



# Molecular Detection of Multidrug-Resistant *Klebsiella pneumoniae* and Screening of Antimicrobial Resistance Genes from Chicken Samples: A comparison study between Kunshan, China, and Nairobi, Kenya

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DOI: <https://dx.doi.org/10.4314/ajhs.v38i1.1>

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## Abstract

**Background:** Resistance to antimicrobial (AMR) agents has been recognised as one of the global public health threats and a significant development concern. Misuse of antimicrobial agents in veterinary and human medicine has been reported as one of the factors accelerating the AMR threat. *Klebsiella pneumoniae*, a member of the *Bacteriaceae*, is mostly used as a community indicator due to its ability to acquire and transfer genes including potential AMR-genes including *Extended-Spectrum-Beta-Lactamases* (ESBLs), *Carbapenemases*, and colistin, across species, which may result in a public health concern. This study aimed to provide prevalence data for *K. pneumoniae* in poultry, assess antimicrobial resistance patterns, and determine resistance genes harboured.

**Materials and Methods:** We carried out a cross-sectional study in Kunshan City, China, and Nairobi, Kenya. A total of 385 cloacal swabs were collected from live chickens, 192 from Kunshan, and 193 from Nairobi. Samples were cultured, isolated, and identified using standard media, and their identity was confirmed by Polymerase Chain Reaction and gel electrophoresis. Susceptibility of *K. pneumoniae* isolates towards antimicrobial agents was determined through the Disk diffusion technique, and results were determined according to the Clinical and Laboratory Standards Institute (CLSI-M100-2022). The prevalence of resistance genes was also determined by PCR amplification alongside gene-specific primers.

**Results:** The prevalence of *K. pneumoniae* was higher in Kunshan (46.1%) compared to the prevalence recorded in Nairobi (35.9%). Resistance was reportedly high against Ampicillin, 81.7%, Ciprofloxacin, 60.8%, and Aztreonam, 48.7%. *BlaOXA-1* was the predominant resistance gene in both sites, while *blaCTX-M* and *blaNDM-1* were highly noted in Nairobi isolates, with a significant difference of  $p=0.037$ , showing an existing variation in resistance genes based on region.

**Conclusion:** There was a high prevalence and resistance of isolates towards antimicrobial agents from both sites; however, the presence of resistant genes was predominant in isolates from Nairobi. The study highlights the need to invest in programs that will promote best practices, improve awareness, and establish surveillance programs exploring a One Health framework.

**Keywords:** Antimicrobial Resistance, *Extended Spectrum Beta Lactamase*, *Klebsiella pneumoniae*, *Carbapenemases*, Multi-drug Resistant strains

[*Afr. J. Health Sci.* 2025;38(1): Article 1. <https://doi.org/10.4314/ajhs.v38i1.1>]



## Introduction

Antimicrobial resistance (AMR) is one of the fastest-rising global health threats (1). Global data shows AMR claims about 1.2 million lives annually, with a projection of 10 million by 2050 (2,3). Studies have raised concerns about increased threats, especially post-COVID-19 (4). The impact of resistance is severe in developing nations due to competing social and economic issues and poor infrastructure (5). Studies have reported the interconnectivity of life on earth, contributing to the transfer of infectious agents between humans, animals, and the environment. Thus, eradication requires a multi-sectoral approach involving healthcare practitioners, veterinary officers, and environmental scientists (6). Among other contributors to AMR emergence, inappropriate prescription and misuse of antimicrobial agents in animal and poultry feeds significantly contribute to rising infection rates (7,8). At least 50% of antimicrobial medications used in animal feeds have been reported as potential causes of resistance passed on to humans (9).

Members of the Enterobacteriaceae family, including *Klebsiella pneumoniae*, have widely spread in the environment. Most strains are commensal but can harbour genes that confer resistance to antimicrobial agents, including broad-spectrum, first-line drugs (10). These strains are referred to as superbugs, indicators of community-based infection. Their presence may lead to ineffective treatment of bacteria that cause common infections (11), resulting in adverse outcomes like prolonged illness or death. Like other Enterobacteriaceae, *K. pneumoniae* rapidly multiplies, occupying about 80% of the environment (12). When it reaches the bloodstream and internal organs, it may become pathogenic, causing pneumonia, wounds, and urinary tract infections, and in extreme cases, severe organ failure.

*K. pneumoniae* encodes genes that confer resistance to first-line antimicrobial agents, including broad-spectrum drugs. These include *Extended-Spectrum Beta-Lactamases (ESBLs)*, *Carbapenemases*, and colistin-resistant genes, presenting difficulties in treatment (13). Resistance has been linked to unrestrained antimicrobial use in humans, animals, and their environment, poor water and sanitation services, inadequate infection control, and improper food handling. Resistance genes can be transferred between humans, animals, and the environment, as life systems are interconnected (14). Detection of resistant bacteria in samples from companion and domesticated animals raises concern due to possible transmission between animals, humans, and the environment, contaminating water and soils, a public health concern, as poultry may act as AMR reservoirs (15).

Uncontrolled use of antimicrobials in veterinary medicine and as growth promoters also contributes to resistance across species. Resistance genes, including  $\beta$ -lactamases like *blaTEM*, *blaCTX-M*, *blaOXA*, and *blaSHV*, carbapenemases like *VIM*, *NDM*, and *IMP*, and colistin resistance gene *mcr-1*, have been reported in clinical and veterinary samples (16). Hence, there is a need to explore a One Health approach to understanding infections. The One Health framework promotes interdisciplinary action to halt the spread of resistant agents. Most antimicrobials used in veterinary medicine are also used in humans. Of 30 antimicrobial classes globally, only 14 are for animals, and only a third are actively available. With AMR's emergence, vets may be left unable to treat life-threatening diseases (17).

Despite increased public awareness, policy enforcement remains weak, the surveillance data is limited, and treatment plans are compromised. This study uses a cross-sectional approach to investigate the prevalence and AMR patterns of *K. pneumoniae* in poultry



and determine the presence of resistance genes. Sub-Saharan Africa, including Kenya, has limited AMR data in food animals, while surveillance systems are developing. These challenges have slowed information flow and limited understanding of AMR and One Health dynamics. China, however, has developed regulatory controls over antimicrobial use in livestock and poultry, with several areas already applying best practices. This study will offer findings to support AMR surveillance, guide targeted policy action, and promote a One Health framework for future research and implementation. These findings are essential for effective stewardship, combating AMR linked to meat consumption, and advancing the UN Vision 2030 and sustainable health and food safety goals.

## Materials and Methodology

### Study sites

This study took place in two sites: Kunshan area in China, a high-income region, and Nairobi area in Kenya, a middle-income region. Samples were processed at the Duke Kunshan University at the Global Health Centre in China, the Kenya Institute of Primate Research (KIPRE) in Kenya, and the One-Health Centre in Kenya.

### Sample size determination

Fisher's sample size determination by Fisher's exact formula:

$$n = \frac{Z^2 PQ}{SE^2}$$

$$q=0.5 \text{ SE}=0.05, Z=1.96 \text{ and } p=0.5$$

$$n = ((1.96)^2 \times 0.5(0.5)) / (0.05)^2$$

$$(3.8416 \times 0.25) / 0.0025$$

$$0.9604 / 0.0025$$

384.16, thus, 384 samples.

n = required sample size,

Z = the critical value of the normal distribution, t  
95 % CI,

P = estimated sample proportion, standard deviation

d = margin of error of prevalence estimate at 5 % (0.05),

$\alpha$  = level of significance at 5 %, SE is the margin of error, 0.05.

### Sampling criteria

A random sampling technique was used in both sites. Ten live chicken vendor shops were selected from each site, each located at a significant distance apart to ensure even distribution and avoid clustering. Vendors voluntarily consented to allow at least 20 live chickens to be randomly selected, coded, and screened using a simple random number generator.

### Sample collection

A total of 385 cloacal swabs samples were collected: 193 from Kunshan (China) and 192 from Nairobi (Kenya), representing *K. pneumoniae* prevalence and resistance patterns. Swabs were collected with sterile cotton swabs, placed in 2 ml sterile tubes containing Dulbecco's Modified Eagle Medium (DMEM), and transported under cold chain and sterile conditions to maintain integrity and prevent contamination.

### Isolation and identification of *K. pneumoniae*

#### *pneumoniae*

Collected swabs were cultured on MacConkey and Eosin Methylene Blue (EMB) agars, incubated at 37°C for 18–24 hours, and identified based on colony morphology. Mucoid pink colonies on MacConkey and purplish pink on EMB were preliminarily identified as *K. pneumoniae*. Confirmation was done using the polymerase chain reaction technique (PCR) (18). *K.pneumoniae* isolates were confirmed by PCR amplification of the *rpoB* gene using species-specific primers, performed as under the cycling conditions below. Initial denaturation at 95°C/5min, 35 cycles at 95°C/30seconds, annealing at 58°C /30seconds, and extension at



72°C/45seconds, followed by a final extension at 72°C/7minutes. Amplicons visualised on a 1.5% agarose gel, using a reference strain, *K. pneumoniae* ATCC 700603.

### Antimicrobial sensitivity and susceptibility testing

Revived isolates were cultured on Mueller-Hinton agar (MHA) and incubated for 18 hours at 37°C. Colonies were emulsified in normal saline to a 0.5 McFarland standard before MHA inoculation. Antimicrobial discs were placed, and plates were left for 15 minutes, then incubated at 37°C for 18–24 hours. Disc diffusion was performed using the Kirby-Bauer method per CLSI 2022 guidelines (19). Antimicrobials tested included agents from the following classes, Betalactams or Penicillins represented by Ampicillin (AMP, 10 µg) and Tigecycline (TGC, 10 µg), Florouquinolones represented by Ciprofloxacin (CIP, 5 µg), Monobactams, represented by Aztreonam (ATM, 30 µg), Fourth generation Cephalosporins represented by Cefepime (FEP, 30 µg), Third-generation Cephalosporins agents represented by Cefotaxime (CTX, 30 µg), Carbapenems represented by Imipenem (IPM, 10 µg), and Zones of inhibition were measured and interpreted as susceptible (S), intermediate (I), or resistant (R) per CLSI M100–2022 breakpoints (20). Table 1 shows interpretive criteria (zone diameter in mm) used to categorise isolates as susceptible (S), intermediate (I), or resistant (R) to each antimicrobial agent tested.

### Molecular analysis

DNA was extracted using the TAKARA commercial kit, while PCR assays were performed using a HotStarTaq DNA Polymerase kit (Qiagen, Valencia, CA), which included a Hot-taq master mix, PCR buffer, primers, and DNA template. Thermocycling involved pre-denaturation at 95°C/15min, followed by 30 cycles (94°C/1min, 55°C/1min, 72°C/1min), and a final extension at 72°C/10min. Electrophoresis was done on a 1.5% agarose gel using 1×TAE buffer, SYBR safe, loading dye, and a 100bp DNA ladder. Resistant genes were identified in isolates previously resistant to first-line drugs using gene-specific primers. All tests included the *K. pneumoniae* positive control, KPN 700603 (Table 2). This table shows gene-specific primers used to detect resistance genes in isolates previously identified as resistant to the tested antimicrobial agents. Reference strain KPN 700603 served as the positive control.

### Statistical analysis

Descriptive statistics and analysis of variance (ANOVA) with Tukey's HSD were carried out with SPSS 26.0 Statistical Software. A value of  $p < 0.05$  was considered a statistically significant association using logistic regression.

### Ethical approval

This study was approved by the Institutional Animal Care & Use Committee (IACUC), Duke Kunshan University, China, and the Institute of Primates Research (IPR-ISERC/01/23), Kenya.

**Table 1**  
*CLSI M100-2022 Antimicrobial Susceptibility Breakpoints for Tested Antibiotics*

| Class                                   | Antimicrobial agent | Disc code     | S   | I     | R   |
|-----------------------------------------|---------------------|---------------|-----|-------|-----|
| Betalactams/ Penicillins                | Ampicillin          | AMP-10 µg     | >17 | 14-16 | <13 |
| Cephalosporins (4th gen Cephalosporins) | Cephems             | FEP-30 µg     | >25 | 19-24 | <22 |
| Third-generation Cephalosporins         | Cefotaxime          | CTX-30 µg     | >26 | 23-25 | <22 |
| Monobactams                             | Aztreonam           | ATM-30 µg     | >23 | 18-20 | <17 |
| Carbapenems                             | Imipenem/Meropenem  | IPM/MPM-30 µg | >23 | 20-22 | <19 |
| Colistin                                | Colistin            | C-30 µg       | >16 | 17-21 | <15 |
| Florouquinolones                        | Ciprofloxacin       | CIP-5 µg      | >26 | 22-25 | <21 |
| Betalactam                              | Tigecycline         | TGC           | >20 | 15-17 | <14 |



## Results

### Microbiological analysis

**Culture and identification of *Klebsiella pneumoniae*.** A total of 385 cloacal swab samples were collected, 193 from Nairobi and 192 from Kunshan. Confirmed *K. pneumoniae* were 89 (46.1%) in Kunshan, and 69 (35.9%) in Nairobi.

### Antimicrobial sensitivity testing

#### Comparison of antimicrobial resistance patterns in Kunshan and Nairobi.

All confirmed positive isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method. All isolates, except one from Kunshan, exhibited resistance to at least one antimicrobial agent. The highest overall resistance rate was observed against Ampicillin (AMP, 81.7%), Ciprofloxacin (CIP, 60.8%), and Aztreonam (ATM, 48.7%). Most isolates remained susceptible to fourth-generation

cephalosporins (Cefepime, FEP, 8.2%), Tigecycline (TGC, 10.1%), and Imipenem (IMP, 11.4%). Intermediate resistance was recorded in Cefotaxime (CTX, 29.8), a third-generation cephalosporin.

When antimicrobial resistance (AMR) rates were compared between Kunshan and Nairobi, findings noted a high resistance prevalence of about 99.4% of isolates exhibiting resistance. Resistance was reported in at least 1 antibiotic agent by isolates from both locations. The overall prevalence of resistant isolates did not, however, differ significantly between the two sites ( $p > 0.05$ ).

Despite the prevalence and resistance burden being comparable, isolates from Kunshan generally exhibited higher resistance rates across most individual antibiotics, except Imipenem, which showed higher resistance in Nairobi.

**Table 2**

PCR-based detection of resistance genes in multidrug-resistant isolates

| Target                       | Target Gene       | Primer  | Oligonucleotide Sequence (5'→3') | Product Size (bp) | Annealing Temp (°C) | References |      |
|------------------------------|-------------------|---------|----------------------------------|-------------------|---------------------|------------|------|
| <i>Klebsiella pneumoniae</i> | <i>Rpo B</i> gene | Forward | CAACGGTGTGGTTACTGACG             | 108               | 55                  | (21)       |      |
|                              |                   | Reverse | TCTACGAAGTGGCCGTTTTC             |                   |                     |            |      |
| ESBL resistance gene         | <i>blaOXA-1</i>   | Forward | GGCACCAGATTCAACTTTCAAG           | 564               | 61                  | (22)       |      |
|                              |                   | Reverse | GACCCCAAGTTTCCTGTAAGTG           |                   |                     |            |      |
|                              | <i>blaCTX-M</i>   | Forward | ATGATGAAAAATCGTTATGC             | 489               | 57                  | (23)       |      |
|                              |                   | Reverse | CAGCATCTCCCAGCCTAAT              |                   |                     |            |      |
|                              | <i>blaSHV</i>     | Forward | CCTGTTAGCCACCCTGCC               | 768               | 60                  |            |      |
|                              |                   | Reverse | CCGCAGATAAATCACCAC               |                   |                     |            |      |
|                              | <i>blaTEM</i>     | Forward | ATTTCCGTGTCGCCCTTAT              | 759               | 54.5                |            |      |
|                              |                   | Reverse | CTACGATACGGGAGGGCTTA             |                   |                     |            |      |
| Carbapenem resistance genes  | <i>blaIMP</i>     | Forward | CATGGTTTGGTGGTTCTTGT             | 488               | 53                  |            | (24) |
|                              |                   | Reverse | ATAATTTGGCGGACTTTGGC             |                   |                     |            |      |
|                              | <i>blaVIM</i>     | Forward | AGTGGTGAGTATCCGACA               | 280               | 52                  |            |      |
|                              |                   | Reverse | ATGAAAGTGCGTGAGAC                |                   |                     |            |      |
|                              | <i>blaNDM1</i>    | Forward | GGCGGAATGGCTCATCACGA             | 287               | 55                  |            |      |
|                              |                   | Reverse | CGCAACACAGCCTGACTTTC             |                   |                     |            |      |
| Colistin resistance gene     | <i>mcr-1</i>      | Forward | CGGTCAGTCCGTTTGTTTC              | 309               | 55                  | (25)       |      |
|                              |                   | Reverse | CTTGGTCGGTCTGTAGGG               |                   |                     |            |      |
|                              |                   | Reverse | AAGTAAGTGA                       |                   |                     |            |      |

### Distribution of resistant compared with sensitive isolates by antibiotic

Ampicillin and Ciprofloxacin showed high resistance to *K. pneumoniae* isolates, while Imipenem and Cefepime had comparatively lower resistance rates. Resistance in Ampicillin was 83.2% in Kunshan vs. 79.7% in Nairobi, and that of Ciprofloxacin was 86.2% vs. 66.7% in Kunshan and Nairobi, respectively. In contrast, resistance to Imipenem (IMP) was higher in Nairobi (15.9%) when compared to that of Kunshan (7.9%), and that of Cefepime was slightly higher in Kunshan (11.2%) than in Nairobi (4.4%). Resistance to Ticarcillin was 10.1% vs. 10.2%, while Imipenem was 7.9% vs. 15.9% in Kunshan and Nairobi, respectively. (Figure1).

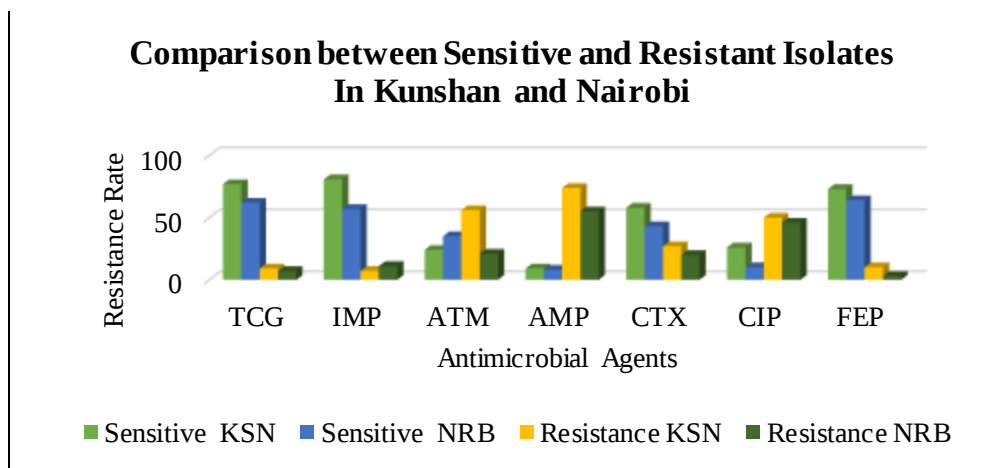
### Site-specific differences in AMR profiles of bacterial isolates from Kunshan and Nairobi

Statistically significant differences ( $p < 0.05$ ) were observed in the resistance profiles of specific antimicrobial agents across both sites. High levels of resistance were recorded for AMP, CIP, and Aztreonam (ATM), while moderate to low resistance was observed for Cefotaxime (CTX), Imipenem (IMP), Tigecycline (TGC),

and Cefepime (FEP). Significant differences ( $p < 0.05$ ) were observed in resistance to AMP, CIP, and ATM compared to CTX, IMP, TGC, and FEP. The overall AMR burden between Kunshan and Nairobi was not significantly different ( $p > 0.05$ ). This pattern highlights a concerning resistance trend against commonly used first-line and second-line antibiotics, particularly  $\beta$ -lactams and fluoroquinolones, which are frequently relied upon for empirical treatment. (Figure 2).

### Prevalence of antimicrobial resistance genes in *Klebsiella pneumoniae* isolates from Kunshan and Nairobi

The average resistance gene prevalence across both study sites was 28.9%, with Nairobi exhibiting a higher rate (35.5%) compared to Kunshan (27.9%). Most of the resistance genes identified were associated with *Extended-Spectrum Beta-Lactamase-producing* (ESBL-KP) and *Carbapenem-Resistant K. pneumoniae* (CR-KP) strains. Among these, *blaOXA-1* was the most frequently detected gene, with high prevalence in both Kunshan (48.3%) and Nairobi (53.6%). This was followed by *blaCTX-M* and *blaNDM-1*, which together had an average prevalence of 39.2%.



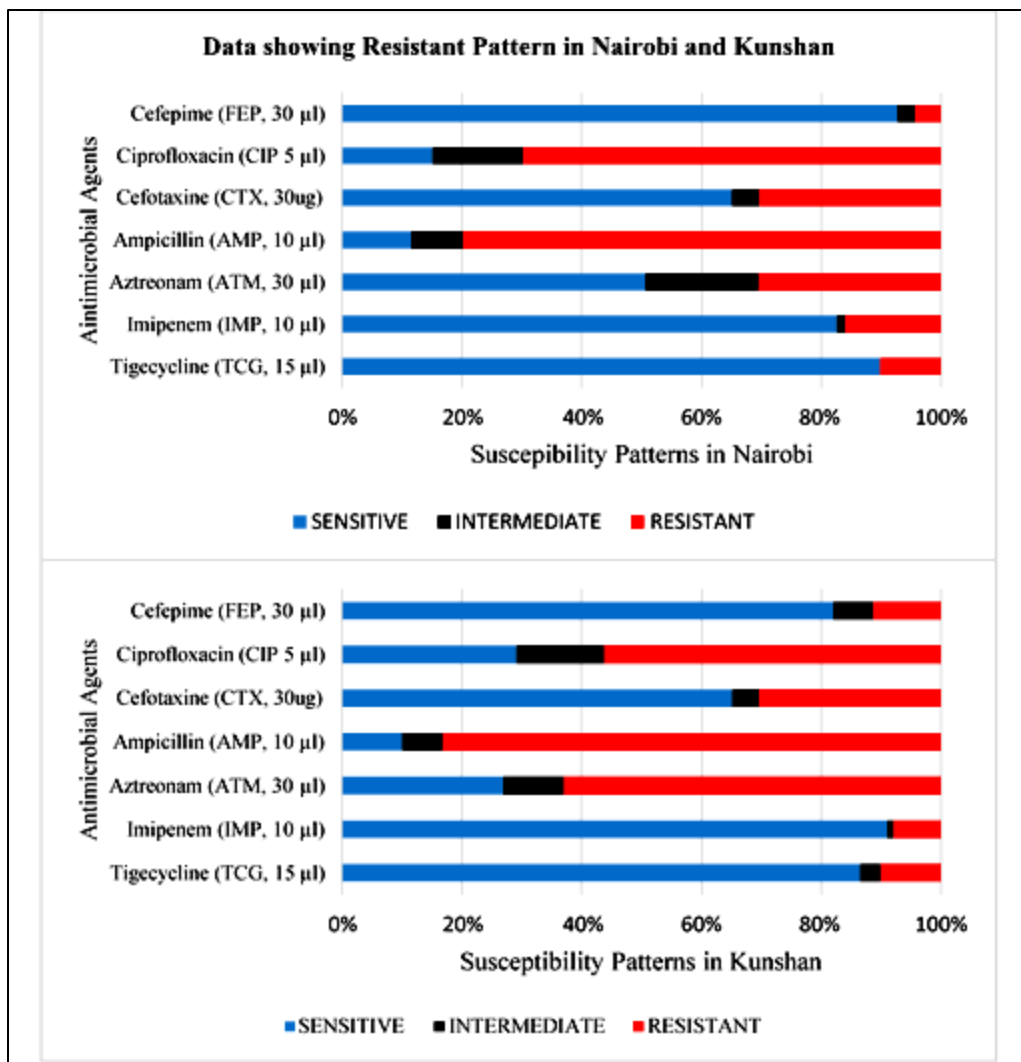
**Figure 1**

Distribution of resistant and sensitive isolates across antibiotics for all samples combined from both sites

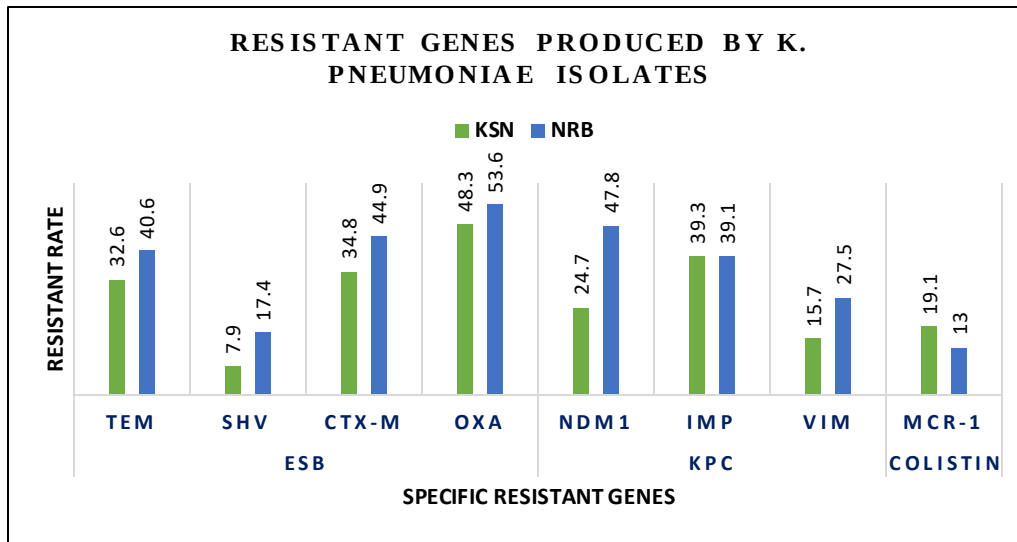


Nairobi isolates exhibited higher resistance gene detection across nearly all genes, including *mcr-1*, which is associated with colistin resistance. Differences in the prevalence of genes related to Imipenem resistance were not statistically significant ( $p>0.05$ ), suggesting no meaningful site-specific variation for those particular markers. Genes such as *blaSHV* and *mcr-1* showed relatively low overall prevalence, at 12% and 16.5%, respectively. Overall, the *K. pneumoniae* isolates were found to be multidrug-

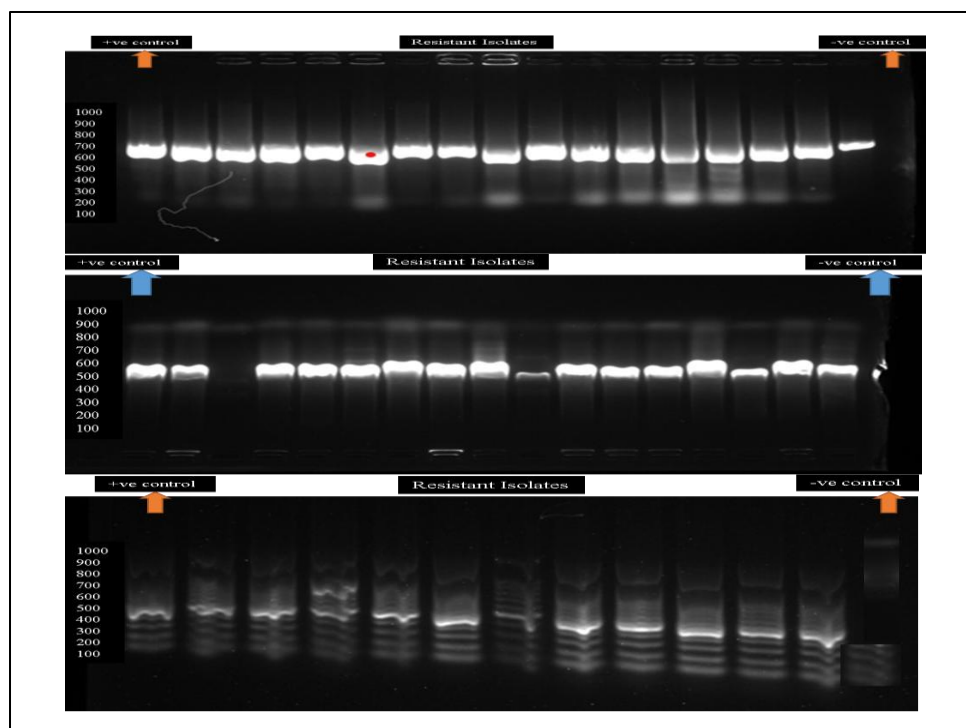
resistant (MDR), exhibiting resistance to at least one agent in three or more antimicrobial categories. Most isolates were positive for *blaOXA-1*, *blaCTX-M*, and *blaNDM-1*, highlighting the presence of clinically significant resistance mechanisms. The prevalence of *blaOXA-1* was significantly higher than that of other genes ( $p<0.05$ ). However, when grouped by resistance class (*ESBL-KP*, *CR-KP*, and *COLR-KP*), the differences in overall prevalence were not statistically significant ( $p>0.05$ ).



**Figure 2**  
Site-specific differences in Antimicrobial Susceptibility Patterns of bacterial isolates from both Nairobi and Kunshan.



**Figure 3**  
Prevalence of antimicrobial resistance genes in *Klebsiella pneumoniae* isolates from Kunshan and Nairobi.



**Figure 4**  
Gel electrophoresis of PCR amplification products for *bla*TEM, *bla*OXA, and *bla*IMP genes in *K. pneumoniae* isolates



## Discussion

A combined prevalence of 40.8% was observed across both sites, with Kunshan recording a prevalence of 52.8% and Nairobi a prevalence of 48.4%. Previous studies noted a prevalence of 4.67% in China (26) and a slightly lower prevalence in Kenya (27). These findings concur with findings presented by a systematic review study in Kubang Keria in Malaysia. The study noted an estimated pooled prevalence of 32.8% of *K. pneumoniae* that conferred resistance to at least three antimicrobial agents (28).

Findings from Kunshan, China, however, contradict previous findings noted in Shandong Province in China, which reported a significantly low prevalence rate (4.6%) in chicken samples, suggesting variance in prevalence rates in different parts of China. These findings could be attributed to improved adherence to controlled practices and enactment of high safety standards in Shandong province (29). In their findings, the team noted that intervention targeting healthcare-associated microbes was effective through adherence to prescribed antimicrobial use and recommended application of behaviour change principles.

Studies in India recorded a relatively high prevalence rate, between 43.8% and 72.3%, which demonstrates that the prevalence of microbial agents varies from one geographical location and environment to another. Findings show that the prevalence in Nairobi (48.4%) was slightly higher than in a previous study (30). A study in Uganda reported the presence of enterobacteria, including

*K. pneumoniae*, in chicken samples (31), while Kenya's national AMR surveillance reported the presence of the *Enterobacteriaceae* family, including *Escherichia coli* and *K. pneumoniae*, in common livestock (32), despite

the limited existing data. An increase in the prevalence rate in Nairobi may be associated with the unregulated use of antimicrobial agents in both humans and animals, poor practices, and a lack of public awareness. The current study, therefore, provides reference data that will significantly contribute to the fight against AMR.

The current study noted 99% resistance towards antimicrobial agents, demonstrating high resistance towards beta-lactams/Penicillins (Ampicillin), Fluoroquinolones (Ciprofloxacin), and Monobactams (Aztreonam). These classes of antimicrobial agents have been placed on the priority list of restricted drugs because of their potential to develop resistance in the environment, yet they remain among the most readily available options for treating infections in humans. Therefore, if bacterial strains that acquire resistance to these agents are transmitted to humans through contaminated chicken products or other exposure routes, they may compromise treatment efficacy, resulting in limited or costly therapeutic options (33, 34).

Resistance towards Carbapenems (Imipenem) was notably higher in Nairobi (15.9%) when compared to Kunshan (7.9%), which is a potential indicator of misappropriation of antimicrobial agents, pointing to differences in their usage patterns. These findings align with findings from a study in Shandong Province, which noted high exhibition of resistance (87.9%) from poultry samples (35). High resistance in China, contrary to previous studies, which exhibited great efforts to curb the overuse of

Antimicrobial agents, leading to a relative decrease in consumption of antimicrobial agents (36), show continuous unrestrained use of these agents, especially in companion animals (37).



Nairobi reported 96% resistance, findings that are similar to those of another study in Egypt. Both studies have exhibited the presence of multidrug-resistant *K. pneumoniae* from poultry farms, highlighting potential zoonotic infectious agents that are a threat to public health (38). This is attributed to their capacity to confer resistance against last-resort antimicrobials, posing a significant public health threat. The Kenya National Antimicrobial Stewardship Plan identified fluoroquinolone and penicillin resistance as a concern in both humans and animals (39, 40). These findings also present a worrying state that should be addressed through an interdisciplinary approach by exploring a One Health framework. Kenya has experienced a lack of veterinary professionals owing to limited training centres, contributing to a lack of consultation services and unregulated prescription practices (41).

*BlaOXA* was the predominant gene, followed by *BlaCTX-M* and *blaNDM-1*, correlating with high resistance demonstrated by these isolates towards various classes of antimicrobial agents, including the broad spectrum. Studies have shown a high resistance rate in isolates phenotypically resistant to antimicrobial agents (42). The overall high resistance gene burden in Nairobi mirrors previous studies, which noted high resistance in Mukuru, an informal settlement in Nairobi (43). In this study, the author noted that most of the isolates exhibited resistance towards  $\beta$ -lactams and ciprofloxacin, commonly used antimicrobial agents locally. The detection of *blaNDM-1* despite low Imipenem resistance indicates the presence of carbapenemase-producing strains, an emerging threat to first-line therapies. In West Africa, *blaNDM* prevalence in *K. pneumoniae* reached 18.92%, indicating regional spread of  $\beta$ -lactamase producers. Though East African data on *K.*

*pneumoniae* in poultry is limited, detection in Kenyan clinical isolates suggests possible zoonotic transmission, reinforcing the One Health perspective.

Our findings are consistent with those of Karam et al., who reported high resistance among isolates, with most exhibiting phenotypic resistance to  $\beta$ -lactams and even broad-spectrum antimicrobials (44). Similarly, a South African study found that 96.7% of poultry isolates were multidrug resistant (MDR), a result comparable to our observations (45). Notably, the study also identified carbapenem-resistant broiler isolates carrying multiple resistance genes, including *blaKPC*, *blaOXA-48*, and *blaNDM*, underscoring the global MDR burden. Within East Africa, MDR *K. pneumoniae* has been increasingly reported. For instance, a 2020 Kenyan study revealed that over 70% of hospital isolates were MDR, primarily due to ESBL production (46). Together, these trends highlight the likelihood of shared reservoirs and reinforce the urgent need for integrated, cross-sectoral AMR surveillance.

## Study Limitations and Strengths

### Limitations

- The sample size, while statistically adequate, may not fully represent the wider poultry population in Kenya and China, as it was limited to two locations.
- Time constraints restricted the study to live birds; inclusion of human and environmental samples with phylogenetic analysis would offer more insight.
- Some phenotypically resistant isolates lacked tested genes, indicating possible alternative resistance mechanisms not explored.
- The absence of a standardised veterinary drug catalogue meant human clinical prescriptions were used to determine resistance levels.



### Strengths

- This study offers a direct comparison of *K. pneumoniae* in poultry from both a developing and a developed country, revealing geographic resistance differences.
- PCR targeting of the *rpoB* gene ensured accurate isolate identification.
- Antimicrobial susceptibility testing was performed following CLSI standards, ensuring reliable and comparable results.
- The study highlights the role of food animals in AMR, reinforcing the One Health approach.

### Conclusion and Future Perspectives

This study confirms poultry in both locations as potential reservoirs for resistant microbes. Some isolates harboured genes capable of producing ESBLs and Carbapenemases, threatening the efficacy of nearly all clinically used antimicrobials. High resistance to Ampicillin and Ciprofloxacin suggests an urgent need for intervention. The strong gene presence reflects antimicrobial pressure and underscores the animal-human health link. The findings support AMR stewardship and surveillance improvements and provide critical data on resistance gene trends, though they do not address microbial transmission routes to poultry. Broader geographic sampling would enhance generalizability, but this study serves as a valuable reference point.

### Recommendations

- Future research should assess gene transfer between strains and promote the best poultry farming practices.
- Strengthen coordinated AMR surveillance across human and veterinary sectors per WHO guidelines.
- Enforce policies regulating critical antimicrobial use in animals.
- Promote public awareness campaigns on responsible antibiotic use and biosecurity.

### Acknowledgment

Gratitude to Duke Kunshan University Global Health Research Centre, Staff at the Institute of Primate Research (Kenya), Jacob Ochieng, Christian and Victoria.

**Source of Funding.** Funded by Duke Kunshan University.

**Conflict of Interests.** No competing interests declared.

**Data availability.** Data available upon request from the corresponding author. Not publicly available due to ethical restrictions.

### Authors' contributions

Ms. Sophia Kuve led the conceptualisation, research, and manuscript drafting. Prof. Sajid Umar and Dr. Lucy Ochola reviewed and supported site-specific components. Prof. Wu and Prof. Kam reviewed the proposal and approved the final manuscript. All authors made substantial contributions to the development and publication of the manuscript.

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### Reference

1. Aljeldah MM. Antimicrobial resistance and its spread is a global threat. *Antibiotics*. 2022 Aug 9;11(8):1082. DOI: 10.3390/antibiotics11081082
2. Yuan H, Xu J, Wang Y, Shi L, Dong Y, Liu F, Long J, Duan G, Jin Y, Chen S, Zhu J. The longitudinal trend and influential factors exploring of global antimicrobial resistance in *Klebsiella pneumoniae*. *Science of The Total Environment*. 2024 Nov 10; 950:175357. DOI: 10.1016/j.scitotenv.2024.175357



3. Founou RC, Blocker AJ, Noubom M, Tsayem C, Choukem SP, Dongen MV, Founou LL. The COVID-19 pandemic: A threat to antimicrobial resistance containment. *Future Science OA*. 2021 Sep 1;7(8): FSO736. DOI: 10.2144/fsoa-2021-0012
4. Golli AL, Zlatan OM, Cara ML, Olteanu M. Pre- and post-COVID AMR patterns in ICU. *Pharmaceuticals*. 2024 Mar. DOI: 10.3390/ph17040407
5. Tompkins K, Juliano JJ, Van Duin D. Antimicrobial resistance in Enterobacterales and its contribution to sepsis in sub-Saharan Africa. *Frontiers in medicine*. 2021 Jan 26; 8:615649. DOI: 10.3389/fmed.2021.615649
6. Vidovic N, Vidovic S. Antimicrobial resistance and food animals: Influence of livestock environment on the emergence and dissemination of antimicrobial resistance. *Antibiotics*. 2020 Jan 31;9(2):52. DOI: 10.3390/antibiotics9020052
7. Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, Van Duijkeren E, Mateus A, Moreno MA, Pyörälä S, Ružauskas M, Sanders P. Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy*. 2017 Apr 1;72(4):957-68. DOI: 10.1093/jac/dkw481
8. Mancinelli CA, Mattioli S, Twining C, et al. Poultry products as n-3 PUFA sources. *Nutrients*. 2022 May. DOI: 10.1093/jac/dkw481
9. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MA. Antimicrobial resistance: a growing serious threat for global public health. In *Healthcare* 2023 Jul 5 (Vol. 11, No. 13, p. 1946). MDPI. DOI: 10.3390/healthcare11131946
10. Jamrozik E, Selgelid MJ. Drug-resistant infection: Causes, consequences, and responses. In *Ethics and drug resistance: Collective responsibility for global public health* 2020 Oct 27 (pp. 3-18). Cham: Springer International Publishing. DOI: 10.1007/978-3-030-27874-8
11. Reynolds D, Burnham JP, Guillaumet CV, McCabe M, Yuenger V, Betthausen K, Micek ST, Kollef MH. The threat of multidrug-resistant/extensively drug-resistant Gram-negative respiratory infections: another pandemic. *European Respiratory Review*. 2022 Oct 19;31(166). DOI: 10.1183/16000617.0068-2022
12. Elmonir W, Abd El-Aziz NK, Tartor YH, Moustafa SM, Abo Remela EM, Eissa R, Saad HA, Tawab AA. Emergence of colistin and carbapenem resistance in extended-spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae* isolated from chickens and humans in Egypt. *Biology*. 2021 Apr 26;10(5):373. <https://doi.org/10.3390/biology10050373>
13. Li B, Zhao Y, Liu C, Chen Z, Zhou D. Molecular pathogenesis of *Klebsiella pneumoniae*. *Future microbiology*. 2014 Sep 1;9(9):1071-81. DOI: 10.2217/fmb.14.48
14. Azabo, R., Mshana, S., Matee, M. and Kimera, S.I., 2022. Antimicrobial usage in cattle and poultry production in Dar es Salaam, Tanzania: pattern and quantity. *BMC Veterinary Research*, 18(1), p.7. DOI: 10.1186/s12917-021-03056-9
15. Cartoni Mancinelli A, Mattioli S, Twining C, Dal Bosco A, Donoghue AM, Arsi K, Angelucci E, Chiattelli D, Castellini C. Poultry meat and eggs as an alternative source of n-3 long-chain polyunsaturated fatty acids for human nutrition. *Nutrients*. 2022 May 8;14(9):1969. DOI: 10.3390/nu14091969
16. Wu H, Wang M, Liu Y, Wang X, Wang Y, Lu J, Xu H. Characterization of antimicrobial resistance in *Klebsiella* species isolated from chicken broilers. *International Journal of Food Microbiology*. 2016 Sep 2; 232:95-102. DOI: 10.1016/j.ijfoodmicro.2016.06.001
17. Bennani H, Mateus A, Mays N, Eastmure E, Stärk KD, Häsler B. Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. *Antibiotics*. 2020 Jan 28;9(2):49. DOI: 10.3390/antibiotics9020049
18. Anelia N, Suhartono S, Hayati Z. Abundance and phenotypic-genotypic analysis of antibiotic-resistant *Escherichia coli* isolated from wastewater of the Zainoel Abidin Hospital, Banda Aceh, Indonesia.



- Biodiversitas: Journal of Biological Diversity. 2023 May 1;24(5). DOI:10.13057/biodiv/d240502
19. Gaur P, Hada V, Rath RS, Mohanty A, Singh P, Rukadikar A. Interpretation of antimicrobial susceptibility testing using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints: analysis of agreement. *Cureus*. 2023 Mar 31;15(3). DOI: 10.7759/cureus.36977
20. Wayne P.A, Clinical and Laboratory Standards Institute. performance standards for antimicrobial susceptibility testing. inform suppl[Internet]. 2011;31(1):100-121. Available from: doi: 10.3389/fphar.2025.1529854
21. Ebrahim AE, Abd El-Aziz NK, Elariny EY, Shindia A, Osman A, Hozzein WN, Alkhalifah DH, El-Hossary D. Antibacterial activity of bioactive compounds extracted from red kidney bean (*Phaseolus vulgaris* L.) seeds against multidrug-resistant Enterobacterales. *Frontiers in Microbiology*. 2022 Nov 7; 13:1035586. doi: 10.3389/fmicb.2022.1035586
22. Ahmed Hasan S, Mohammed Bakr M. Bacteriological and molecular detection of *Klebsiella oxytoca* and its resistance to antibiotics among clinical specimens from Kirkuk, Iraq. *Archives of Razi Institute*. 2022 Oct 31;77(5):1521-5. DOI: 10.22092/ARI.2022.357753.2095
23. Ogutu JO, Zhang Q, Huang Y, Yan H, Su L, Gao B, Zhang W, Zhao J, Cai W, Li W, Zhao H. Development of a multiplex PCR system and its application in detection of blaSHV, blaTEM, blaCTX-M-1, blaCTX-M-9 and blaOXA-1 group genes in clinical *Klebsiella pneumoniae* and *Escherichia coli* strains. *The Journal of antibiotics*. 2015 Dec;68(12):725-33. DOI: 10.1038/ja.2015.68
24. Elmonir W, Abd El-Aziz NK, Tartor YH, Moustafa SM, Abo Remela EM, Eissa R, Saad HA, Tawab AA. Emergence of colistin and carbapenem resistance in extended-spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae* isolated from chickens and humans in Egypt. *Biology*. 2021 Apr 26;10(5):373. DOI: 10.3390/biology10050373
25. Huang X, Yu L, Chen X, Zhi C, Yao X, Liu Y, Wu S, Guo Z, Yi L, Zeng Z, Liu JH. High prevalence of colistin resistance and mcr-1 gene in *Escherichia coli* isolated from food animals in China. *Frontiers in Microbiology*. 2017Apr4;8:562. DOI: 10.3389/fmicb.2017.00562
26. Huang X, Yu L, Chen X, Zhi C, Yao X, Liu Y, Wu S, Guo Z, Yi L, Zeng Z, Liu JH. High prevalence of colistin resistance and mcr-1 gene in *Escherichia coli* isolated from food animals in China. *Frontiers in Microbiology*. 2017 Apr 4;8:562. doi: 10.3389/fmicb.2017.00562
27. Kuve SA, Nyerere AK, Mwaniki JN. Prevalence and risk factors for the transmission of *Helicobacter pylori*, *Escherichia coli* and *Klebsiella* species among patients presenting with gastritis in Nairobi, Kenya. *East African Medical Journal*. 2022 Mar 1;99(3). <https://www.ajol.info/index.php/eamj/article/view/226460>
28. Mohd Asri NA, Ahmad S, Mohamud R, Mohd Hanafi N, Mohd Zaidi NF, Irekeola AA, Shueb RH, Yee LC, Mohd Noor N, Mustafa FH, Yean CY. Global prevalence of nosocomial multidrug-resistant *Klebsiella pneumoniae*: a systematic review and meta-analysis. *Antibiotics*. 2021 Dec 8;10(12):1508. DOI: 10.3390/antibiotics10121508
29. Xu A, Ma J, Guo X, Wang L, Wu J, Zhang J, Bai Y, Xu J, Lu Z, Xu Z, Zhang X. Association of a province-wide intervention with salt intake and hypertension in Shandong Province, China, 2011-2016. *JAMA internal medicine*. 2020 Jun 1;180(6):877-86. DOI: 10.1001/jamainternmed.2020.0904
30. Wanjiku WC. Antimicrobial Susceptibility and Genetic Basis of Resistance of *Klebsiella* Spp Isolated from Diarrheic and Non-Diarrheic Patients at Health Facilities in Mukuru Informal Settlement, Nairobi, Kenya. <http://41.204.187.24/handle/123456789/5959>
31. Claudia JL. Assessment of antibiotic sensitivity patterns of *Salmonella* Typhimurium,



- Pseudomonas Aeruginosa*, *Klebsiella Pneumoniae* Isolates from Barn Swallow Droppings in Ishaka Town, Bushenyi District, Uganda. <https://ir.kiu.ac.ug/items/86846ce8-684a-44e9-bdb8-af5f612a0a9c>
32. Kuve SA, Ouko JO, Kiiru S, Waithiru D, Brian B, Oginga Z, Sang S, Maina J, Oduor N, Mwaniki JN. Epidemiological Analysis and Diversity of Extended-Spectrum  $\beta$ -Lactamases. *Acta Scientific MICROBIOLOGY* (ISSN: 2581-3226). 2023 Apr;6(4). DOI: 10.31080/ASMI.2023.06.1234
33. Nazir A, Zhao Y, Li M, Manzoor R, Tahir RA, Zhang X, Qing H, Tong Y. Structural genomics of repA, repB1-carrying IncFIB family pA1705-qnrS, P911021-tetA, and P1642-tetA, multidrug-resistant plasmids from *Klebsiella pneumoniae*. *Infection and Drug Resistance*. 2020 Jun 22;1889-903. DOI: 10.2147/IDR.S228704
34. Musila L, Kyany'a C, Maybank R, Stam J, Oundo V, Sang W. Detection of diverse carbapenem and multidrug resistance genes and high-risk strain types among carbapenem non-susceptible clinical isolates of target gram-negative bacteria in Kenya. *PLoS One*. 2021 Feb 22;16(2):e0246937. DOI: 10.1371/journal.pone.0246937
35. Yan C, Shi J, Cui P, Chen Y, Wang C, Wang Y, Miao J, Zhang Y, Kong H, Zeng X, Tian G. Characterization of emerging H3N3 avian influenza viruses in poultry in China. *Emerging Microbes & Infections*. 2025 Dec 31;14(1):2509748. DOI: 10.1080/22221751.2025.2509748
36. Mulchandani R, Wang Y, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. *PLOS Global Public Health*. 2023 Feb 1;3(2):e0001305. doi: 10.1371/journal.pgph.0003133
37. Wairimu CW, Odari EO, Makobe CK, Kariuki S. Antimicrobial Susceptibility and Genetic Basis of Resistance of *Klebsiella* spp Isolated from Diarrheic and Non-Diarrheic Children at Health Facilities in Mukuru Informal Settlement, Nairobi, Kenya. *Advances in Microbiology*. 2021 Oct 21;11(10):554-78. DOI: 10.4236/aim.2021.1110041
38. Moussa IM, Eljakee J, Beder M, Abdelaziz K, Mubarak AS, Dawoud TM, Hemeg HA, Alsubki RA, Kabli SA, Marouf S. Zoonotic risk and public health hazards of companion animals in the transmission of *Helicobacter* species. *Journal of King Saud University-Science*. 2021 Sep 1;33(6):101494. doi: 10.1186/s12917-025-04886-7
39. Munyua PM, Njenga MK, Osoro EM, Onyango CO, Bitek AO, Mwatondo A, Muturi MK, Musee N, Bigogo G, Otiang E, Ade F. Successes and challenges of the One Health approach in Kenya over the last decade. *BMC public health*. 2019 May 10;19(Suppl 3):465. DOI: 10.1186/s12889-019-6772-7
40. Shen C, Zhong LL, Yang Y, Doi Y, Paterson DL, Stoesser N, Ma F, El-Sayed MA, Feng S, Huang S, Li HY. Dynamics of mcr-1 prevalence and mcr-1-positive *Escherichia coli* after the cessation of colistin use as a feed additive for animals in China: a prospective cross-sectional and whole genome sequencing-based molecular epidemiological study. *The Lancet Microbe*. 2020 May 1;1(1):e34-43. DOI: 10.1016/S2666-5247(20)30005-7
41. Hawkey J, Wyres KL, Judd LM, Harshegyi T, Blakeway L, Wick RR, Jenney AW, Holt KE. ESBL plasmids in *Klebsiella pneumoniae*: diversity, transmission and contribution to infection burden in the hospital setting. *Genome medicine*. 2022 Aug 23;14(1):97. DOI: <https://doi.org/10.20546/ijcmas.2025.1408.023>
42. Waithiru D, Njeru JM, Maingi JM, Mulinge E, Ngugi B, Maina J, Kiiru J. Unravelling Antimicrobial Resistance Phenotypes and Carriage of Extended-Spectrum  $\beta$ -Lactamase Genes in *Escherichia coli* Isolated from Dairy Farms in Kiambu County, Kenya. *Advances in Microbiology*. 2022 May 9;12(5):295-315. DOI: 10.4236/aim.2022.125021
43. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of multidrug resistance. *Critical Care*. 2016 Jun 22;20(1):136. DOI: 10.1186/s13054-016-1320-7



44. Li Z, Xin L, Peng C, Liu C, Wang P, Yu L, Liu M, Wang F. Prevalence and antimicrobial susceptibility profiles of ESBL-producing *Klebsiella Pneumoniae* from broiler chicken farms in Shandong Province, China. *Poultry Science*. 2022 Sep 1;101(9):102002. DOI: 10.1016/j.psj.2022.102002
45. Khan MT, Formenti N, Tosi G, Guarneri F, Scali F, Saleemi MK, Monti E, Alborali GL. Prevalence of ESBL-Resistant Genes in Birds in Italy—A Comprehensive Review. *Animals*. 2025 May 29;15(11):1598. DOI:10.3390/ani15111598
46. Mukoko J, Wesangula E, Gitonga N, Kusu N, Odhiambo C, Tanui E, Azegele A, Ndanyi R, Joshi MP, Hafner T, Konduri N. Kenya's National Action Plan on antimicrobial resistance: measuring implementation progress. *Frontiers in Tropical Diseases*. 2025 Mar 20;6:1540713. doi.org/10.3389/fitd.2025.1540713