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Original Research Article

Poultry farms as reservoirs of antibiotic-resistant *Klebsiella pneumoniae*: a "one health" concern

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ABSTRACT

Background: The emergence and spread of antibiotic-resistant bacteria have been largely attributed to the indiscriminate use of antibiotics in poultry farming, posing significant risks to human, animal, and environmental health. Increasing evidence links *Klebsiella pneumoniae* with multidrug resistance, highlighting its role as an emerging opportunistic pathogen. The present study aimed to isolate and evaluate antibiotic-resistant bacteria from chicken (*Gallus gallus domesticus*) droppings and assess their potential eco-health implications.
Methods: Droppings were collected from a poultry farm located in Mavelikkara, Kerala, India. Microorganisms were isolated and characterized using standard microbiological protocols. Molecular identification of the isolates was carried out following established procedures. Confirmation of antibiotic-resistant strains was performed using species-specific polymerase chain reaction analysis.
Results: Two antibiotic resistant strains of *Klebsiella pneumoniae PB-01(PX376439)* and *PB-02(PX376441)* strains were identified. Both strains exhibited resistance to commonly used antibiotics, including ampicillin, streptomycin, and erythromycin.
Conclusions: Poultry farms may act as reservoirs of antibiotic resistance genes, facilitating their spread beyond ecological boundaries. This is supported by the isolation of multidrug-resistant *Klebsiella pneumoniae* from chicken fecal samples collected from the farm. The findings highlight the importance of integrated surveillance and prudent antibiotic stewardship to limit the spread of antibiotic resistance within the One Health framework.

Keywords: *Klebsiella pneumoniae*, Antibiotic-resistance, Poultry farm

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative opportunistic pathogen widely distributed in soil ecosystems and associated with a broad spectrum of human infections. Although this bacterium is a normal component of both human and animal gastrointestinal tract flora, its ability to adapt to different ecological niches is demonstrated by its survival and growth in soil.[1] Antimicrobial resistance has emerged as a major global public health challenge, threatening the effective treatment of infectious diseases.[2] The agricultural sector, particularly poultry farming within low-resource settings, contributes significantly to this crisis due to the extensive use of antibiotics for growth promotion, prophylaxis, and disease control.[3] Antibiotic residues and resistant bacteria originating from poultry farms are dispersed into the environment through feces, litter, wastewater, and aerosols, generating complex transmission pathways affecting animals, humans, and ecosystems.[4] The

pathogens known as "ESKAPE" (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.) exhibit rising virulence and multidrug resistance. *K. pneumoniae* is one of these pathogens. Most nosocomial infections are caused by these bacteria, which are also able to "escape" the biocidal effects of antibiotics.[5]

K. pneumoniae, is increasingly recognized as an opportunistic pathogen capable of acquiring multiple antibiotic resistance determinants.[6] Though traditionally associated with clinical settings, recent studies have reported its presence in food animals and environmental sources. The bacterium is known to harbor plasmid-borne resistance genes, facilitating horizontal gene transfer across bacterial species.[7] Despite growing concern, data on the occurrence and molecular characterization of antibiotic resistance determinants in *K. pneumoniae* from poultry farms remain limited, particularly in developing countries. Understanding its resistance profile and genetic determinants is essential for assessing eco-health risks and developing effective mitigation strategies.[8]

Data has recently surfaced to show a connection between animal antimicrobial resistance and overuse of antimicrobial agents, which contributes to the total burden of antibiotic resistance.[9] Kerala has a high prevalence of *K. pneumoniae*, especially as a multi-drug resistant and extensively drug-resistant pathogen in clinical settings.[10] Over the past ten years, poultry has grown to be a significant agricultural sector in India. India has a relatively high rate of infectious illness and antibiotic resistance.[11] However, there are few investigations on this *E. coli* resistance in chickens.[12] Kerala is the first Indian state to implement an antimicrobial resistance action plan. Extensive study is essential to achieving the plan's strategies.[13] Such studies will shed light on the limitations and necessary actions, as there are currently only a few published works on the antimicrobial resistance of diseases from Kerala's poultry environment. The present study aimed to isolate microorganisms from poultry farms in Mavelikkara and evaluate their antibiotic susceptibility profiles in order to assess their eco-health implications within the One Health framework.

METHODS

Study area and collection of samples

A total of 20 fresh faecal samples (droppings) from chickens (*Gallus gallus domesticus*), a commonly domesticated avian species, were collected over a six-month period (from August to December, 2024) from a commercial poultry farm located in Mavelikkara, Alappuzha district, Kerala, India. Care was taken to avoid contamination during collection. Poultry dropping sample was collected in sterilized bottles transferred immediately on ice and processed within 2 hours of collection.

Isolation and identification of bacteria

Samples were enriched in buffered peptone water and cultured on MacConkey agar and eosin methylene blue agar. Lactose-fermenting, mucoid colonies were selected and subjected to standard biochemical tests including indole, methyl red, Voges–Proskauer, citrate utilization, urease, and sugar fermentation tests.

Sample preparation

One gram of the sample was weighed and homogenized using a sterile mortar and pestle under a laminar airflow cabinet. The serial dilution was conducted according to standard procedures under aseptic conditions. Five test tubes were prepared, each containing 9 ml of sterile distilled water. 1 ml of the sample was aseptically transferred into the first test tube and mixed thoroughly. From the first test tube 1 ml of sample was taken and added to 9 ml of distilled water taken in second test tube. Subsequently, serial dilutions were carried out following the same procedure. From the final three dilutions, 1 ml samples were taken and inoculated onto solidified agar plate (Mac Conkey agar) containing approximately 10-15 ml of agar media. Sample poured on surface of agar plate were evenly spread using bent glass rod to make a spread plate. The inoculated plates were incubated at 37 °C for 24 to 48 hours.

Indole test

A test tube containing 5 ml of tryptone broth was prepared and sterilized. The bacterial isolate to be identified was inoculated into the broth and incubated at 37 °C for 24–48 hours. Following incubation, 0.5 ml of Kovac's reagent was added to the culture. The formation of a red-colored ring at the surface, indicated a positive indole reaction, whereas the absence of a color change was interpreted as a negative result.

Methyl red test

Two test tubes containing methyl red-Voges-Proskauer (MR–VP) broth were inoculated with the pure culture of the test microorganism under aseptic conditions. The inoculated tubes were incubated at 35 °C for 4 days. Following incubation, 5 drops of methyl red indicator were added to first tube. The development of a red color within a few minutes was interpreted as a positive methyl red (MR) reaction and if not, it is negative.

Voges-Proskauer test

A tube containing MR–VP broth was inoculated with the test culture and incubated at 35 °C for 24 hours. Following incubation, a 1 ml aliquot of the broth culture was transferred to a clean test tube. To this, 10 drops of 5% α-naphthol solution were added, followed by 2–3 drops of 40% potassium hydroxide. The contents were gently shaken and exposed to atmospheric oxygen, and the tube

was left undisturbed for 10–15 minutes for color development. The development of a cherry red to rose coloration within 15–30 minutes was interpreted as a positive reaction. In contrast, the appearance of a yellow-brown color indicated a negative result.

Citrate test

Sterilized Simmons citrate agar medium containing the required salts was dispensed into test tubes and allowed to solidify in a slanted position to prepare agar slants. The test culture was inoculated onto the surface of the agar slant by streaking under aseptic conditions. The inoculated slants were incubated at 37 °C for 18–24 hours prior to observation. Growth of test microorganisms with a deep blue color indicated citrate utilization, whereas absence of growth and lack of colour change indicated the inability to utilize citrate.

Antibiotic susceptibility testing

The confirmed *K. pneumoniae* isolates were tested for their susceptibility to a panel of antibiotics using the disc diffusion method. The antibiotics tested included Ampicillin (10 mcg), Streptomycin (25 mcg) and Erythromycin (15 mcg). The zone of inhibition was measured in mm using a calibrated ruler.

The test colonies were cultured overnight in nutrient broth medium at 37 °C. Sterile agar plates were prepared and appropriately labeled prior to inoculation. The inoculum was evenly spread across the surface of the agar plates using a sterile cotton swab to produce a uniform bacterial lawn. The inoculated plates were allowed to dry for 5 minutes at room temperature. The antimicrobial sensitivity discs were aseptically placed at equal distances using sterile forceps. A sterile disc without antimicrobial agent was used as a control.

The inoculated plates were incubated in an inverted position at 37 °C for 24–48 hours. Following incubation, the plates were examined for the presence of zones of inhibition surrounding the antimicrobial discs. The diameter of each zone of inhibition was and recorded for analysis.

Molecular confirmation

The identified bacterial strains were designated as PB01 and PB02. The molecular identification of the selected strains done using 16S RNA sequencing at Rajiv Gandhi Centre for Biotechnology, Trivandrum. Sequencing analysis done by using basic local alignment search tool (BLAST) to determine their closest phylogenetic relatives based on sequence similarity. Following confirmation of their taxonomic identity, the nucleotide sequences were deposited in the GenBank database, and accession numbers were assigned.

Statistical analysis

Data were analyzed using descriptive statistics. Antibiotic resistance frequencies were calculated and expressed as percentages. Zone of inhibition data were subjected to statistical analysis using a two-way analysis of variance (ANOVA) in statistical package for the social sciences (SPSS) software to assess the effects of bacterial strain, antibiotic type, and their interaction.

RESULTS

Isolation and identification

Out of 20 samples analyzed, 2 isolates were presumptively identified as *Klebsiella sp.* namely *PB-01(PX376439)* and *PB-02(PX376441)* (Figure 1 and Table 1).

Table 1: Results of biochemical tests.

Test	Result
Gram staining	–
Indole (I)	–
Methyl red (MR) (M)	–
Voges-Proskauer (VP) (V)	+
Citrate utilization (C)	+

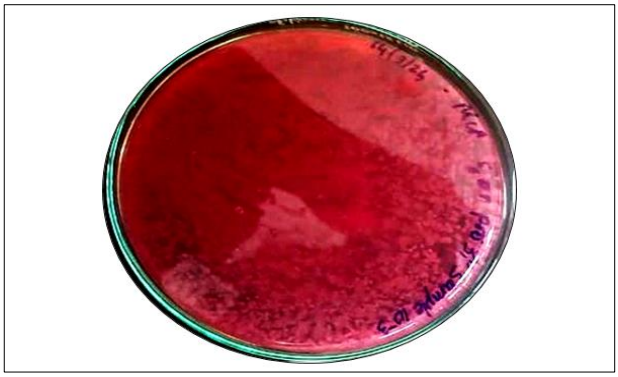

Figure 1: Plate showing Mac Conkey agar assay.

Antibiotic susceptibility pattern

The isolated *K. pneumoniae* strain was evaluated for antimicrobial susceptibility using the disc diffusion (Kirby–Bauer) method. The antibiotics selected for this assay included Streptomycin (25 µg), Erythromycin (15 µg) and Ampicillin (10 µg). The results of the susceptibility testing are presented in Table 2. To assess the effects of strain, antibiotic type, and their interaction, zone of inhibition data was subjected to a two-way analysis of variance (ANOVA). The model included strain (two levels) and antibiotic (three levels) as fixed factors. Mean comparisons were conducted at a significance level of $p<0.05$. All statistical analyses were performed using standard ANOVA procedures.

Table 2: Statistical analysis of antibiotic resistance using ANOVA.

Source of variation	Sum of squares	df	F value	P value	Source of variation
Strain	37.18	1	10.77	0.0066	Strain
Antibiotic	61.72	2	8.94	0.0042	Antibiotic
Strain × antibiotic	23.03	2	3.34	0.0705	Strain × antibiotic
Error	41.42	12	—	—	Error

The two-way ANOVA revealed a statistically significant main effect of strain on the zone of inhibition (p=0.0066), indicating that strain A and strain B differ significantly in their overall antibiotic resistance profiles. Across all antibiotics tested, strain B generally exhibited smaller inhibition zones, reflecting higher resistance compared to strain A.

A significant effect of antibiotic was also observed (p=0.0042), demonstrating that the antibiotics differed significantly in their inhibitory efficacy against *K. pneumoniae*. Among the tested antibiotics, erythromycin produced comparatively larger zones of inhibition, whereas streptomycin and ampicillin showed reduced effectiveness, indicating a higher level of resistance to these agents. The strain × antibiotic interaction was not statistically significant (p=0.0705). This suggests that, although inhibition zones varied among antibiotics and between strains, the relative pattern of antibiotic effectiveness remained consistent across both strains. In other words, both strains responded similarly to changes in antibiotic type, even though their overall levels of resistance differed.

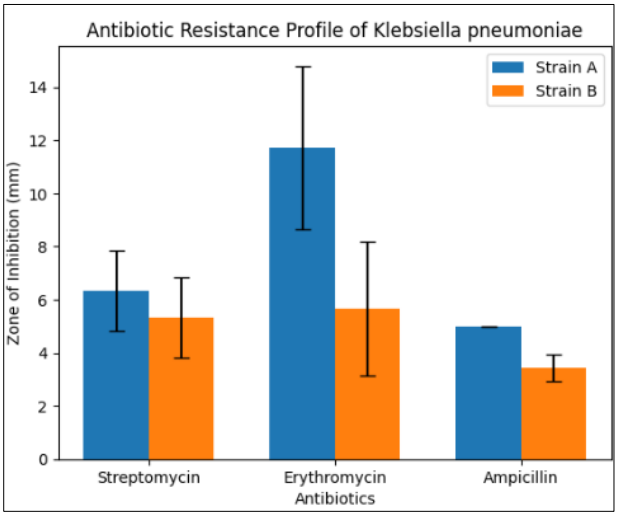

Figure 2: Antibiotic resistance profile of *K. pneumoniae* strains A and B against Streptomycin (25 µg), Erythromycin (15 µg), and Ampicillin (10 µg). Bars represent mean zone of inhibition (mm) and error bars indicate ±standard deviation (n=3).

DISCUSSION

The detection of multidrug-resistant (MDR) *K. pneumoniae* in poultry farms underscores the expanding role of food animal production systems as significant reservoirs and dissemination points of antimicrobial resistance (AMR).[14] In poultry, *K. pneumoniae* is increasingly recognized as an emerging respiratory pathogen capable of causing severe respiratory symptoms, septicemia, and high mortality, especially under intensive production conditions.[10] The extensive resistance patterns observed in this study reflect the strong selective pressures created by the routine use—and often misuse—of antibiotics in poultry rearing practices.

In the present investigation, two *K. pneumoniae* strains isolated from poultry sources were examined for their susceptibility to streptomycin, erythromycin, and ampicillin using the disc diffusion method. Both strains displayed consistently small inhibition zones across all tested antibiotics, indicating widespread resistance. The two-way ANOVA revealed significant main effects of strain and antibiotic type, suggesting inherent variability in resistance levels; however, the absence of strain × antibiotic interaction implies that both strains follow a similar resistance pattern across antibiotic classes. This uniformity may indicate shared resistance determinants or exposure to similar selective pressures within the farm environment. A recent study has reported a high prevalence of *K. pneumoniae* in dairy farm soil in Thrissur, along with considerable resistance to colistin, indicating that such environments may act as significant reservoirs with implications for environmental, animal, and human health.[10]

The absence of a significant strain–antibiotic interaction is consistent with previous reports indicating that *K. pneumoniae* strains from poultry commonly exhibit conserved resistance mechanisms.[14] Such resistance is increasingly being reported not only in clinical settings but also in environmental and food-chain isolates, suggesting widespread dissemination of resistance genes.[14] These findings indicate the potential for multidrug-resistant *K. pneumoniae* to spread from fresh poultry feces to surrounding environments and agricultural fields, particularly where untreated poultry litter is applied as manure. Consistent with the present findings, antibiotic-resistant *K. pneumoniae* has also been reported in chicken meat swab samples from Tamil Nadu, India.[15] Multidrug-resistant *K. pneumoniae* isolates have also been reported in oropharyngeal swab samples of chickens from Indonesia.[16] However, the presence of another microorganism, Enterobacteriaceae in Malaysian chicken and poultry meat highlights the significance of disposing of poultry waste properly and practicing good hygiene while handling poultry and poultry meats.[17] In contrast,

despite the occurrence of multidrug-resistant *K. pneumoniae* in poultry sources, the continued susceptibility of certain isolates to alternative antibiotics suggests the possibility of minimizing dependence on carbapenems, which are widely used for treating severe infections.[18]

These highlight the fact that chicken farms act as significant reservoirs of antibiotic-resistant *K. pneumoniae*, highlighting the potential role of poultry farms in the persistence and environmental dissemination of antibiotic-resistant bacteria. World Health Organization lists *K. pneumoniae* as one of the urgent priority pathogens because of its growing resistance to several classes of antibiotics, therefore the presence of multidrug-resistant isolates in this study is quite concerning.[19] The probability of horizontal gene transfer between environmental bacteria and human diseases is increased by the presence of resistance determinants in poultry isolates. Antibiotic resistance was previously considered to be primarily confined to clinical settings such as hospitals; however, recent studies indicate that food-producing animals serve as significant reservoirs of MDR microorganisms.[20] Studies have shown compelling evidence that close contact with farm animals increases the risk of acquiring antibiotic-resistant bacteria.[21] Prolonged exposure to antibiotics, especially at sub-therapeutic levels, can promote the development of resistance among bacteria commonly associated with poultry diseases.[22]

Additionally, the litter, feed, water supplies, and faecal matter present on chicken farms create an environment conducive to the survival and proliferation of bacteria. Animal products, contaminated equipment, and farm workers may act as potential carriers, facilitating the transmission of resistant bacteria from farms to the broader community. Such transmission pathways align with the One Health concept, which recognizes the interconnectedness of environmental, animal, and human health.

Therefore, the findings of this study highlight the need for cautious use of antibiotics in poultry production. The implementation of strict biosecurity measures, improved farm hygiene practices, and effective antimicrobial stewardship programs may help prevent the emergence and spread of resistant bacteria. Furthermore, regular monitoring of antimicrobial resistance in chicken farms is essential to identify potential threats and mitigate risks to public health.

From an eco-health perspective, the high resistance observed across all tested antibiotics is particularly concerning. *K. pneumoniae* is increasingly recognized as an important environmental and zoonotic reservoir of antimicrobial resistance genes, which can be transferred to other pathogenic bacteria through horizontal gene transfer.

Therefore, the findings of this study highlight the urgent need for judicious antibiotic use, continuous surveillance of antimicrobial resistance, and the adoption of integrated One Health approaches that link human, animal, and environmental health.

CONCLUSION

In summary, the present findings add to the growing body of evidence indicating that poultry farms serve as important reservoirs of multidrug-resistant *K. pneumoniae*. The conserved resistance patterns observed among the isolates suggest the circulation of common resistance determinants within the farm environment. These observations underscore the need for integrated One Health strategies that link human, animal, and environmental health sectors to effectively address the multifaceted challenge of antimicrobial resistance.

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