Original Research Article
DOI: https://www.doi.org/10.62418/ijvph.11.2.2025.58-64

# Factors Influencing the Occurrence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Animal and Environmental Reservoirs within Veterinary Hospitals in Central Kerala, India

**Asif M. Hebbal [(1)], Binsy Mathew [(1)]*, B. Sunil [(1)], C. Sethulekshmi [(1)], Radhika G. [(2)], Justin Davis K. [(3)], G.K. Sivaraman[(4)]**

[(1)]Department of Veterinary Public Health, College of Veterinary and Animal Sciences, [(2)]Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, [(3)]Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Thrissur, Kerala, India-680651; [(4)]Microbiology, Fermentation and Biotechnology Division, ICAR- Central Institute of Fisheries Technology, Kochi, Kerala, India- 682029

*(Received: 14th October 2025 | Accepted: 23rd December 2025)*

## Abstract

This study aimed to determine the prevalence and the specific factors influencing the distribution of two critical ESKAPE pathogens, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, in Veterinary facilities in Kerala, India. A total of 450 animal and environmental samples were collected from 50 veterinary facilities across three districts (Thrissur, Ernakulam, Idukki) between July 2023 and June 2024 and screened for these bacteria. Isolates were identified using standard culture-based methods and confirmed by PCR. A Generalised Linear Mixed-Effects Model (GLMM) was used to identify factors associated with bacterial presence. The overall occurrence of *K. pneumoniae* was 34.0%, significantly higher than *P. aeruginosa* at 18.9%. A McNemar's test on paired samples from bovine nasal and rectal swabs showed that *P. aeruginosa* was 5.5 times more likely to be isolated from the nasal cavity ($p = 0.0265$). The GLMM identified bacterial species, sample type, and host species as significant predictors of occurrence. *P. aeruginosa* was significantly less likely to be detected than *K. pneumoniae* (OR = 0.38). Samples from goats and those from contact surfaces had significantly lower odds of being positive compared to soil samples. Host species, anatomical site, and environmental reservoir were identified as significant predictors of occurrence, with soil acting as a primary reservoir. These findings highlight the need for targeted, risk-based surveillance to inform infection control strategies in veterinary settings.

**Keywords:** *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; ESKAPE pathogens; Veterinary; Epidemiology; Risk factors

## Introduction:

The *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species (ESKAPE) group of pathogens are notorious for their capacity to evade the action of antimicrobial drugs. Among these, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are of paramount importance in the context of antimicrobial resistance (AMR) due to their high frequency of multi-drug resistance (MDR) and their ability to cause severe, life-threatening infections in both clinical and community settings. Their clinical significance is further underscored by their classification as 'Critical' priority pathogens on the World Health Organization (WHO) list, particularly due to rising resistance to carbapenems (WHO, 2017). This escalating resistance threatens the efficacy of many essential antimicrobials, posing a substantial threat to both animal and public health at the human-animal-environment interface. Veterinary facilities, in particular, can act as hotspots for the selection of resistant organisms and the horizontal transmission of antimicrobial resistance genes (ARGs). In India, the burden of AMR is particularly high, with recent surveillance data from the Indian Council of Medical Research (ICMR) indicating that carbapenem resistance in *K. pneumoniae* has exceeded 40% in clinical isolates, posing a severe challenge to public health (ICMR, 2023).

Despite the global threat, comprehensive surveillance data from veterinary settings in regions such as Kerala, India, remain limited. Few studies have concurrently investigated the prevalence of these pathogens across both clinical and environmental sources within the same facilities. Therefore, this study was designed to determine the prevalence and distribution of *K. pneumoniae* and *P. aeruginosa* isolated from varied animal and environmental sources within veterinary facilities across three districts of Kerala, India. The objectives were to: (i) determine and compare the overall prevalence of each bacterium from the collected samples and (ii) identify the factors, such as host species and anatomical site that significantly influence the likelihood of their occurrence.

## Materials and Methods:

### *Study design and sample collection*

A total of 50 veterinary facilities were randomly selected across Thrissur, Ernakulam, and Idukki during the study period of 2023–2024. From these locations, 450 samples were collected. Animal sampling involved the collection of nasal and rectal swabs from cattle, goats, and dogs (n=50 per species) presented for clinical treatment. Environmental samples included soil, water, and swabs from contact surfaces like examination tables and instruments. All samples were collected aseptically and transported to the laboratory under refrigeration.

### *Sample processing and biosafety*

All samples were processed in a Biosafety Level II (BSL-II) laboratory. Following a pre-enrichment step in Tryptone Soy Broth (TSB) incubated at 37°C for 24 hours (ICMR, 2019) samples were inoculated onto specialised media for the selective culture of the target pathogens.

### *Isolation and identification of isolates*

For *K. pneumoniae*, enriched cultures were streaked onto modified MacConkey Agar (MCA) with carbenicillin and incubated at 37°C for 24 hours. For *P. aeruginosa*, cultures were streaked onto modified Cetrimide Agar (CA) with nalidixic acid. Characteristic colonies (Figure 1A, B) were selected, and pure isolates were presumptively identified using a panel of biochemical tests (Table 1).

### *Molecular confirmation of isolates*

Genomic DNA was extracted from isolates using a thermal lysis method (Singh et al., 2022). Species-level confirmation was performed by Polymerase Chain Reaction (PCR) targeting the *rpoB* gene for *K. pneumoniae* and the *oprI* gene for *P. aeruginosa* (Douraghi et al., 2014; Bobbadi et al., 2020). The primers used are detailed in Table 2. PCR amplification was performed in a total reaction volume of 25 µL (Table 3) using a T100™ Thermal Cycler (Bio-Rad, USA). The thermal cycling conditions are detailed in Table 4. The amplified PCR products were analysed by 1.5% agarose gel electrophoresis and visualised using a gel documentation system (Syngene, USA) (Figure 2A, B).

### *Statistical analysis*

Data were analysed using R software (Version 4.4.2). Descriptive statistics were calculated to determine the occurrence of *K. pneumoniae* and *P. aeruginosa*. McNemar's test was used for paired sample analysis. To identify factors associated with bacterial presence, a Generalised Linear Mixed-Effects Model (GLMM) was employed. The model included bacterial species, sample type, and district as fixed effects, while the hospital ID (Veterinary Hospital VH) was treated as a random effect to account for clustering. For all inferential tests, a p-value $< 0.05$ was considered statistically significant.

## Results and Discussion:

This study provides valuable insight into the occurrence and distribution of two critical ESKAPE pathogens within veterinary facilities in Kerala, India. Of the 450 samples processed, the overall occurrence of *K. pneumoniae* was 34.0% (153/450), while the occurrence of *P. aeruginosa* was 18.9% (85/450). A McNemar's test on the paired data for each sample showed this difference to be statistically significant, indicating that *K. pneumoniae* was the more frequently isolated bacterium. This finding aligns with other surveillance studies, potentially reflecting its robust nature as a commensal of the gastrointestinal tract and its ability to thrive in various environmental niches (Gonzalez-Ferrer et al., 2021). The occurrence of both bacteria varied considerably across different sample types and districts (Table 5). The highest rates were generally observed in soil samples from Thrissur and Ernakulam districts (Figure 3, 4).

Analysis of co-occurrence revealed that in most sample subgroups, it was most common for neither pathogen to be present. The isolation of *K. pneumoniae* alone was the next most frequent outcome, particularly in animal samples. The co-occurrence of both pathogens in the same sample was relatively rare across all subgroups (Figure 5).

The multivariate model revealed that host species, sample site, and the specific bacterium are the key factors influencing distribution. Paired analysis of bovine samples showed that *P. aeruginosa* was 5.5 times more likely to be found in a nasal swab than a rectal swab from the same animal (McNemar's $\chi^2 = 4.92$, $p = 0.0265$). This predilection of *P. aeruginosa* for the bovine respiratory tract is consistent with its known ecology (Sadikot et al., 2005). While prevalence provides a baseline, the application of a Generalised Linear Mixed-Effects Model (GLMM) allowed for the identification of specific predictors that influence bacterial distribution while accounting for clustering at the hospital level (Figure 6).

*Pseudomonas aeruginosa* had significantly lower odds of being detected compared to *K. pneumoniae* (OR = 0.38, $p < 0.001$). The GLMM further identified soil as a primary environmental reservoir; animal-derived samples, particularly rectal swabs and those from goats, had significantly lower odds of being positive compared to soil. Compared to the reference category (Soil), samples from bovine rectum (BRS), canine rectum (DRS), goat nasal (GNS), and goat rectum (GRS) all had significantly lower odds of being positive. Most notably, samples from contact surfaces had extremely low odds of being positive

(OR = 0.03, $p < 0.001$), which is an encouraging finding, suggesting that cleaning protocols may be effective at reducing contamination on these surfaces.

While the model did not find a statistically significant difference in occurrence between the districts, the descriptive data (Table 5, Figure 3, 4) suggest potential regional variations that may warrant further investigation with larger sample sizes. A significant strength of this study is the use of a GLMM to account for the non-independence of samples from the same hospital. However, the study is limited by its cross-sectional design and a warning of model convergence failure was noted, which suggests the model may be complex for the data and that the interpretations should be made with caution. These findings underscore the complex ecology of ESKAPE pathogens in veterinary settings and highlight the need for targeted, risk-based surveillance, particularly focusing on soil as a major reservoir to inform effective infection control strategies.

## Conclusion:

In conclusion, this study demonstrates that the occurrence of *K. pneumoniae* and *P. aeruginosa* in veterinary facilities in central Kerala is non-random, with *K. pneumoniae* being the more prevalent organism. Soil appears to be a major environmental reservoir, while host species and anatomical site significantly influence the likelihood of colonisation. These findings underscore the complex ecology of ESKAPE pathogens in veterinary settings and highlight the need for targeted, risk-based surveillance, with a particular focus on environmental reservoirs like soil, to inform effective infection control strategies.

## Conflicts of interest:

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## Data availability:

The data presented in this study are available on request from the corresponding author.

## Authors' contribution:

**Asif M. Hebbal:** Conceptualisation, Investigation, Methodology, Writing – Original Draft. **Binsy Mathew:** Conceptualisation, Supervision, Project Administration, Writing – Review & Editing. **B. Sunil:** Conceptualisation, Supervision, Resources. **C. Sethulekshmi:** Investigation, Resources. **Radhika G.:** Formal Analysis, Visualisation. **Justin Davis K.:** Formal Analysis, Software. **G.K. Sivaraman:** Writing – Review & Editing.

## Ethical approval:

This study did not involve any experimental procedures on animals. All samples (nasal and rectal swabs) were collected by qualified veterinarians as part of routine diagnostic sampling from animals presented to veterinary facilities, with informed consent from the owners. The collection was performed with the utmost consideration for animal welfare, ensuring minimal distress, and adhered to the standard guidelines for veterinary practice in India.

## Acknowledgements:

The authors wish to express their sincere gratitude to the Kerala Veterinary and Animal Sciences University (KVASU) for providing the necessary facilities to carry out this research. Special thanks are extended to the Dean and the Director of Academics and Research for their administrative support and encouragement.

## References:

Bobbadi S, Kiranmayi Chinnam B, Nelapati S, Tumati SR, Kandhan S, Gottapu C, Boddu SV. Occurrence and genetic diversity of ESBL producing Klebsiella species isolated from livestock and livestock products. Journal of Food Safety. 2020; 40(1): e12738.

Douraghi M, Ghasemi F, Dallal MS, Rahbar M, Rahimiforoushani A. Molecular identification of Pseudomonas aeruginosa recovered from cystic fibrosis patients. Journal of preventive medicine and hygiene. 2014; 55(2): 50.

Gonzalez-Ferrer S, Peñaloza HF, Budnick JA, Bain WG, Nordstrom HR, Lee JS, Van Tyne D. Finding order in the chaos: outstanding questions in Klebsiella pneumoniae pathogenesis. Infection and immunity. 2021; 89(4): 10-128.

Indian Council of Medical Research. Annual Report of the Antimicrobial Resistance Surveillance Network. New Delhi: Indian Council of Medical Research; 2023.

Indian Council of Medical Research. Guidelines for Sample Collection and Transport for Microbiological Investigations. New Delhi: Indian Council of Medical Research; 2019.

Sadikot RT, Blackwell TS, Christman JW, Prince AS. Pathogen–host interactions in Pseudomonas aeruginosa pneumonia. American journal of respiratory and critical care medicine. 2005; 171(11): 1209-23.

Singh A, Sharma P, Kumar S. A simple and rapid thermal lysis method for bacterial genomic DNA extraction for PCR. MethodsX. 2022; 9: 101633.

World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

**Table 1: Biochemical characteristics for presumptive identification**

| Biochemical Test | *Klebsiella pneumoniae* | *Pseudomonas aeruginosa* |
|---|---|---|
| Gram's staining | Gram negative | Gram negative |
| Oxidase test | Negative | Positive |
| Catalase test | Positive | Positive |
| Indole test | Negative | Negative |
| Methyl Red test | Negative | Negative |
| Voges-Proskauer test | Positive | Negative |
| Citrate test | Positive | Positive |
| Motility | Negative | Positive |
| Urease test | Positive | Negative |
| Acetamide broth | Not applicable | Positive |

**Table 2: Primers used for molecular identification of isolates**

| Organism | Gene | Primer | Primer Sequence | Size (bp) | Reference |
|---|---|---|---|---|---|
| *K. pneumoniae* | *rpoB* | F | 5'-CAACGGTGTGGTTACTGACG-3' | 108 | Bobbadi et al., 2020 |
| | | R | 5'-TCTACGAAGTGGCCGTTTTC-3' | | |
| *P. aeruginosa* | *oprI* | F | 5'-TTCGAGGTTGGTTTCGTGGT-3' | 336 | Douraghi et al., 2014 |
| | | R | 5'-CTGGATGCGCACACTTTCAC-3' | | |

**Table 3: Components of PCR mixture for molecular identification**

| Reagent | Quantity (µL) |
|---|---|
| PCR master mix (2X) | 12.5 |
| Forward primer (10 pmol/µL) | 1 |
| Reverse primer (10 pmol/µL) | 1 |
| Nuclease-free water | 7.5 |
| Template DNA (50 ng/µL) | 3 |
| Total Volume | 25 |

**Table 4: Cyclic conditions for PCR for molecular identification**

| Step | Temperature (°C) | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 5 min | 1 |
| Denaturation | 95 | 45 sec | 34 |
| Annealing (*rpoB*) | 58.0 | 1 min | |
| Annealing (*oprI*) | 58.6 | 1 min | |
| Extension | 72 | 1 min | |
| Final Extension | 72 | 5 min | 1 |

**Table 5: Occurrence of *K. pneumoniae* and *P. aeruginosa* across sample types and study districts**

| Sample Type | Thrissur (n=153) | | Ernakulam (n=198) | | Idukki (n=99) | |
|---|---|---|---|---|---|---|
| | KP (%) | PA (%) | KP (%) | PA (%) | KP (%) | PA (%) |
| BNS (Nasal Swab – Bovine) | 29.4 | 17.6 | 59.1 | 36.4 | 27.3 | 36.4 |
| BRS (Rectal Swab – Bovine) | 29.4 | 5.9 | 45.5 | 13.6 | 27.3 | 18.2 |
| DNS (Nasal Swab – Dog) | 52.9 | 11.8 | 40.9 | 18.2 | 45.5 | 36.4 |
| DRS (Rectal Swab – Dog) | 58.8 | 17.6 | 27.3 | 22.7 | 27.3 | 27.3 |
| GNS (Nasal Swab – Goat) | 29.4 | 11.8 | 27.3 | 18.2 | 18.2 | 0 |
| GRS (Rectal Swab – Goat) | 23.5 | 0 | 22.7 | 0 | 27.3 | 9.1 |
| Soil | 64.7 | 41.2 | 59.1 | 45.5 | 27.3 | 0 |
| Water | 47.1 | 41.2 | 36.4 | 27.3 | 27.3 | 27.3 |
| Contact Surface | 5.9 | 0 | 0 | 0 | 5.9 | 0 |

KP: *Klebsiella pneumoniae* PA: *Pseudomonas aeruginosa*

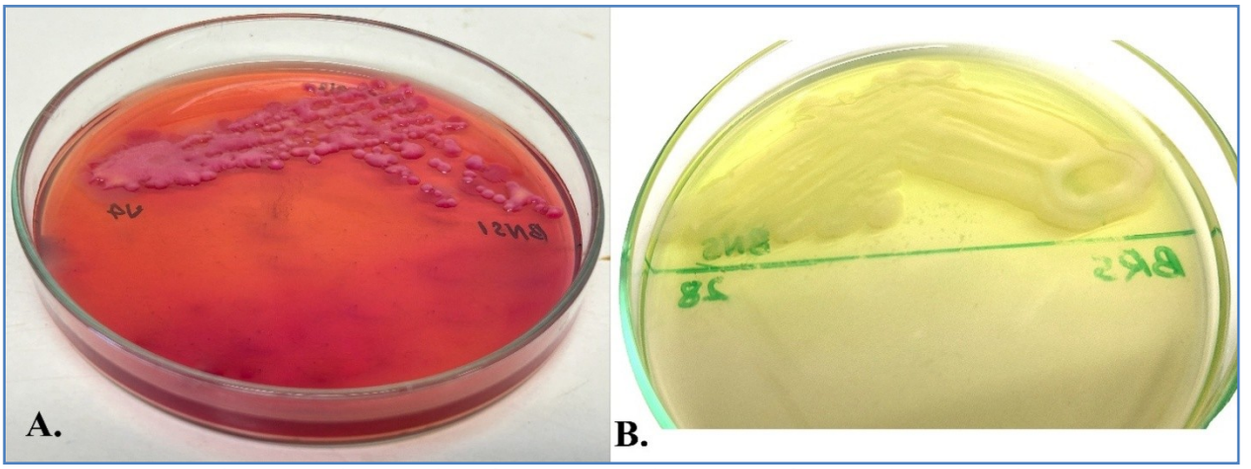


**Figure 1: (A) Large, pink, mucoid colonies of *K. pneumoniae* on MacConkey Agar (B) Pigmented colonies of *P. aeruginosa* on Cetrimide Agar**

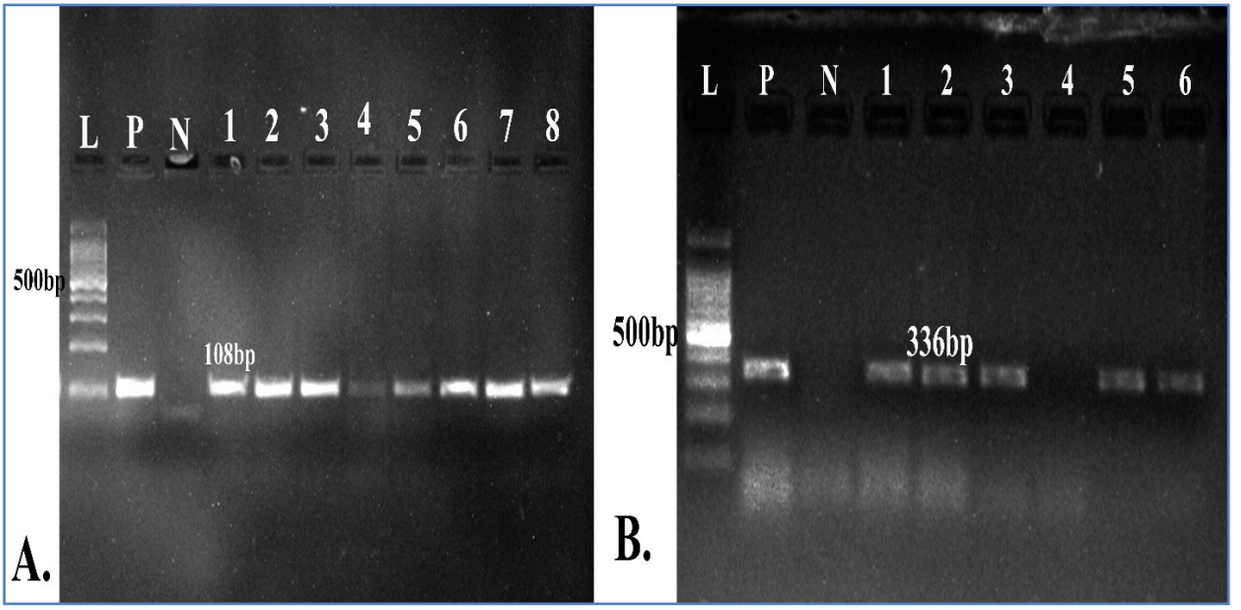


**Figure 2: Agarose gel electrophoresis of PCR products for (A) *rpoB* gene (108 bp) of *K. pneumoniae* and (B) *oprI* gene (336 bp) of *P. aeruginosa* [lanes for the DNA ladder (L), positive control (P), negative control (N), and Samples (1 to 8)]**

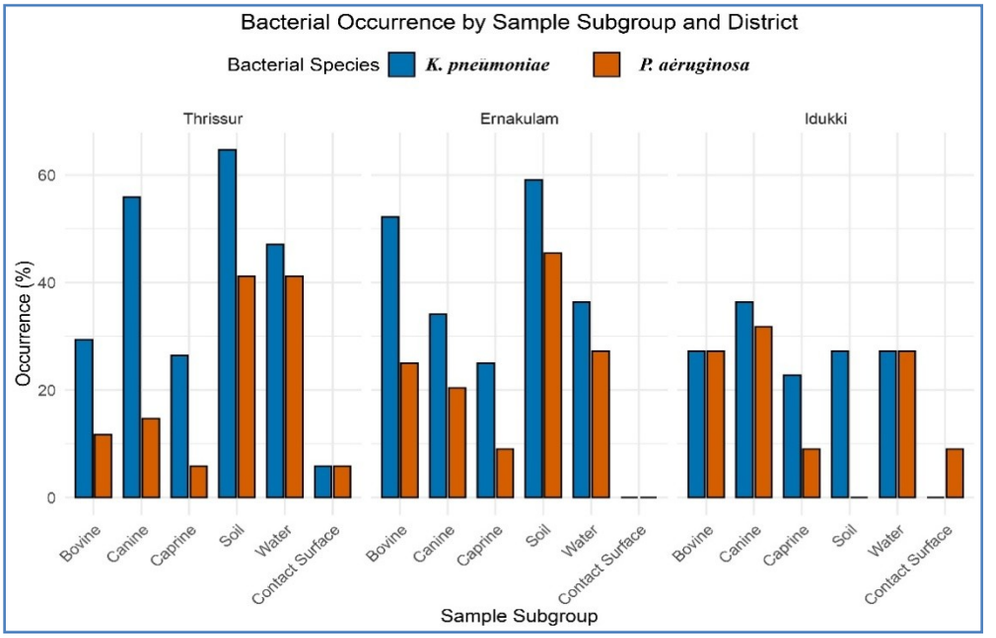


**Figure 3: Occurrence of *K. pneumoniae* and *P. aeruginosa* by sample subgroup and district**

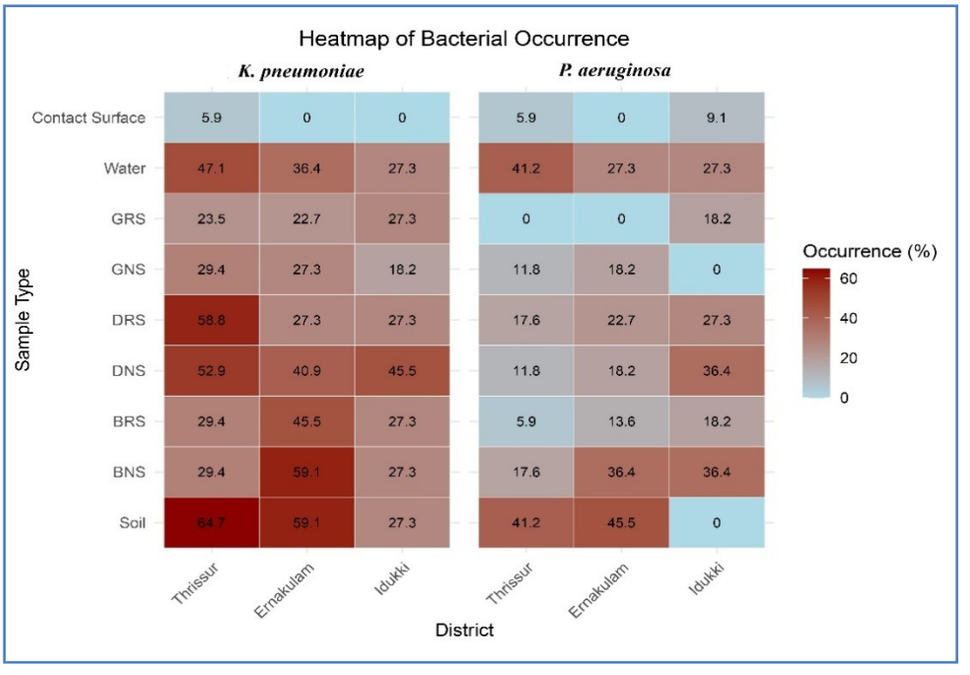


**Figure 4: Heatmap illustrating the percentage occurrence of *K. pneumoniae* and *P. aeruginosa* across different sample types and districts**

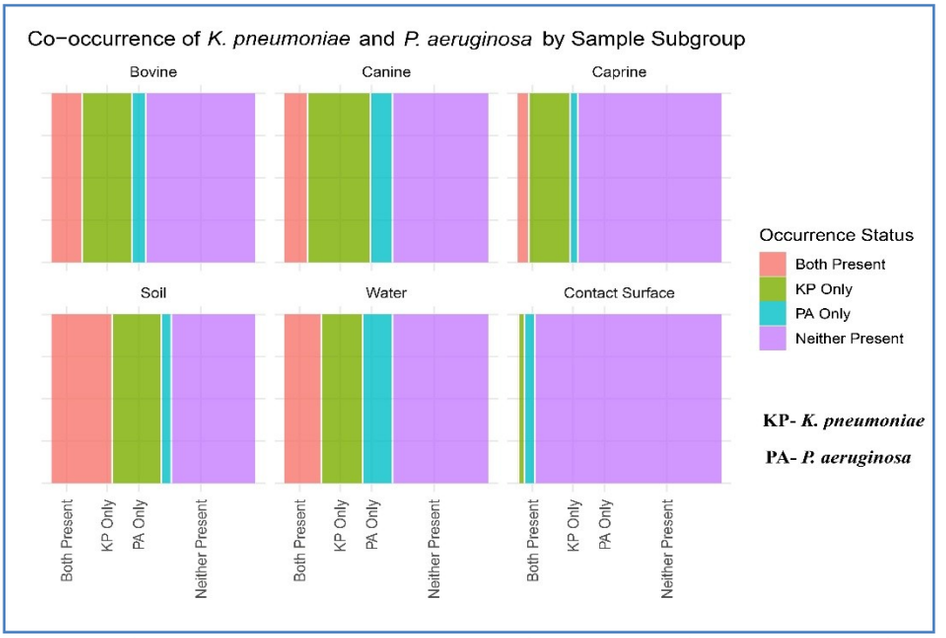


**Figure 5: Mosaic plot showing the co-occurrence patterns of *K. pneumoniae* and *P. aeruginosa* across different sample subgroups**

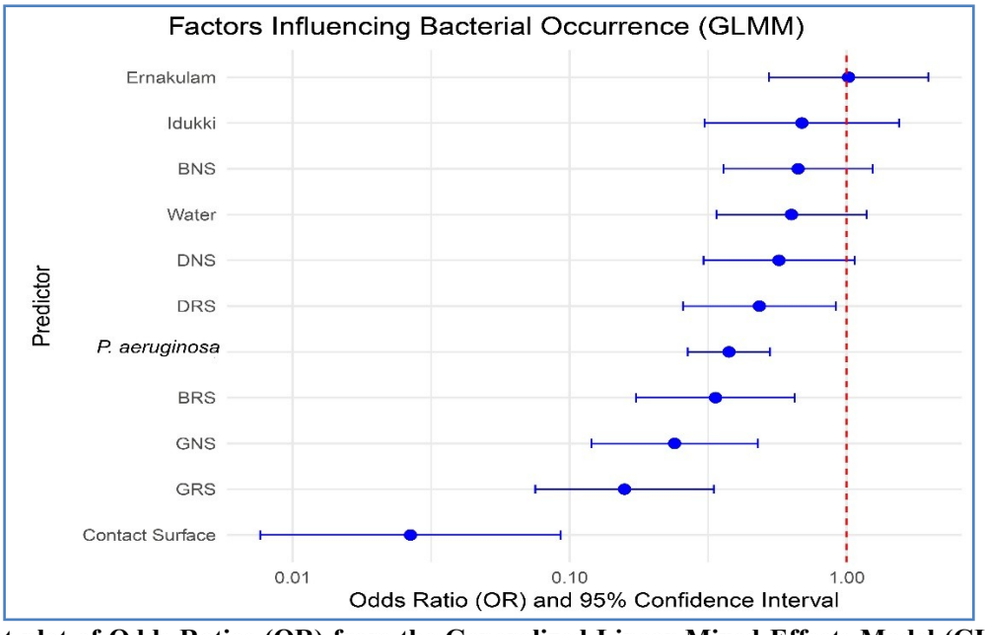


**Figure 6: Forest plot of Odds Ratios (OR) from the Generalized Linear Mixed-Effects Model (GLMM) showing factors influencing bacterial occurrence**

***Corresponding author's email ID:** binsymathew@kvasu.ac.in

**Citation:** Hebbal AM, Mathew B, Sunil B, Sethulekshmi C, G Radhika, K Justin Davis, Sivaraman GK. Factors Influencing the Occurrence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Animal and Environmental Reservoirs within Veterinary Hospitals in Central Kerala, India. Indian Journal of Veterinary Public Health. 2025; 11(2): 58-64.

DOI: https://www.doi.org/10.62418/ijvph.11.2.2025.58-64