterial isolates. Of these isolates, 15 (88.2%) and 12 (70.6%) were resistant to trimethoprim and tetracycline respectively, while imipenem and meropenem had the best antibacterial activity. Biofilm formation was observed in 13 (76.5%) isolates. The isolate strains were found to produce capsule (100%), proteinase activity (11.7%) and hemolysin (35.3%). Regarding virulence genes, mrkD was detected in all isolates (100%), followed by t1fim (94.1%), magA (58.8%), and hly (35.3%). The results suggest that K. pneumoniae is a significant bacterial pathogen of surgical wound infections after plastic surgery. The frequency of biofilm, capsule formation and virulence genes such as mrkD demonstrates its virulence. Thus, it is crucial to routinely identify bacteria, test antimicrobial susceptibility and assess virulence factors for optimal plastic and reconstructive surgery infection prevention and proper antimicrobial stewardship.

Keywords: K. pneumoniae; surgical site infections; plastic surgery; antimicrobial resistance; virulence factors

Introduction

Infection of the surgical incision is the most common surgical complication. Estimated rates of surgical site infection (SSI) worldwide are 2–11% of surgical patients (World Health Organization. 2018). Rates as high as 25–30% have been reported in several low- and middle-income countries (LMIC) (Monahan et al., 2020). SSI increases length of hospital stay and costs the health system by increasing the need for additional operations and delays in adjuvant therapies (Khan et al., 2023). Infection control and prevention strategies are as important in surgical practice as aseptic technique. Plastic and reconstructive surgical procedures are at increased risk for SSI, as many of the procedures in this specialty involve the use of foreign bodies, tissue undermining and devascularization, long operative times and procedures on compromised and infected tissue (Le Couteur et al., 2025). Reconstruction of a defect with the use of a free flap involves undermining of a large area of skin, which leaves the overlying tissue ischemia and a compromised immune barrier. If infection were to occur, an unsightly and potentially disfiguring scar may result (Jung et al., 2025).

The microbiology of surgical site infections (SSIs) is changing. In various surgical units, isolates of Gram-negative bacteria, including K. pneumoniae, are increasing. High rates of capsule-producing K. pneumoniae have been reported in surgical site wound infections (Humada et al., 2024). The rapid increase of resistance in K. pneumoniae is attributed to horizontal gene transfer and the acquisition of resistance determinants. Additionally, K. pneumoniae produces adhesins, which play a role in biofilm formation, and is capable of immune evasion [7]. Klebsiella pneumoniae is one of the most common ESKAPE organisms isolated in Iraq. Other reports from Egypt and Croatia also documented high rates of ESKAPE organisms in surgical site wound infections (Abebe et al., 2026). Although most studies describe the antibiograms of the ESKAPE organisms, very few studies have described the virulence determinants of these organisms. The reason for this is that phenotypic analyses of bacteria may not always correlate with their pathogenicity.

This study will analyze K. pneumoniae wounds from a major tertiary care center in Iraq. It will describe the antibiotic resistance of K. pneumoniae, other phenotypic virulence factors, and the presence of magA, mrkD, hly and t1fim. It is hypothesized that the genotypic analyses of K. pneumoniae will provide additional information beyond antibiotic sensitivity testing and traditional microbiology.

Materials and methods

Study design and participants

This study was conducted at Azadi Hospital, Duhok, Iraq from March to October 2026. It included 150 patients who developed post-operative wound infection signs including purulence, inflammation and induration, dehiscence and/or partial or non-healing of the surgical wound, within the first post-operative month after undergoing a reconstructive or aesthetic surgical procedure. Priority was given to the inclusion of cases with recent wound infection signs. Patients with infected non-surgical wounds and patients on antibiotic therapy at the time of sample collection were not included in the study. Prior to sample collection, participants signed an informed consent form. Other data including name, address and age were also collected. Sample collection and other procedures were performed before any modification to the antibiotic therapy. Samples were delivered to the Microbiology Laboratory of the hospital for analysis and processing

Culture and isolation

Swab samples were transported to the laboratory in a transporter medium and inoculated in 5% Human Blood Agar, MacConkey Agar, EMB Agar and Mannitol Salt Agar. Plates were read at 24 to 48 hours and lactose fermentation, pigment and haem production by the organisms were documented. Other observations made included colonies morphology and effects of the

media. If required, pure cultures of the organisms were made and preserved in brain heart infusion broth with 20% glycerol for long term storage at -20°C (Sirisha, 2025; Cheesbrough, 2006).

Identification of isolates

Isolates were identified through biochemical tests and the DL microbial ID system (Zhuhai DL Biotech Co., Ltd./China). Following the manufacturer's instructions, 0.5 McFarland standards were prepared from suspensions of the tested isolates. 200 µL of the standards were added to biochemical, antimicrobial and broth tests, and cards were then incubated at 37°C. After 18-24 hours, tests were read.

Antimicrobial susceptibility tests

The modified Kirby-Bauer method was used to determine antimicrobial susceptibility. Mueller-Hinton agar (4 mm deep) was inoculated with a 0.5 McFarland standard. Antibiotic disks (Liofilchem) were allowed to touch the agar and included the following (µg): penicillin, ampicillin-sulbactam, tetracycline, cefixime, cefotaxime, cefepime, ceftriaxone, imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin, amikacin, and trimethoprim. A Clinical and Laboratory Standards Institute (CLSI) reference strain of Enterobacterales (E. coli ATCC 25922) was included as a control. After 16-18 hours of incubation at 37 °C, inhibition zones were measured and determined as sensitive or insensitive to each antibiotic (Tille, 2021).

Virulence Testing

Strains producing a black, dried biofilm on Congo red agar were considered biofilm producers. The production of a capsule was tested using India ink and strains producing a cleared zone on blood agar were considered haemolysin producers. Liquefaction was observed on nutrient gelatin after 48 hours at 37°C and 4°C. Hyaluronidase and proteinase activities resulted in clear halos and zones of casein hydrolysis respectively. All tests were performed in duplicate, and each test was evaluated independently by two people (Clinical and Laboratory Standards Institutem 2025; Tille, 2021).

DNA extraction, primers and PCR

Genomic DNA was isolated from overnight broths using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). The quantity and quality of the DNA was assessed by agarose gel electrophoresis. The DNA was stored at -20°C until use. For each gene, PCR was performed in a reaction volume of 25 µL using GoTaq Green PCR mix (MacFaddin, 2000). Cycling conditions were: an initial denaturation at 96°C for 4 min; 35 cycles at 96°C for 15 sec, annealing at the temperatures specified in Table 1 for 20 sec, and extension at 72°C for 55 sec; a final extension at 72°C for 5 min. The products of PCR were analyzed on a 1.5% agarose gel. The gel was stained with ethidium bromide and a 100 bp DNA ladder was used as a molecular weight standard. A negative control was included for each reaction (Sambrook & Russell, 2001).

Table 1. Oligonucleotide primers and amplification conditions used for the detection of virulence genes in K. pneumoniae.

Species / gene	Primer	Sequence (5′→3′)	Amplicon (bp)	Annealing (°C)
K. pneumoniae mrkD	F	GGGAGAGCGGCGGTAACCCG	477	65
	R	CACCGGCGAGGTGGAGTCGC		
K. pneumoniae hly	F	CGGCTATCTCTGGAAGCTGG	277	62
	R	GCGCCGCGCTTCGCCCCTTC		
K. pneumoniae t1fim	F	GCAAAACGGCCACCGGGGCG	472	66
	R	CCGCCGGTGGGGACCACCAC		
K. pneumoniae magA	F	GGTGATTCAAGCACTATACC	360	56
	R	CGGACTGGCCATATTGCTCC		

F, forward; R, reverse. All reactions: 35 cycles, 96 °C/15 s, annealing 20 s, 72 °C/55 s, after initial denaturation at 96 °C for 4 min.

Statistical analysis

Data were analysed with SPSS version 25. Descriptive results are presented as numbers and percentages or as mean ± standard deviation. Associations between categorical variables (sex, age group, anatomical site) were tested with the chi-square test; a two-sided $P < 0.05$ was considered statistically significant. Exact 95% confidence intervals (CI) for gene prevalence were calculated by the Wilson method.

Results

Characteristics of the study sample

The sample included 150 patients. Of these, 91 (60.7%) were males and 59 (39.3%) were females. The urban patients were 110 (73.3%). The mean age of the males was 49.20 (9.20) and of the females was 37.20 (7.40). The age group of 31-50 years was represented by 92 (62.0%) patients. The group of 41-50 years was represented by 47 (31.3%) patients. The age groups of the male and female patients were differently distributed ($P < 0.05$). The females were under-represented in the age group of 41 and above, as shown in Table 2.

Table 2. Demographic characteristics of the 150 patients with infected post-plastic surgery wounds.

Characteristic	Male (n = 91)	Female (n = 59)	Total (n = 150)	P
Age, years (mean ± SD)	49.20 ± 9.20	37.20 ± 7.40	44.50 ± 9.80	<0.05
Age group, n (%)				
<20 years	7 (7.7)	4 (6.8)	11 (7.3)	<0.05
21–30 years	14 (15.4)	16 (27.1)	30 (20.0)	<0.05
31–40 years	25 (27.5)	21 (35.6)	46 (30.7)	<0.05
41–50 years	34 (37.4)	13 (22.0)	47 (31.3)	<0.05
≥51 years	11 (12.1)	5 (8.5)	16 (10.7)	<0.05
Urban residence	—	—	110 (73.3)	NS
Rural residence	—	—	40 (26.7)	NS

SD, standard deviation; NS, not significant. P values refer to the chi-square test for the association between age group and sex.

Bacterial isolates

Cultures were positive in 85 of the 150 specimens (56.7%); 65 (43.3%) yielded no growth. K.

pneumoniae was recovered from 17 specimens (11.3% of all specimens and 20.0% of isolates).

Distribution by anatomical site

A total of 17 isolates of K. pneumoniae were recovered from all the anatomical sites of the wounds studied. These sites include the abdomen (5 isolates; 29.4%), foot (7 isolates; 41.2%), thoracic (3 isolates; 17.6%), skin graft (1 isolate; 5.9%), and hand (1 isolate; 5.9%).

Phenotypic Virulence Factors

Capsule was evident in all 17 K. pneumoniae isolates (100%). Biofilm was produced by 12 isolates (70.6%). Producing proteinase and gelatinase was demonstrated by 12 isolates (70.6%) as well. Hyaluronidase was demonstrated by 1 isolate (5.9%). Haemolysin was demonstrated by 6 isolates (35.3%).

Antimicrobial Resistance

The K. pneumoniae isolates were comparatively less resistant to the penicillin group (35.3% to penicillin and 41.2% to ampicillin) and more resistant to trimethoprim (88.2%) and tetracycline (70.6%). On the other hand, high levels of susceptibility (94.1% to both) was demonstrated against imipenem and meropenem, Table 3.

Table 3. Antimicrobial resistance profiles of K. pneumoniae (n = 17) isolated from infected post-plastic surgery wounds, n (%).

Antimicrobial agent	K. pneumoniae (n = 17)	
	R	S
Penicillin (100 µg)	6 (35.3)	11 (64.7)
Ampicillin (10 µg)	7 (41.2)	10 (58.8)
Tetracycline (10 µg)	12 (70.6)	5 (29.4)
Cefixime (75 µg)	7 (41.2)	10 (58.8)
Cefotaxime (30 µg)	8 (47.1)	9 (52.9)
Cefepime (30 µg)	7 (41.2)	10 (58.8)
Ceftriaxone (30 µg)	6 (35.3)	11 (64.7)
Imipenem (10 µg)	1 (5.9)	16 (94.1)
Meropenem (10 µg)	1 (5.9)	16 (94.1)
Ciprofloxacin (5 µg)	9 (52.9)	8 (47.1)
Levofloxacin (5 µg)	7 (41.2)	10 (58.8)
Gentamicin (10 µg)	6 (35.3)	11 (64.7)
Amikacin (30 µg)	5 (29.4)	12 (70.6)
Trimethoprim (75 µg)	15 (88.2)	2 (11.8)

R, resistant; S, susceptible. Interpreted by CLSI disk-diffusion criteria.

VIRULENCE GENES

Of the 17 K. pneumoniae isolates, mrkD was identified in 100% (95% CI 80.5-100.0), t1fim in 94.1% (95% CI 73.0-99.0), magA in 58.8% (95% CI 36.0-78.4) and hly in 35.3% (95% CI 16.3-61.2) of the isolates, as shown in Figures (1-4).

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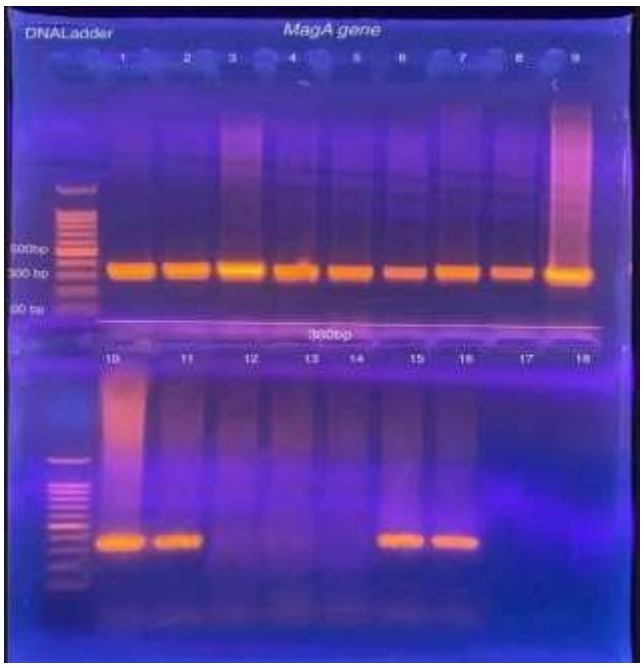

Figure 1. PCR amplification of the Klebsiella pneumoniae magA virulence gene. Specimens were resolved on a 1.5% agarose gel at 80 V for 1 hour. Lane 1 (far left) contains a 100 bp DNA ladder, followed by amplified sample lanes.

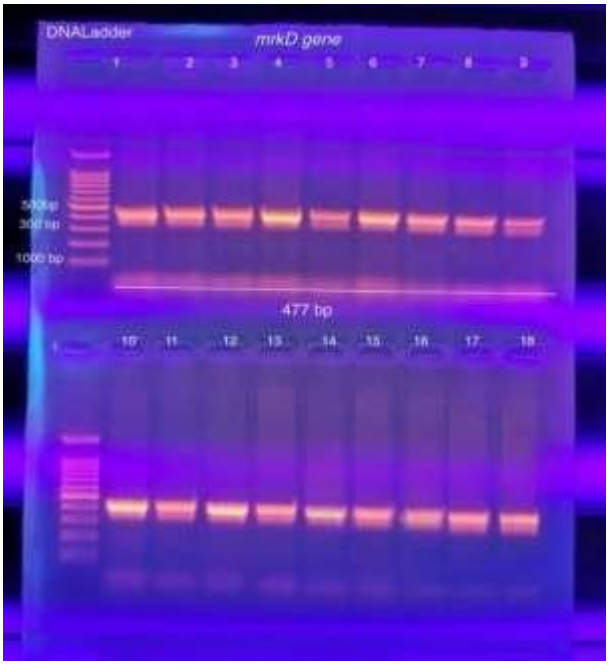

Figure 2. PCR amplification of the Klebsiella pneumoniae mrkD virulence gene. Specimens were resolved on a 1.5% agarose gel at 80 V for 1 hour. Lane 1 (far left) contains a 100 bp DNA ladder, followed by amplified sample lanes.

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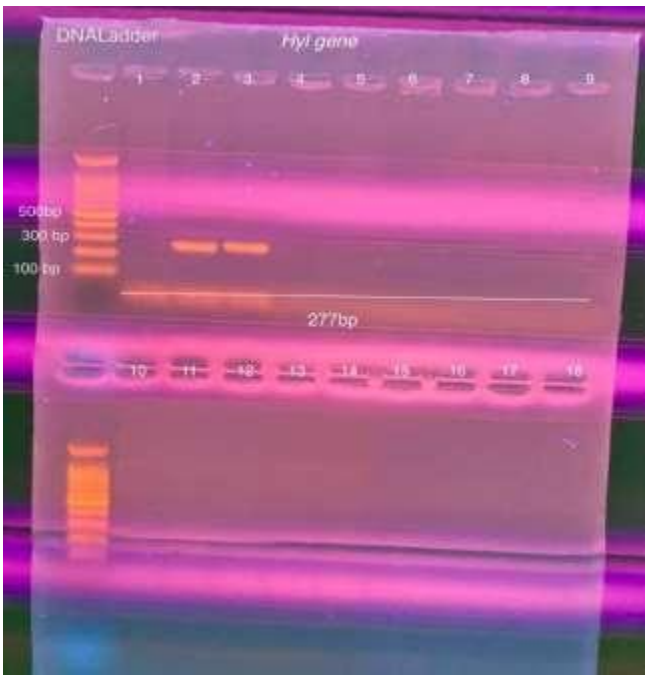

Figure 3. PCR amplification of the Klebsiella pneumoniae hly virulence gene. Specimens were resolved on a 1.5% agarose gel at 80 V for 1 hour. Lane 1 (far left) contains a 100 bp DNA ladder, followed by amplified sample lanes.

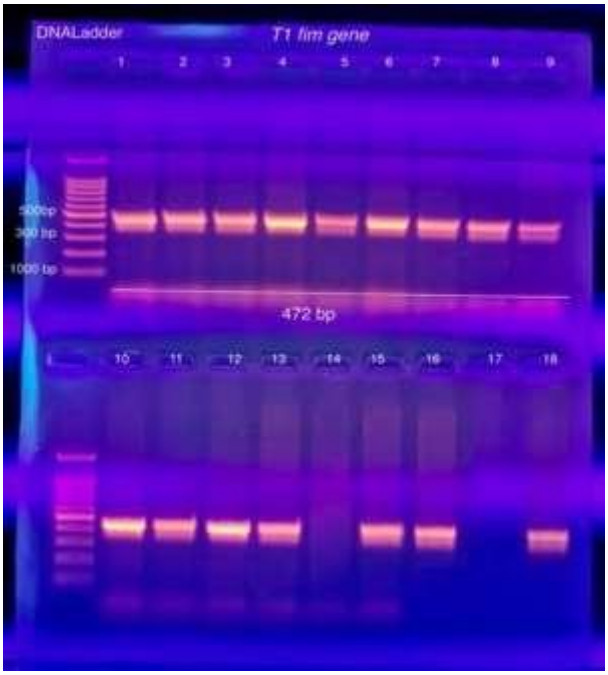

Figure 4. PCR amplification of the Klebsiella pneumoniae t1fim virulence gene. Specimens were resolved on a 1.5% agarose gel at 80 V for 1 hour. Lane 1 (far left) contains a 100 bp DNA ladder, followed by amplified sample lanes.

Discussion

Klebsiella pneumoniae was one of the most prevalent microorganisms recovered from infected wound sites. Within the spectrum of K. pneumoniae, there was high levels of resistance to tetracycline and trimethoprim, combined with the presence of genes for capsule synthesis and adhesins. Of the 150 infected surgical site wounds, 84 (56.7%) yielded a pathogen. The

presence of K. pneumoniae in our sample may be explained by high level of resistance to carbapenems, combined with the presence of adhesins and capsule genes (Kelishomi et al., 2025). The culture positivity observed in our series is similar to previously reported series from the same geographical area. It has been reported that culture positivity may be as high as 75%, and is dependant on sampling depth (Monahan et al., 2020). The 43.3% culture negativity may be explained by the fact that culture does not necessarily reflect the true infection status (Le Couteur et al. 2025). The characteristics of our sample (prevalence of males, patients between 31-50 years, urban residents) is similar to the general reconstructive surgery population (Her, 2026).

Approximately 70.6% of K. pneumoniae isolates from the clinical samples tested positive for biofilm formation, which is in agreement with previously published studies that have reported the presence of biofilms in both chronic and surgical wounds (Mohammed and Zgair, 2025). Tissue damage is attributable to proteinase (70.6%) and gelatinase (70.6%) activities. Strong immune evasion is attributed to 100% capsular expression (Abebe et al., 2026). Most isolates (64.7%) were resistant to Oxytetracycline (35.3%–41.2%) and all were susceptible to Carbapenems (94.1%). The regional studies have shown low frequency of Carbapenemase production by K. pneumoniae (Hussein et al., 2025).

All isolates (100%) had mrkD and 94.1% had t1fim. Type 3 fimbriae, of which mrkD is a part, are biofilm-forming adhesins and are found on surgical equipment and prosthetic materials (Maqsoodi et al., 2026). Therefore, mrkD is a rapid biofilm former and a capsule biosynthesis regulator (Maqsoodi et al., 2026; Monteiro et al., 2024). Hypermucoviscous K1 capsule, of which magA was found in 58.8% of the isolates, and hlyA in 35.3% of the isolates, are indicators of toxin production. However, K. pneumoniae isolates use capsular and adhesin mediated immune evasion (Abebe et al., 2026; Monteiro et al., 2024).

Finally, it is important to consider the following limitations when interpreting the results: t1fim is not present in all K. pneumoniae isolates; there are other factors besides mrkD that can mediate biofilm formation; and phenotypic and genotypic assays for mucoviscinosity may differ.

Study limitations

Considering the limitations, first, the study was single center and cross sectional. As such, the authors could not associate the genotypes to clinical failure to infection. In addition, there was no follow up to assess the status of the healed wounds. Second, swab samples were less ideal as deep tissue samples to assess infection. Third, there were several limitations to assess antibiotic resistance. The authors relied on disk diffusion, and measuring minimum inhibitory concentrations (MICs) and sequencing resistance genes was beyond the scope of the study. Lastly, determining virulence genes by polymerase

Conclusion

The results of this study indicate that Klebsiella pneumoniae is a significant bacterial pathogen in plastic and reconstructive surgery surgical wound infections at the study centre. Its detection and antimicrobial resistance profiles are indicative of its importance in postoperative infections and their development and persistence. The higher level of antibacterial activity of imipenem and meropenem against the tested isolates did not deter from the fact that resistance to the commonly used antibiotics highlights the need to constantly monitor antimicrobial susceptibility and use antibiotics rationally. Moreover, these mrkD and t1fim genes were also found in high numbers, suggesting that fimbrial adhesins play an important role in bacterial adhesion and colonization in wounds. The detection of capsular production in all the isolates and the high occurrence of biofilm production further supports the pathogenic potential of these isolates because these virulence factors may enable the bacteria to survive, persist and resist host immune response and antimicrobial drugs. The presence of other virulence factors such as magA and hly further indicates that multiple virulence mechanisms are likely playing a role in the involvement of wound infection. The results of this study provide

information on the significance of correct and timely identification of K. pneumoniae, routine antimicrobial susceptibility testing, and molecular characterization of its virulence genes. Therefore, robust infection prevention and control, better surgical wound management, good hygiene and effective antimicrobial stewardship programmes are crucial to minimise surgical site infection. More extensive and larger scale studies, with multiple healthcare centers and extensive molecular investigations, are recommended to better understand the epidemiology, mechanisms of antimicrobial resistance, and virulence characteristics of K. pneumoniae associated with postoperative wound infections.

References

[1] World Health Organization. (2018). Global guidelines for the prevention of surgical site infection (2nd ed.). World Health Organization.
[2] Monahan, M., Jowett, S., Pinkney, T., Brocklehurst, P., Morton, D. G., Abdali, Z., et al. (2020). Surgical site infection and costs in low- and middle-income countries: A systematic review of the economic burden. PLOS ONE, 15(6), e0232960.
[3] Khan, O. H., Zakaria, A. D., Hashim, M. N., Khan, A. H., AlQarni, A., AlGethamy, M., et al. (2023). The burden of surgical site infection at Hospital Universiti Sains Malaysia and related postoperative outcomes: A prospective surveillance study. Antibiotics, 12(2), 208.
[4] Le Couteur, J., et al. (2025). Systematic review of surgical site infection prevention guideline recommendations for maintenance of homeostasis in the perioperative period. Anesthesiology, 142(6), 1150–1165.
[5] Jung, S., Lee, S., & Eun, S. (2025). Free flap reconstruction of traumatic skin defects of the entire hand dorsum. Journal of Clinical Medicine, 14(4), 1308.
[6] Humada, Y. H., Khalid, R. W., & Hussein, F. K. (2024). Molecular study of K1, K2, and magA genes in highly virulent Klebsiella pneumoniae in Kirkuk City, Iraq. South Asian Research Journal of Biology and Applied Biosciences, 6(6), 242–245.
[7] Abebe, A. A., Worku, G., & Moges, F. (2026). Antibiotic resistance and virulence mechanisms in Klebsiella pneumoniae: A systematic review. Microorganisms, 14(2), 245–262.
[8] Sirisha, M. (2025). ESKAPE pathogens identified in wound infections and their antibiotic susceptibility pattern. International Journal of Medical and Pharmaceutical Research, 6(4), 1092–1097.
[9] Cheesbrough, M. (2006). District laboratory practice in tropical countries: Part 2 (2nd ed.). Cambridge University Press.
[10] Tille, P. M. (2021). Bailey & Scott's diagnostic microbiology (15th ed.). Elsevier.
[11] Clinical and Laboratory Standards Institute. (2025). Performance standards for antimicrobial susceptibility testing (35th ed., CLSI supplement M100). CLSI.
[12] MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria (3rd ed.). Lippincott Williams & Wilkins.
[13] Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.
[14] Kelishomi, F. Z., et al. (2025). Antimicrobial resistance patterns and virulence gene profiles of Klebsiella pneumoniae isolated from surgical site infections. Surgical Infections, 26(8), 567–575.
[15] Monahan, M., Jowett, S., Pinkney, T., Brocklehurst, P., Morton, D. G., Abdali, Z., et al. (2020). Surgical site infection and costs in low- and middle-income countries: A systematic review of the economic burden. PLOS ONE, 15(6), e0232960.
[16] Le Couteur, J., et al. (2025). Systematic review of surgical site infection prevention guideline recommendations for maintenance of homeostasis in the perioperative period.

Anesthesiology, 142(6), 1150–1165.

[17] Her, H. A (2026). Antimicrobial susceptibility and prevalence of multidrug-resistant Klebsiella pneumoniae in pediatric patients in Karbala, Iraq: A two-year retrospective study. Iraqi National Journal of Medicine, 8(2), 178–185.

[18] Mohammed, M. T., & Zgair, A. K. (2025). Multidrug-resistant Klebsiella pneumoniae isolated from an infected wound in Iraq. World Journal of Experimental Biosciences, 13, 11–14.

[19] Abebe, A. A., Worku, G., & Moges, F. (2026). Antibiotic resistance and virulence mechanisms in Klebsiella pneumoniae: A systematic review. Microorganisms, 14(2), 245–262.

[20] Hussein, N., Muhammad, A., & Abozait, H. (2025). Antibiotic resistance in Klebsiella pneumoniae in Iraq: A narrative review. Journal of Life Bio-Science Research, 6(2), 64–69.

[21] Maqsoodi, M., Tajbakhsh, E., & Momtaz, H. (2026). Virulence genes and antimicrobial resistance profiles of Klebsiella pneumoniae isolated from urinary tract infections. BMC Urology, 26(1), 150.

[22] Monteiro, A. D. S., Cordeiro, S. M., & Reis, J. N. (2024). Virulence factors in Klebsiella pneumoniae: A literature review. Indian Journal of Microbiology, 64(3), 389–401.