Natural Resources for Human Health
eISSN: 2583-1194

Original Research

**Received** 24 May 2026
**Revised** 27 June 2026
**Accepted** 19 July 2026

Natural Resources for Human Health 2026, Vol. 6 (9s): 118–125

**KEYWORDS**:
Klebsiella pneumoniae, antimicrobial resistance, environmental water, multidrug resistance, water microbiology, Kirby-Bauer disk diffusion.

# To the Study of Occurrence, Identification & Antimicrobial Resistance Profile of Klebsiella Pneumoniae Isolated from Environmental Surface Water Sources

Harsha Sharma[1], Navneet Kumar[1], Amandeep Singh[1], Lakshay Vashishtha[2], Manik Sharma[3]

[1]Rayat Bahra University, Mohali, Punjab
[2]ICT, Mumbai, Maharashtra
[3]Vydehi Institute of Medical Sciences, Bengaluru

**ABSTRACT:** Antimicrobial resistance (AMR) is a major global public health challenge, with environmental water bodies increasingly recognized as reservoirs for antimicrobial-resistant bacteria. Klebsiella pneumoniae is an opportunistic Gram-negative pathogen capable of surviving in aquatic environments and acquiring resistance to multiple antimicrobial agents. This study aimed to isolate and identify K. pneumoniae from different surface water sources and evaluate its antimicrobial susceptibility and multidrug resistance (MDR) patterns. A total of 60 environmental water samples were collected from four surface water sources: river water, pond water, irrigation canals, and temple water tanks (15 samples each). Isolation and identification were performed using standard microbiological methods, including selective culture, Gram staining, and biochemical characterization. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method according to CLSI guidelines. Pearson's Chi-square test was used to assess the association between sampling source and bacterial isolation. K. pneumoniae was isolated from 33 (55.0%) samples. The highest isolation rate was observed in temple water tanks (66.7%), followed by ponds (60.0%), while river and canal water each showed 46.7%. No significant association was observed between sampling source and bacterial isolation ($\chi^2 = 1.818$, $p = 0.611$). High resistance was observed to cefotaxime (57.6%), ceftriaxone (54.5%), amoxicillin-clavulanic acid (51.5%), and ceftazidime (48.5%). Meropenem (97.0%), imipenem (93.9%), and amikacin (90.9%) showed the highest activity. Overall, 12 (36.4%) isolates were MDR. These findings highlight surface water as a potential reservoir of antimicrobial-resistant K. pneumoniae and support the need for environmental surveillance, improved water management, and antimicrobial stewardship.

## 1. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges of the twenty-first century. The rapid emergence and dissemination of antimicrobial-resistant bacteria have significantly reduced the effectiveness of commonly used antibiotics, resulting in prolonged hospital stays, increased healthcare expenditure, therapeutic failures, and higher mortality worldwide [1,2]. According to the World Health Organization (WHO), AMR is among the top ten global public health threats requiring coordinated efforts involving surveillance, infection prevention, antimicrobial stewardship, and environmental monitoring [1,3]. Environmental ecosystems are increasingly recognized as critical reservoirs for antimicrobial-resistant microorganisms and antimicrobial resistance genes. Rivers, ponds, lakes, irrigation canals, and other freshwater bodies receive untreated municipal wastewater, hospital effluents, industrial discharge, agricultural runoff, and livestock waste, creating favorable ecological conditions for the persistence and dissemination of resistant bacteria [3,4]. These aquatic environments

facilitate horizontal gene transfer and contribute to the spread of antimicrobial resistance between environmental, animal, and human populations, highlighting the importance of the One Health approach [5].

Among the members of the family Enterobacteriaceae, Klebsiella pneumoniae has gained considerable clinical importance because of its ability to cause both community-acquired and health-care-associated infections. It is responsible for pneumonia, urinary tract infections, bloodstream infections, wound infections, liver abscesses, and neonatal infections, particularly among immuno-compromised individuals [6]. The organism possesses several important virulence factors, including a polysaccharide capsule, fimbriae, siderophore production, lipopolysaccharide, and biofilm-forming ability, which facilitate environmental persistence and enhance pathogenicity [4,5].

In addition to clinical settings, K. pneumoniae has been increasingly reported from environmental water sources, including rivers, ponds, lakes, wastewater, irrigation water, and drinking water distribution systems [4-6]. The global emergence of multidrug-resistant (MDR), extended-spectrum β-lactamase (ESBL)-producing, and carbapenem-resistant K. pneumoniae has further complicated treatment options and increased the burden on healthcare systems worldwide [6,7,8]. Environmental contamination with antimicrobial-resistant K. pneumoniae is of particular concern because these water bodies may serve as reservoirs for resistant strains and resistance genes, thereby facilitating their transmission to humans through domestic, agricultural, recreational, and food production activities [9].

Despite the increasing recognition of environmental water bodies as important reservoirs of antimicrobial-resistant bacteria, relatively few studies have investigated the occurrence and antimicrobial susceptibility profile of environmental K. pneumoniae isolated from different surface water sources in India. Furthermore, comparative information regarding the distribution of multidrug-resistant environmental isolates from diverse aquatic ecosystems remains limited. This knowledge gap highlights the need for continuous environmental surveillance to understand the prevalence and resistance characteristics of environmental K. pneumoniae and to support effective public health interventions.

Therefore, the present study was undertaken to isolate and identify Klebsiella pneumoniae from different environmental surface water sources and to evaluate their antimicrobial susceptibility patterns and multidrug resistance profiles using standard microbiological methods. In addition, the association between sampling source and bacterial isolation was statistically evaluated to determine whether significant differences existed among the investigated environmental water sources.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

The present study was a laboratory-based cross-sectional study conducted to isolate, identify, and determine the antimicrobial susceptibility profile of Klebsiella pneumoniae recovered from different environmental surface water sources. A total of 60 environmental surface water samples were collected from different locations in Haridwar, Uttarakhand, India, between October 2025 to July 2026. The study was designed to evaluate the occurrence of K. pneumoniae and compare its distribution among selected environmental water bodies.

A total of 60 environmental surface water samples were collected from four different sampling sources, namely river water, pond water, irrigation canal water, and temple water tanks, with 15 samples collected from each source. Water samples were aseptically collected in sterile 500 mL screw-capped bottles according to the Standard Methods for the Examination of Water and Wastewater [10]. All samples were properly labeled and transported to the microbiology laboratory under refrigerated conditions for bacteriological analysis. Microbiological processing was initiated within 6 h of sample collection.

### 2.2 Isolation of Klebsiella pneumoniae

Each water sample was mixed thoroughly before processing. A measured volume of the sample was inoculated onto MacConkey agar and Eosin Methylene Blue (EMB) agar (HiMedia Laboratories, Mumbai, India) using standard microbiological techniques. EMB agar was used as a selective and differential medium for the isolation of lactose-fermenting Gram-negative bacilli, thereby facilitating the presumptive identification of Klebsiella pneumoniae based on its characteristic colony morphology [11,12]. The inoculated plates were incubated aerobically at 37°C for 24 h. Colonies showing characteristic morphology suggestive of Klebsiella pneumoniae were selected and sub-cultured on nutrient agar to obtain pure cultures.

### 2.3 Identification of Bacterial Isolates

Presumptive isolates were identified by colony morphology, Gram staining, and conventional biochemical tests. The biochemical characterization included catalase, oxidase, indole, citrate utilization, urease, methyl red (MR), Voges-Proskauer (VP), Triple Sugar Iron (TSI), and motility tests. Identification was based on standard microbiological procedures described in Bailey & Scott's Diagnostic Microbiology and Microbiology: A Laboratory Manual [11,12].

### 2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following the recommendations of the Clinical and Laboratory Standards Institute (CLSI M100, 34th edition) [13]. Fresh bacterial suspensions equivalent to 0.5 McFarland turbidity standard were prepared, and sterile cotton swabs were used to inoculate the surface of Mueller-Hinton agar plates.

The antibiotic discs used in the study included:

- Amoxicillin-clavulanic acid
- Cefotaxime
- Ceftriaxone
- Ceftazidime
- Ciprofloxacin
- Levofloxacin
- Gentamicin
- Amikacin
- Imipenem
- Meropenem

After incubation at 37°C for 18-24 h, the diameters of inhibition zones were measured in millimeters and interpreted as Susceptible (S), Intermediate (I), or Resistant (R) according to CLSI guidelines [13].

### 2.5 Determination of Multidrug Resistance (MDR)

Multidrug resistance (MDR) was defined according to the international criteria proposed by Magiorakos et al. [14], in which an isolate is considered multidrug-resistant when it exhibits resistance to at least one antimicrobial agent in three or more antimicrobial classes.2.4 Analytical Methods

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam erat volutpat. Vivamus luctus egestas leo.

2.6 Statistical Analysis

The collected data were entered into Microsoft Excel 2021 and analyzed using JASP software (Version 0.18.1). Descriptive statistics were used to summarize the frequency and percentage of positive and negative isolates obtained from different environmental water sources.

The association between sampling source and the occurrence of Klebsiella pneumoniae was evaluated using Pearson's Chi-square test. A p-value $<0.05$ was considered statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation of Klebsiella pneumoniae from Environmental Surface Water Samples

A total of 60 environmental surface water samples were collected from four different sampling sites, including river water, pond water, irrigation canal water, and temple water tanks (15 samples from each source). Among these, 33 samples were confirmed positive for Klebsiella pneumoniae, resulting in an overall isolation rate of 55.0%. The highest isolation frequency was recorded

in temple water tanks (66.7%), followed by pond water (60.0%), whereas river water and irrigation canal water each showed an isolation rate of 46.7%.

**Table 1.** Isolation of Klebsiella pneumoniae from different environmental water sources

| Water Source | Samples Examined (n) | Positive Isolates (n) | Isolation Rate (%) |
|---|---|---|---|
| River | 15 | 7 | 46.7 |
| Pond | 15 | 9 | 60.0 |
| Irrigation Canal | 15 | 7 | 46.7 |
| Temple Water Tank | 15 | 10 | 66.7 |
| | 60 | 33 | 55.0 |

Figure 1. Isolation rate (%) of Klebsiella pneumoniae recovered from different environmental surface water sources. Temple water tank samples showed the highest isolation rate (66.7%), followed by pond water (60.0%), whereas river water and irrigation canal water each exhibited an isolation rate of 46.7%.

### 3.2 Statistical Analysis of Isolation Rate

To determine whether the isolation of Klebsiella pneumoniae differed significantly among the four sampling sites, a Pearson Chi-square test was performed using JASP software. Although temple water tanks demonstrated the highest isolation rate, no statistically significant association was observed between sampling source and bacterial isolation ($\chi^2 = 1.818$; df = 3; $p = 0.611$). Furthermore, the calculated Cramer's V (0.174) indicated only a weak association between sampling source and isolation frequency.

**Table 2.** Pearson Chi-square analysis of Klebsiella pneumoniae isolation

| Statistical Parameter | Value |
|---|---|
| Pearson Chi-square ($\chi^2$) | 1.818 |
| Degrees of Freedom (df) | 3 |
| p-value | 0.611 |
| Cramer's V | 0.174 |
| Statistical Significance | Not Significant ($p > 0.05$) |

### 3.3 Biochemical Characterization of Klebsiella pneumoniae

All 33 isolates exhibited the characteristic biochemical profile of Klebsiella pneumoniae. The isolates were Gram-negative bacilli and showed positive reactions for catalase, Voges-Proskauer, citrate utilization and urease tests. Conversely, all isolates were negative for oxidase, indole, methyl red and motility tests. On Triple Sugar Iron (TSI) agar, all isolates produced an acid/acid (A/A) reaction with gas production and no hydrogen sulfide ($H_2S$).

**Table 3.** Biochemical characteristics of Klebsiella pneumoniae isolates

| Biochemical Test | Positive (n) | Negative (n) |
|---|---|---|
| Gram-negative bacilli | 33 | 0 |
| Catalase | 33 | 0 |
| Oxidase | 0 | 33 |

| | | |
|---|---|---|
| Indole | 0 | 33 |
| Methyl Red | 0 | 33 |
| Voges-Proskauer | 33 | 0 |
| Citrate Utilization | 33 | 0 |
| Urease | 33 | 0 |
| Motility | 0 | 33 |
| TSI (A/A + Gas, $H_2S$-) | 33 | 0 |

### 3.4 Antimicrobial Susceptibility Profile

The antimicrobial susceptibility profile of the confirmed Klebsiella pneumoniae isolates demonstrated variable resistance patterns among the tested antimicrobial agents. The highest resistance was observed against cefotaxime (57.6%), followed by ceftriaxone (54.5%), amoxicillin-clavulanic acid (51.5%), and ceftazidime (48.5%). In contrast, meropenem (97.0%), imipenem (93.9%), and amikacin (90.9%) showed the highest antimicrobial activity against isolates. Gentamicin (75.8%) and levofloxacin (72.7%) also demonstrated high susceptibility rates.

**Table 4.** Antimicrobial susceptibility profile of Klebsiella pneumoniae isolates

| Antibiotic | Sensitive n (%) | Intermediate n (%) | Resistant n (%) |
|---|---|---|---|
| Amoxicillin-Clavulanic acid | 12 (36.4) | 4 (12.1) | 17 (51.5) |
| Cefotaxime | 11 (33.3) | 3 (9.1) | 19 (57.6) |
| Ceftriaxone | 13 (39.4) | 2 (6.1) | 18 (54.5) |
| Ceftazidime | 14 (42.4) | 3 (9.1) | 16 (48.5) |
| Gentamicin | 25 (75.8) | 2 (6.1) | 6 (18.2) |
| Amikacin | 30 (90.9) | 1 (3.0) | 2 (6.1) |
| Ciprofloxacin | 19 (57.6) | 2 (6.1) | 12 (36.4) |
| Levofloxacin | 24 (72.7) | 2 (6.1) | 7 (21.2) |
| Imipenem | 31 (93.9) | 1 (3.0) | 1 (3.0) |
| Meropenem | 32 (97.0) | 0 (0.0) | 1 (3.0) |

### 3.5 Distribution of Multidrug-Resistant (MDR) Isolates

Based on the predefined criteria for multidrug resistance, 12 of the 33 confirmed isolates (36.4%) were classified as multidrug-resistant (MDR), whereas 21 isolates (63.6%) were categorized as non-MDR.

**Table 5.** Distribution of multidrug-resistant Klebsiella pneumoniae isolates

| **Category** | **Number of Isolates** | **Percentage (%)** |
|---|---|---|
| MDR | 12 | 36.4 |
| Non-MDR | 21 | 63.6 |
| Total | 33 | 100.0 |

### 3.5 Discussion

The present study investigated the occurrence, biochemical characteristics, and antimicrobial susceptibility profile of Klebsiella pneumoniae isolated from environmental surface water sources. The findings demonstrated that environmental aquatic ecosystems may serve as important reservoirs of antimicrobial-resistant Klebsiella pneumoniae, supporting the growing concern regarding the environmental dissemination of antimicrobial resistance within the One Health framework [1–3].

Among the 60 environmental water samples examined, 33 (55.0%) were positive for Klebsiella pneumoniae. Similar findings have been reported in environmental surveillance studies, where K. pneumoniae has frequently been recovered from rivers, ponds, lakes, wastewater, and recreational water bodies, suggesting that contaminated aquatic environments play an important role in the maintenance and transmission of clinically significant bacteria [6,14]. The increasing contamination of surface waters with municipal sewage, hospital effluents, and agricultural runoff creates favorable ecological conditions for the persistence and dissemination of antimicrobial-resistant Enterobacterales [1,2].

In the present investigation, temple water tanks exhibited the highest isolation rate (66.7%), followed by pond water (60.0%), whereas river water and irrigation canal water each demonstrated an isolation rate of 46.7%. However, Pearson's Chi-square analysis showed no statistically significant association between sampling sources and bacterial occurrence ($p = 0.611$), indicating that K. pneumoniae was widely distributed across all investigated environmental water sources. This finding suggests that contamination was not restricted to a specific aquatic ecosystem but was prevalent throughout the study area. Similar observations have been reported in previous environmental monitoring studies that documented the widespread occurrence of K. pneumoniae in different aquatic environments without significant source-specific variation [6,9].

All recovered isolates exhibited the characteristic biochemical profile of K. pneumoniae, including catalase positivity, citrate utilization, urease production, Voges-Proskauer positivity, oxidase negativity, indole negativity, and non-motility. These findings are consistent with the classical diagnostic characteristics described in standard microbiology references and support the reliability of conventional biochemical methods for the identification of environmental K. pneumoniae isolates [11,12]. Antimicrobial susceptibility testing demonstrated high resistance to cefotaxime, ceftriaxone, ceftazidime, and amoxicillin–clavulanic acid, whereas meropenem, imipenem, and amikacin remained highly effective against most isolates. Comparable antimicrobial susceptibility patterns have been reported in previous studies involving both environmental and clinical K. pneumoniae isolates, where resistance to third-generation cephalosporins was common while carbapenems retained good activity [5,6,9]. Although carbapenems remained highly effective in the present study, continuous surveillance is warranted because carbapenem-resistant K. pneumoniae has been increasingly reported worldwide [1,2].

A particularly important finding of the present study was the identification of 12 multidrug-resistant (MDR) isolates (36.4%). Environmental water bodies are increasingly recognized as reservoirs of multidrug-resistant bacteria and antimicrobial resistance genes that may spread through horizontal gene transfer among environmental microorganisms [2,3,15]. The presence of MDR K. pneumoniae in surface water represents a significant public health concern because these water sources may be directly or indirectly associated with domestic use, agriculture, livestock, and recreational activities. These observations further emphasize the importance of continuous environmental surveillance as an integral component of the One Health strategy for controlling antimicrobial resistance [1-3].

The findings of the present investigation have important public health implications. Continuous microbiological monitoring of environmental water sources, implementation of effective wastewater treatment systems, rational use of antimicrobial agents, and integration of environmental surveillance into national antimicrobial resistance monitoring programs are essential to reduce the dissemination of multidrug-resistant bacteria. Future studies incorporating molecular characterization of resistance determinants and genomic epidemiology will further improve our understanding of the environmental transmission dynamics of K. pneumoniae and support the development of effective public health interventions.

### Study Limitations

The present study has certain limitations. Seasonal variation in bacterial occurrence was not evaluated, which may have influenced the observed isolation rates. The sample size was relatively small and limited to selected environmental water sources. Molecular characterization of antimicrobial resistance genes, virulence determinants, and sequence typing of the isolates was not performed. Therefore, future investigations involving larger sample sizes, multicentric sampling, and molecular techniques

such as PCR and whole-genome sequencing are recommended to better understand the epidemiology and resistance mechanisms of environmental K. pneumoniae.

## 4. CONCLUSION

The present study confirmed the occurrence of Klebsiella pneumoniae in different environmental surface water sources, with an overall isolation rate of 55.0%. Although temple water tanks showed the highest recovery rate, no statistically significant association was observed between sampling source and bacterial occurrence. The isolates demonstrated considerable resistance to several β-lactam antibiotics, while carbapenems and amikacin remained highly effective. The identification of multidrug-resistant isolates highlights the environmental dissemination of antimicrobial resistance and underscores the need for regular surveillance, effective wastewater treatment, and rational antibiotic use. Future studies integrating molecular epidemiology and genomic characterization will further strengthen our understanding of environmental K. pneumoniae and support public health interventions.

## ACKNOWLEDGEMENTS

The authors would like to thank the Department of Microbiology and the laboratory staff for providing the facilities and technical support required for this study. The authors also acknowledge all individuals who assisted during sample collection and laboratory investigations.

## AUTHOR CONTRIBUTIONS

**Harsha Sharma:** Conceptualization, Investigation.

**Amandeep Singh:** Methodology, Laboratory Analysis.

**Navneet Kumar :** Data Curation, Formal Analysis.

**Lakshay Vashistha :** Supervision, Writing

Manik Sharma : Review & Editing.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## ETHICS STATEMENT

Since the present study involved environmental water samples only and did not include human participants, animals, or patient data, formal Institutional Ethics Committee approval was not required.

## DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

## REFERENCES

[1] World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2023. https://www.who.int/publications/i/item/9789240083478

[2] Murray CJL, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019. https://doi.org/10.1016/S0140-6736(21)02724-0

[3] Hernando-Amado S, Coque TM, Baquero F, Martínez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. https://doi.org/10.1038/s41564-019-0503-9

[4] Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens. https://doi.org/10.1128/CMR.11.4.589

[5] Martin RM, Bachman MA. Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. https://doi.org/10.3389/fcimb.2018.00004

[6] Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. https://doi.org/10.1093/femsre/fux013

[7] Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant *Enterobacteriaceae*. https://doi.org/10.1093/infdis/jiw282
[8] Wyres KL, Holt KE. *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. https://doi.org/10.1016/j.tim.2016.09.007
[9] Giri S, Shekar M, Shetty AV, et al. Antibiotic resistance and RAPD typing of *Klebsiella pneumoniae* isolated from clinical and water samples. https://doi.org/10.1002/wer.1603
[10] American Public Health Association. Standard Methods for the Examination of Water and Wastewater. https://www.standardmethods.org
[11] Tille PM. Bailey & Scott's Diagnostic Microbiology. https://www.us.elsevierhealth.com/bailey-and-scotts-diagnostic-microbiology-9780323829976.html
[12] Cappuccino JG, Welsh CT. Microbiology: A Laboratory Manual. https://www.pearson.com/en-us/subject-catalog/p/microbiology-a-laboratory-manual/P200000003551
[13] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. https://clsi.org/standards/products/microbiology/documents/m100/
[14] Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria. https://doi.org/10.1111/j.1469-0691.2011.03570.x
[15] Tacconelli E, Carrara E, Savoldi A, et al. WHO priority list of antibiotic-resistant bacteria. https://doi.org/10.1016/S1473-3099(17)30753-3