Research Article
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Isolation and characterization of environmental *Klebsiella spp.* from surface swabs for understanding their distribution and ecological role

Abstract

Environmental surfaces can serve as reservoirs for opportunistic pathogens such as *Klebsiella* spp., which are linked to both hospital and community acquired infections. This study aimed to isolate, enumerate and characterize *Klebsiella* spp. from surface swabs collected within university campus. A total of twenty surface swab samples were processed using selective culture methods followed by biochemical identification. Colony-forming unit (CFU) counts were determined using the plate count method. Our findings revealed a presence of the bacterial culture with an average of 110 CFU/swab in positive samples, emphasizing the need for targeted hygiene practices to mitigate potential health risks. These data provide a basis for developing effective control strategies against environmental reservoirs of *Klebsiella* spp. Despite its common presence in aquatic environments the virulence potential is mostly unknown. Hence, in this study, *Klebsiella* spp. isolated from the various sources and screened for various characteristics. Finally, the study indicated that *Klebsiella* is ubiquitous in the environment.

Keywords: *klebsiella* spp., surface swabs, environmental reservoirs, isolation, enumeration, public health

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Introduction

Klebsiella spp. is Gram-negative bacteria commonly associated with human infections.[1] Generally pathogenic microbial consortium growth is fostered and transmitted through wastewater.[2] The exploring of adverse impact on sanitation and hygiene is crucial. The genus of *Klebsiella* belongs to the family *Enterobacteriaceae* which are named after Edwin Klebs, a nineteenth century German microbiologist[3]. Recent studies have highlighted the role of environmental surfaces as reservoirs for these pathogens, thereby contributing to their transmission and persistence in public areas.[3,4] Due to having the potential of farming biofilms *Klebsiella* may be agents for opportunistic infections for immunocompromised individuals. This study was designed and conducted at the Department of Microbiology, Bundelkhand University, Jhansi, UP, India to isolate and enumerate *Klebsiella* spp. from surface swabs with the goal holistic study about potential health risks. In connection to this for better understanding and exploring information the future trend of antimicrobial resistance in *Klebsiella* can be known with integrated of machine learning (ML) models.[5] The bacterial communities in aquatic ecosystems intrinsically possess a diverse assortment of antimicrobial resistance genes.[6] It is well documented that pathogens originally susceptible to antibiotics can subsequently attain resistance from environmental bacterial populations. Multiple studies have established that wastewater show a significant reservoir of antibiotic resistance genes and detected in the discharged effluents of wastewater treatment facilities despite being filtered and processed for disinfection in conventional ways.[7,8] Water plays a vital role for humans and animals for consumption, irrigation and various recreational activities. It's widely accepted that healthcare, communal and built-environments can act as fomites, where microbes accumulate via contact, aerosol deposition or moisture films.[9] The presence of *Klebsiella* spp. from such surfaces can provide a casement into environmental reservoirs of these pathogens. The recent studies have shown that *K. pneumoniae* can survive on dry surfaces in biofilm-like structure for extended periods, maintaining viability and potential for transfer from one to another generation. Isolation and enumeration of *Klebsiella* from surface swabs serve pivotal roles like quantifying the burden of environmental contamination and enabling characterization of isolates phylogeny, antimicrobial resistance and virulence to assess the posing threat by them.[10] Finally, understanding where and how *Klebsiella* persists outside the human body will inform cleaning and disinfection protocols, monitoring frameworks and public health risk assessments for better health and life.

Materials and methods

Sample collection

The study was conducted from July to December, 2025. A total of 20 surface swab samples were collected from various areas like around the water coolers and laboratory equipment across the university campus following the standard procedures.[11,12] Sterile cotton swabs moistened with phosphate buffered saline (PBS) were used for sampling. The samples were collected in sterile bottles and transported to the laboratory under aseptic conditions for bacterial culturing and detailed studies. The figures 1, 2, 3, 4 and 8 were created using NotebookLM and further refined by the authors for better presentation and ameliorating the knowledge.[13]

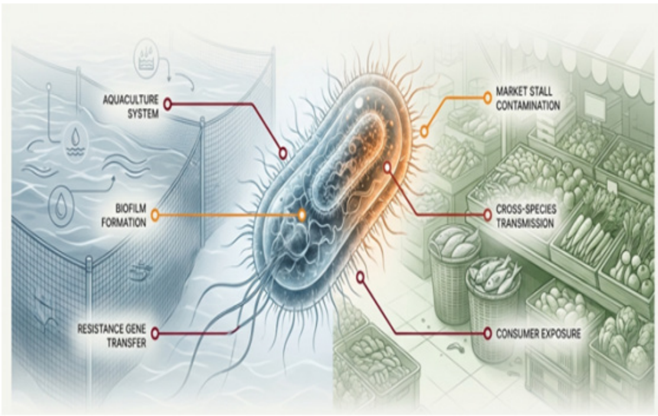

Figure 1 Schematic tracking of the environmental spread of multi drug resistant *Klebsiella* spp through food supply chain in the hidden reservoir.

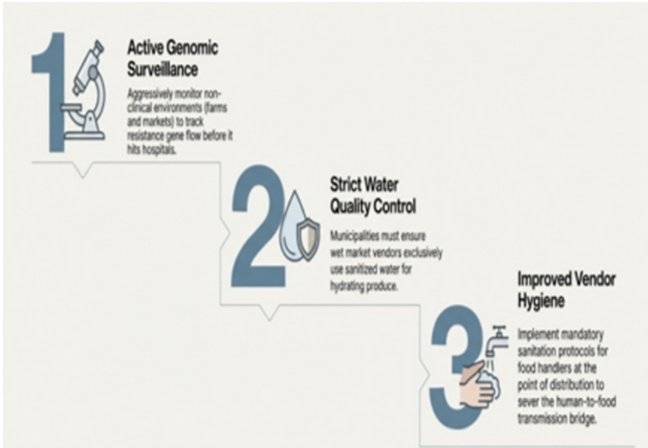

Figure 2 Cutting edge ways to break the chain of *Klebsiella* spp. transmission to avoid contamination in daily life exposure.

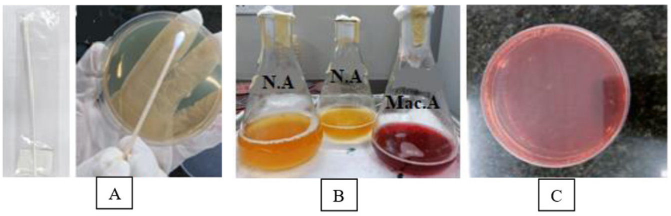

Figure 3 A) sterilized cotton swab B) nutrient agar and macConkey agar C) Prepared media Petri plate of MacConkey- agar.

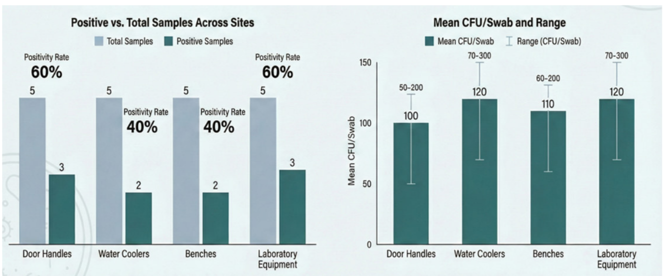

Figure 4 Distribution and Enumeration of *Klebsiella* spp. across the environmental surfaces.

Isolation of *Klebsiella* spp.

To see the growth of bacterial colony on Petriplate spread plate method was used for isolation of *Klebsiella* from the collected samples. Swabs were inoculated into 10 mL of buffered peptone water and incubated at for overnight at room temperature for enrichment in terms of increasing the number and mass. A loopful sample from enriched broth was streaked on well lawn of MacConkey agar plates.[14,15] Colonies exhibiting characteristic pink, large, and mucoid appearances were selected for further analysis. which gives the colonies their appearance on agar plates diffusing red pigment.[16,17] These bacteria might produce certain toxins which can be responsible for causing different types of illness. The obtained *Klebsiella* isolates were purified on MacConkey agar as well and sub-cultured on Eosin Methylene Blue (EMB) agar plates using the streaking method.[18]

Microscopic appearance

It is well explored that Gram staining is a differential bacterial staining technique used to differentiate bacteria into Gram Positive and Gram-Negative types according to their cell wall composition. The all twenty isolates were identified by Gram's staining having the reaction and features under the microscopic evaluation following the standard procedure.

Biochemical based study

Isolated colonies were subjected to a series of biochemical tests like staining, indole, Voges-Proskauer and citrate utilization tests etc to confirm the identity of *Klebsiella* spp.

Antibiotic Sensitivity Test (AST)

AST was performed using the standard disc diffusion method against commonly used antibiotics in micrograms as Amikacin (20), Ampicillin (10), Ceftriaxone (20), Chloramphenicol (20)

Ciprofloxacin (10), Cotrimoxazole (10), Erythromycin (15), Gentamicin (10), Tetracycline (20)

Ofloxacin (10). The mentioned antibiotics were selected on the basis of reported performance.[19]

Results

Isolation and enumeration of *Klebsiella* spp

The plate count method was employed for enumeration. Serial dilutions of the pre-enrichment broth were prepared, and 100 µL aliquots were spread onto MacConkey agar.[20] After incubation, the number of colonies was counted and expressed as CFU per swab.[21] Out of the twenty surface swab samples ten were positive for *Klebsiella* spp.

The statistical analysis of environmental contamination by examining the presence of *Klebsiella* on shared surfaces tested different specific locations, including door handles, water coolers, laboratory equipment, and benches to determine how frequently these areas hosted by these microbes. The data provides quantitative visualization of *Klebsiella spp* prevalence and microbial load on high touch facility surfaces.[4] The findings emphasize potential of these surfaces to serve as bacterial reservoir (Figure 4). Overall positivity rate was seen across fifty percent among twenty samples with door handles and lab tools showing the highest frequency of contamination. Additionally, the findings quantify the bacterial load at each site, measuring the average number of colony-forming units found on positive swabs. By highlighting these high-traffic areas, the study illustrates the

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potential for microbial spread in communal settings. This information is valuable for establishing effective sanitation protocols to limit the transmission of such opportunistic agents (Figure 4).

Microscopic appearance

The prepared slides underwent an examination under a microscope using oil immersion. All the isolates of *Klebsiella* were found as Gram negative, rods in straight form as being arranged one by one but majority in pairs. Such characteristics are usually considered on *Klebsiella*. This technique quickly categorized bacteria as gram-negative with composition and morphology of their cell wall (Figure 5).[22]

Figure 5 Showing the rod shape of *K. pneumoniae* by light microscopic examination.

Biochemical based study

Catalase test was done to isolates which exhibited a positive reaction. For proper testing three percent hydrogen peroxide (H_2O_2) was used which showed vigorous effervescence in the form of bubble formation. This reaction confirmed the presence of the catalase enzyme, which facilitates the breakdown of hydrogen peroxide into water and oxygen (Figure 6). This result reveals as a primary characteristic of members of the *Enterobacteriaceae* like *Klebsiella* spp. Triple Sugar Iron for Triple Sugar Iron (TSI) Agar Test carbohydrate fermentation patterns of the isolates was observed. This Acid/Acid (A/A) reaction indicated by the entire medium turning yellow. This signifies that the isolates are capable of fermenting glucose as well as lactose and sucrose, leading to significant acid production. Furthermore, the presence of gas production was evidenced by the displacement of the agar medium. The absence of blackening in the medium confirms that the isolates are negative for hydrogen sulfide (H_2S) production. This specific profile (A/A, Gas positive, H_2S negative) is characteristic of the metabolic activity of *Klebsiella* spp. In Voges-Proskauer (VP) Test the isolates yielded a positive result indicated by the development of a distinct pink-to-red colour. This positive reaction demonstrates that the isolates utilize the butylene glycol pathway to produce a neutral end-product of glucose fermentation. The VP-positive indication is a key diagnostic marker used to differentiate *Klebsiella* from other closely related enteric bacteria like *E. coli*. The collective results of positive Catalase, TSI and Voges-Proskauer tests combined with the earlier identification provide definitive biochemical confirmation that the isolates are *Klebsiella* spp.

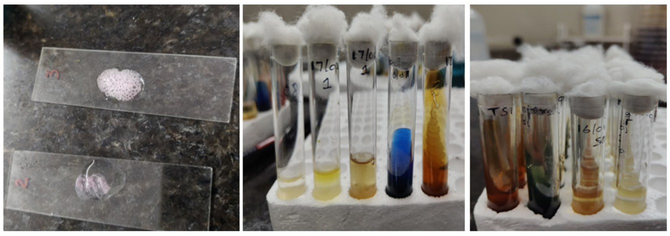

Figure 6 A-Catalase test B-Voges-Proskauer (VP) C- Triple Sugar Iron (TSI) agar test.

Antibiotic sensitivity test

The antimicrobial resistance test was performed for the isolates of *Klebsiella* spp against selective antibiotics (Figures 7 & 8). The data given away that the bacteria generally remain highly susceptible to most of the tested antibiotics. Erythromycin showed the largest zone of inhibition at 20 mm, followed by Tetracycline at 19 mm. Moderate effectiveness was seen across several other antibiotics like Ceftriaxone and loramphenicol both resulting in a 16 mm zone. A unswerving inhibitory zone of 15 mm was observed for Amikacin, Cotrimoxazole, and Gentamicin, all categorized as Sensitive. Reduced susceptibility was observed for three specific antibiotics, which were classified as having a Moderate impact Ampicillin, Ciprofloxacin and Ofloxacin. The findings indicate a favourable susceptibility profile for the *Klebsiella* isolates recovered from high-touch surfaces. The high sensitivity to Erythromycin and Tetracycline suggests these remain potent inhibitors of the growth of isolates. Furthermore, the sensitivity to Gentamicin and Amikacin provides multiple potential pathways for effective antimicrobial intervention. The 12 mm zone for Ofloxacin represents the lowest level of inhibition, marking it as the least effective agent among the tested antibiotics. These results highlight the necessity of monitoring resistance of the microbial consortium in environmental reservoirs to prevent the adverse impact on human health.

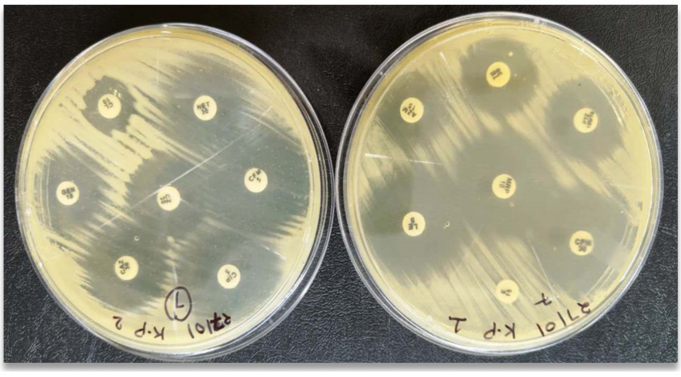

Figure 7 Antibiotic resistance patterns of the *K. pneumoniae* isolates.

Data analysis

Data were analyzed using descriptive statistics and inferential statistics CFU counts were calculated for each sample, and the prevalence of *Klebsiella* spp. was determined as a percentage of the total samples.

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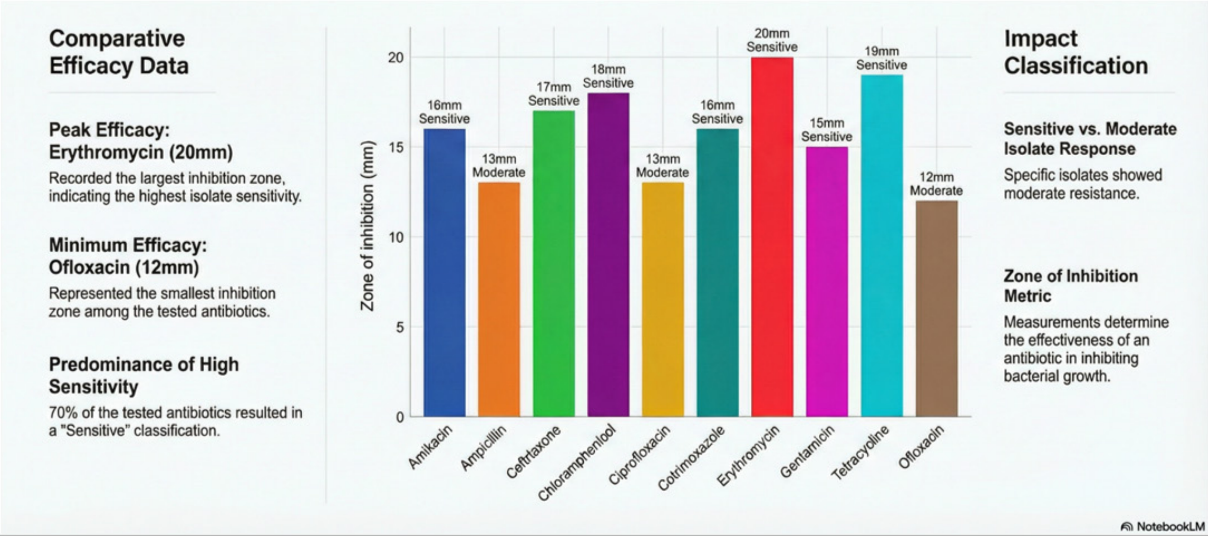

Figure 8 Antimicrobial resistance profile with zone of inhibition analysis (Evaluating isolates susceptibility by measuring inhibition zones (mm) against various antibiotics (µg) classifying responses as sensitive or moderate to determine efficacy).

Discussion

The detection of *Klebsiella* spp. in approximately thirty percent of the surface swab samples indicates that commonly used areas within the university environment serve as significant reservoirs for these bacteria. High-touch surfaces such as door handles, desks, and computer keyboards play a critical role in harboring and facilitating the spread of opportunistic pathogens, thereby contributing to their persistence in public settings. These findings are consistent with earlier reports highlighting environmental contamination by opportunistic microbes in shared spaces [Singh P, Smith]. The recorded microbial load, reaching up to 300 CFU per swab, further supports the possibility that such surfaces can sustain sufficient bacterial populations to enable transmission to individuals.[23] The isolates were conclusively identified using a combination of microscopic and biochemical characterization. They exhibited typical Gram-negative rod morphology along with positive reactions in standard biochemical tests, confirming their identity as *Klebsiella* spp. These diagnostic features reflect the organism's metabolic adaptability, which allows it to survive in diverse environmental niches. Such adaptability enhances its persistence on inanimate surfaces and increases the likelihood of human exposure. Antimicrobial susceptibility profiling revealed that the isolates were highly sensitive to erythromycin, tetracycline, and gentamicin. However, reduced susceptibility was observed for ofloxacin, ampicillin, and ciprofloxacin. This emerging pattern is concerning, as it may indicate the gradual accumulation of resistance traits within environmental reservoirs, potentially driven by exposure to antimicrobial resistance determinants present in wastewater, moist environments, or human-associated contamination. Given the well-documented association of *Klebsiella* spp. with multidrug resistance and severe infections, this observation aligns with previous findings emphasizing its public health significance.[24,25] The presence of such organisms on frequently contacted surfaces poses a notable risk, particularly for immunocompromised individuals and those with prolonged exposure to shared environments. These findings highlight the urgent need for strengthened sanitation measures, including regular cleaning and effective disinfection of high-contact areas, along with strict adherence to hand hygiene practices. Furthermore, the implementation of routine environmental surveillance programs is essential for monitoring contamination trends and evaluating the effectiveness of infection control strategies within academic institutions.[26]

Conclusion

This study highlights the presence of *Klebsiella* spp. on frequently touched surfaces within Bundelkhand University, with a thirty percent of positivity rate and notable CFU counts among positive samples. The data underscore the role of environmental surfaces as reservoirs for these bacteria and the necessity for effective mitigation strategies. Regular monitoring and improved disinfection practices are vital to reducing the risk of *Klebsiella* transmission in academic settings.

Acknowledgments

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Conflicts of interest

The author declares that there are no conflicts of interest.

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