Research Article

# Phenotypic and Molecular Characteristics of Some *Enterobacteriaceae* Associated with Diarrheic Chickens: Insights from a Murine Infection Model

**Roua J. Mohammed[1*], Ikram A. Al Sammaraa[1], Mohammed S. Qasim[2], Haider Mohammed Ali Al-Rubaie[3]**

*[1]Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq; [2]Department of Zoonotic unite, College of Veterinary Medicine, University of Baghdad, Iraq; [3]Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq.*

**Abstract** | Diseases caused by members of the family *Enterobacteriaceae* represent a major threat to poultry health and productivity worldwide, leading to significant economic losses and posing potential zoonotic risks to humans through contaminated food products. This research attempt to establish the phenotypic and molecular characteristics of *Klebsiella pneumoniae, Escherichia coli*, and *Shigella* spp. isolated from diarrheic chickens in Baghdad, Iraq, and to evaluate their pathogenicity using a murine infection model. One hundred (100) fecal samples yielded a total of 67 *Enterobacteriaceae* isolates. *Klebsiella pneumoniae* was predominant, and *Shigella sonnei* was most virulent in the mouse infection model. Species identification by molecular means was confirmed by 16S rRNA sequencing with 99% homology for *K. pneumoniae* and *E. coli*, and 86% for *S. sonnei*. The results point towards the dominance of *K. pneumoniae* in poultry and the high pathogenic potential of *S. sonnei*. Experimental infection in mice established the infective doses as $3.0 \times 10^8$ CFU for *K. pneumoniae* and $6.0 \times 10^8$ CFU for both *E. coli* and *S. sonnei*. Clinical signs included depressed activity, reduced feed intake, and low mortality at these standard dosages, confirming their pathogenicity. Histopathology revealed a variably severe lesion between the bacterial species. *K. pneumoniae* caused moderate hepatic congestion, splenic hyperplasia, and villous intestinal thickening, with renal tissues mostly normal. *E. coli* caused focal hepatocellular necrosis, renal vascular congestion, and mild intestinal hyperplasia, but the spleen was spared. *S. sonnei* induced massive hepatic necrosis, renal tubular degeneration, splenic hemorrhage, and severe intestinal changes, confirming its higher virulence. The findings confirm the zoonotic potential of *Enterobacteriaceae* from Iraqi poultry. Using both phenotypic and molecular methods for identification ensures accurate detection of the pathogens. Overall, these findings support the claim that poultry can be a zoonotic reservoir of *Enterobacteriaceae* and place emphasis on sustained molecular surveillance in monitoring the emergence of pathogenic strains.

**Keywords** | *Enterobacteriaceae, Klebsiella pneumoniae, Escherichia coli, Shigella sonnei,* Poultry, Iraq

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***Correspondence** | Roua J. Mohammed, Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq; **Email:** ruaa.jassem1103a@covm.uobaghdad.edu.iq
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## INTRODUCTION

The *Enterobacteriaceae* family, constituted of Gram-negative, facultatively anaerobic bacilli, include members that are important pathogens for human and animal health including *Klebsiella pneumoniae, Escherichia coli, and Shigella* spp. These bacteria are increasingly responsible for diarrheal diseases in poultry, especially

hens, and have long been linked to gastrointestinal diseases (Alhassan and Abdul-Kareem, 2025; Karmoker *et al*., 2023; Yang *et al*., 2024). Surveillance and field surveys at present report enteritis prevalence of *Enterobacteriaceae* in poultry and chicken products: as, for example, several Egyptian studies have detected *E. coli* (including ESBL producers) to be present in 15–33% of chicken flocks or samples with frequent carriage of multidrug-resistance genes (Salem *et al*., 2023). Isolation rates of *Salmonella* and other *Enterobacteriaceae* from chicken meat, eggs and retail products (results are variable ranging from low single figures through to ~26% in some surveys of fresh chicken) reflect local contamination and transmission risks (Al-Esawi *et al*., 2024; Kaspersen *et al*., 2023). Regional meta-analyses and reviews indicate ESBL-producing *Enterobacteriaceae* are widespread in the food chain, with the pooled ESBL-producer prevalence estimates for poultry or poultry products between ~20% (pooled ESBL) and higher pooled prevalences of ESBL *E. coli* for some food categories (some meta-analyses report pooled estimates up to ~40% in chicken meat), with evidence of high heterogeneity but definite global presence (Rahaman *et al*., 2025).

Both phenotypic and molecular methods rely on correct identification and description of these agents. In terms of phenotype, lactose fermenters (like *E. coli* and *K. pneumoniae*) and non-fermenters (like *Shigella*) can be distinguished by differential growth on MacConkey agar, while VITEK® 2 systems permit the quick identification of the species and antibiotic susceptibility testing (Kahin *et al*., 2024; Kot and Witeska, 2024). Routine diagnostic testing is invaluable in guiding antimicrobial treatment and tracking resistance trends. They are deficient at times in distinguishing among closely related species or strains. On the other hand, phenotypic identification is complemented by taxonomic resolution provided by 16S rRNA gene sequencing, which permits both species-level identification and phylogenetic analysis (De Souza *et al*., 2025). Animal models, such as murine infection models, reveal the probable pathogenicity of avian-borne bacterial isolates. They allow controlled manipulation to investigate bacterial virulence, host immune mechanisms, and disease progression information critical to public health risk assessment and in the formulation of control strategies (Karmoker *et al*., 2023; Mohammed, 2025).

Significant research conducted in Iraq suggests that such thorough phenotypic and molecular characterizations of *Enterobacteriaceae* associated with poultry are feasible. As an illustration, consider a recent study conducted in the province of Thi-Qar that found that 29.5% of broiler intestinal prevalence rates of *K. pneumoniae* were hv-*K. pneumoniae* strains, of which 26.9% were identified by PCR using the 16–23S rDNA ITS region and VITEK® II. These strains were identified by their typical virulence genes, such as iucA, crmpA, rmpA2, iroB, and peg-344 (Musa, 2025; Zhou *et al*., 2024). Another study conducted in Najaf reported detection of various *Enterobacteriaceae* like *Proteus mirabilis*, *Enterobacter cloacae* complex, *Salmonella enterica* subsp. Diarizonae and *Enterobacter aerogenes*, from raw chicken meat sold in retail establishments (Al-Esawi *et al*., 2024). These findings show the extensive prevalence of potentially virulent and antimicrobial-resistant *Enterobacteriaceae* within the poultry value chain in Iraq, and highlight the efficiency and significance of phenotypic-molecular combination strategies for strict pathogen detection. The novelty of the present study lies in applying a murine model of infection to determine the histopathological and immunological impact of poultry-associated *Enterobacteriaceae* isolates (*Klebsiella pneumoniae, Escherichia coli,* and *Shigella* spp.). An application of this kind allows for controlled experimental investigation into bacterial virulence factors, immune response, and tissue modifications of three aspects still missing in avian-associated bacteria. The potential of the approach has been demonstrated by previous murine studies: *E. coli*-induced septicemia models revealed organ-specific immune dysregulation (Shao *et al*., 2023); *Klebsiella pneumoniae* respiratory and systemic infections and infection models revealed new virulence determinants (Iqbal *et al*., 2024; Wasbotten *et al*., 2022); and *Shigella sonnei* and *Shigella flexneri* murine intestinal infection models identified the immunopathological mechanisms of shigellosis, including mucosal inflammation, cytokine induction, and epithelial barrier dysfunction (Ridelfi *et al*., 2025; Sharma *et al*., 2016). Coupling these models with poultry derived isolates, this research bridges the gap between bacteriological description and pathophysiological evaluation, yielding new insights of veterinary and zoonotic relevance.

This research aims to establish the phenotypic and molecular characteristics of *Klebsiella pneumoniae*, *Escherichia coli*, and *Shigella* spp. recovered from diarrheic chickens in Baghdad, Iraq, and evaluate their pathogenicity through a murine infection model.

## MATERIALS AND METHODS

### Experimental study

The study was designed as an experimental laboratory study between October 2024 and February 2025 to detect and isolate the *Enterobacteriaceae* (*Klebsiella pneumoniae, Escherichia coli*, and *Shigella* spp.) of diarrheic chickens in Baghdad, Iraq. Phenotypic and molecular identification and murine infection model for determining pathogenicity were part of the study. All experiments were conducted in triplicate to produce robust and reproducible results. All experimental procedures were performed in triplicate to achieve result reproducibility and reliability.

### Isolation and identification of bacteria

Between October 2024 and February 2025, 100 samples of chicken excrement were collected from different locations throughout Baghdad. In less than two hours, the collected samples were then placed in ice and transported to the laboratory. In a sterile test tube, ten milliliters of normal saline were combined with one gram of each fecal sample, thoroughly mixed, and then allowed to sit for one minute. The MacConkey's agar was inoculated with 0.1 ml of the sample liquid. After allowing the plates to harden at room temperature, they were incubated for 24 to 48 hours at 37°C. Gram-staining was used to examine the questionable colonies under a microscope, followed by Vitek 2 Compact system identification and PCR.

## Identification of *Enterobacteriaceae* spp. by PCR

### DNA purification, concentration, and extraction

Fill a 2-milliliter tube with 1–2 milliliters of the cultivated cells. To compress the cells, spin them for one minute at 13,000 rpm. The gene-spin extracted DNA kit (Intron Biotechnology, cat. no. 17045). Electrophoresis was also utilized to assess the DNA fragments following extraction and to assess the results of the PCR encounter in the presence of reference DNA to ascertain the size of the PCR interaction on the Agarose gel. The concentration and purity of DNA are ascertained by measuring the absorbance of micro-volume samples using the Nano Drop spectrophotometer (Nabi/Korea). 1.80 to 2.00 is the optimal range for the 260/280 ratio, which is used to gauge purity.

### Primers

The primers were lyophilized and then redissolved in ultrapure ddH2O to produce an initial solution containing one hundred pmol/µl. A workable concentration of 10 pmol/µl might be prepared by maintaining this stock solution at -20°C. 90 µl of ddH2O and 10 µl of stock were used to create the working primer solution, which had a final volume of one hundred µl. The primers were supplied by IDT (also known as Integrated DNA Technologies, USA). The 16S rRNA primer sequences 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-TACGGTTACCTTGTTACGACTT-3' were used to establish the amplification size at 1250 bp (Srinivasan *et al*., 2015).

### Sequence alignment analysis and PCR amplification

Sequence alignment analysis and PCR amplification: iNtRON's Maxime PCR PreMix Kit, which includes dNTPs, PCR buffer, gel loading buffer, and Taq polymerase, was used to perform PCR amplification in a final volume of 25 µl. The reaction mixture consisted of 1 µl of each of the two primers at a concentration of 10 pmol, 1.5 µl of DNA template, and 16.5 µl of distilled water. The amplification process was carried out using a Mastercycler (Eppendorf, Germany). The PCR cycle began with five minutes of denaturation at 94°C, followed by 45 seconds of denaturation at 94°C, 45 seconds of annealing at 56°C, one minute of extension at 72°C, and a final seven-minute extension at 72°C. For one hour and thirty minutes, these PCR products were agarose gel electrophoresed (1.5% agarose at 5 volts/cm2) in 1x TBE buffer together with the forward and reverse primers of the 16S rRNA amplified products. The molecular size marker used was Bioneer's 100 bp DNA ladder (Korea), and an observation technique based on gel documentation was used. After being stained with Red Stain or ethidium bromide, the PCR products were exposed to UV light (302 nm), and 2% agarose gel electrophoresis was employed for analysis. Macrogen Korea was responsible for gene sequencing. The Basic Local Alignment Search Tool (BLAST), which is available from the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov and the BioEdit program, was used to do the homology search.

### Infectious dose

A pilot experiment was performed to standardize the infectious dose of *Klebsiella pneumoniae, Escherichia coli, and Shigella* spp. Bacterial suspensions were adjusted to three concentrations ($3.0 \times 10^8$, $6.0 \times 10^8$, and $9.0 \times 10^8$ CFU/ml) using the McFarland tube technique and confirmed by viable plate counts. For each bacterial species, 24 mice of both sexes were randomly divided into three groups of eight animals each. Group 1 inoculate $3.0 \times 10^8$ CFU orally by gastric gavage, Group 2 inoculate $6.0 \times 10^8$ CFU orally, and Group 3 inoculate $9.0 \times 10^8$ CFU orally. Following inoculation, all mice were observed daily for 14 consecutive days to record clinical signs, behavioral changes, and mortality. This pilot study was conducted to determine an appropriate infectious dose for subsequent experimental infection.

### Histopathological examination

After the mice were put to sleep, tissue samples were taken from the intestines, liver, kidneys, and spleen and washed with phosphate-buffered saline (PBS). For a whole day, the specimens were preserved in 10% formalin. Tissues were cleaned to get rid of extra fixative after fixation, then dehydrated in a succession of ethanol grades, clarified in xylene, and embedded in paraffin wax. After that, sections were produced and stained for histological investigation using hematoxylin and eosin (H and E), as previously explained by Bancroft and Gamble (2013).

### Statistical analysis

The findings obtained from the bacterial isolates were analyzed in terms of percentages in order to determine the

prevalence of every bacterial species. Microsoft Excel was used in performing the calculations and in verifying the validity of the results.

## RESULTS

### Isolation and identification

Out of 100 chicken fecal samples collected from different areas of Baghdad between October 2024 and February 2025, a total of 67 bacterial isolates were obtained. *Klebsiella* spp. was the most frequently isolated bacterium, accounting for 30%, followed by *Escherichia coli* (25%) and *Shigella* spp. (12%). These findings indicate that *Klebsiella pneumonia* and *E. coli* were the predominant enteric bacteria in the examined samples, while *Shigella* showed a lower occurrence. Overall, the isolation rate was 67%, whereas 33% of the samples showed no bacterial growth in the culture medium as shown in (Table 1).

**Table 1:** Number and percentage of bacterial isolates from chicken fecal samples (n=100).

| Bacterial isolates | No. of isolates | Percentage (%) |
|---|---|---|
| *Klebsiella* spp. | 30 | 30% |
| *Escherichia coli* | 25 | 25% |
| *Shigella* spp. | 12 | 12% |
| Total positive | 67 | 67% |

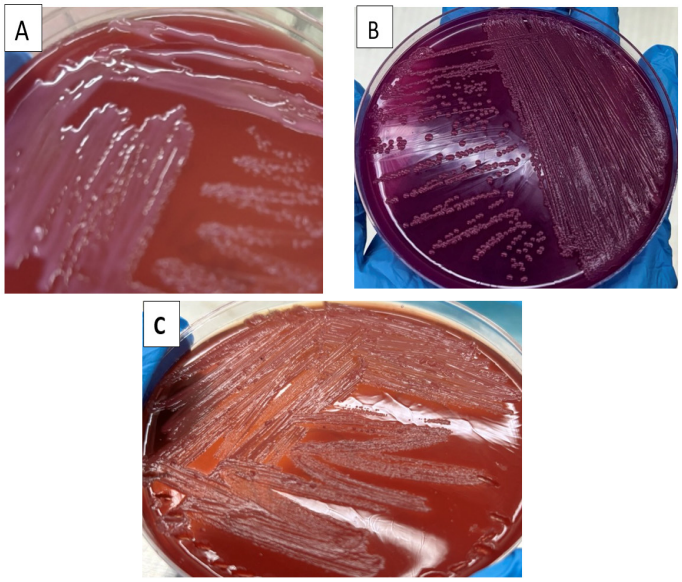


**Figure 1:** (A) *Klebsiella pneumoniae* (B) *Escherichia coli* (C) *Shigella* spp. grown on bacteriological media.

The isolates of *Klebsiella pneumoniae, Escherichia coli*, and *Shigella* spp. manifested as (mucoid pink, Dark pink and pale colonies no lactose ferment) respectively (Figure 1).

The identification and antimicrobial susceptibility testing of *Klebsiella pneumoniae, Escherichia coli*, and *Shigella* spp. were performed using the Vitek 2 Compact system, which showed a 91% probability of correct identification for *Klebsiella pneumoniae* and *Escherichia coli*, and a 94% probability for *Shigella* spp.

The DNA concentrations of *Klebsiella pneumoniae, Escherichia coli*, and *Shigella* spp. were measured at 27.4 ng/ml, 41.2 ng/ml, and 29.5 ng/ml, respectively, with purity ratios of 1.805, 1.960, and 1.850. One isolation from each species was analyzed for the presence of the 16S rRNA gene. The analysis showed successful amplification of a 1250 bp fragment on agarose gel, while the negative control exhibited no amplification (Figures 2 and 3).


**Figure 2:** PCR amplification showing a 1250 bp band. Electrophoresis was performed on 1.5% agarose gel at 5 V/cm$^2$ using 1× TBE buffer for 1.5 hours. Lane L: 100 bp DNA ladder; *K. pneumoniae*; S1 and S2: *E. coli*.


**Figure 3:** PCR amplification showing a 1250 bp band. Electrophoresis was performed on 1.5% agarose gel at 5 V/cm$^2$ using 1× TBE buffer for 1.5 hours. Lane L: 100 bp DNA ladder; S1: *Shigella* species.

The sequence was compared with the NCBI database (http://www.ncbi.nlm.nih.gov). The comparison revealed 99% similarity of the purified strains with the reference strain of *Klebsiella pneumoniae* with accession No. MT516162.1 in GenBank. Likewise, the identical percent of similarity (99%) was recorded with the reference strain of *Escherichia coli* with accession (No. MN208184.1) in GenBank, while revealed 86% similarity to *Shigella sonnei* strain in reference strain on GenBank (Accession No. KU359421.1) (Table 2).

**Table 2:** Sequence analysis for *Enterobacteriaceae.*

| No. | Type of substitution | Location | Nucleotide | Sequence ID with compare | Source | Identities |
|---|---|---|---|---|---|---|
| 1 | Transversion | 439 | G\T | MT516162.1 | *Klebsiella pneumoniae* strain 7609 16S ribosomal RNA gene, partial sequence | 99% |
| 2 | Gap | 27 | A\- | MN208184.1 | *Escherichia coli* strain 148-c pink 16S ribosomal RNA gene, partial sequence | 99% |
| | Gap | 53 | T\- | | | |
| | Gap | 63 | A\- | | | |
| 3 | Transition | 8 | A\G | KU359421.1 | *Shigella sonnei* strain DD5 16S 86% ribosomal RNA gene, partial sequence<br>Sequence ID: KU359421.1 | 86% |
| | Transversion | 24 | A\C | | | |
| | Transversion | 26 | C\G | | | |
| | Transition | 38 | A\G | | | |
| | Transition | 39 | G\A | | | |
| | Multiple | (39-732) | multiple | | | |

## Infectious dose

The inoculum size of $3.0 \times 10^8$ CFU caused mild symptoms such as reduced intake of feed and activity but no mortality in *K. pneumoniae*-inoculated mice, whereas larger inoculum sizes ($6.0 \times 10^8$ and $9.0 \times 10^8$ CFU) caused mortality in two mice in both groups. The appropriate infectious dose for *K. pneumoniae* was thus $3.0 \times 10^8$ CFU. For *Shigella sonnei*. and *E. coli,* $3.0 \times 10^8$ CFU dose did not have any evident clinical manifestations, while the highest dose of $9.0 \times 10^8$ CFU resulted in one animal death in each treatment group. The mice exhibited reduced feed intake and lowered activity but no evident considerable mortality at $6.0 \times 10^8$ CFU. $6.0 \times 10^8$ CFU was therefore regarded as the most suitable infectious dose for *Shigella* spp. and *E. coli*.

## Histopathological examination

Histopathological examination performed 14 days post-challenge showed different but graded lesions among mice infected with *K. pneumoniae, E. coli,* and *S. sonnei.* The severity of the tissue changes increased from *K. pneumoniae* to *E. coli* and was most marked in *S. sonnei* infected mice.

The liver of the mice challenged with *K. pneumoniae* showed dilation and congestion of venous vessels along with perivascular leukocytic aggregation and sinusoidal infiltrations while hepatocytes remained largely normal (Figure 4A). Renal sections showed normal glomeruli with intact tubular epithelium (Figure 4B). The spleen manifested active hyperemia with splenic cord hyperplasia (Figure 4C), while intestinal tissues showed moderate thickening of villi, leukocytic infiltration, and enterocyte hyperplasia (Figure 4D). The hepatic changes were more evident in mice infected with *E. coli*: focal hepatocellular necrosis, aggregation of mononuclear cells, and mild sinusoidal dilation (Figure 5A). The kidneys showed moderate interstitial vascular congestion without affecting the glomerular and tubular architecture (Figure 5B). In the spleen, the normal architecture was maintained, with intact lymphoid follicles and sinusoidal spaces (Figure 5C). Intestinal sections showed mild hyperplasia of the mucosal enterocytes and goblet cells without crypt damage (Figure 5D).

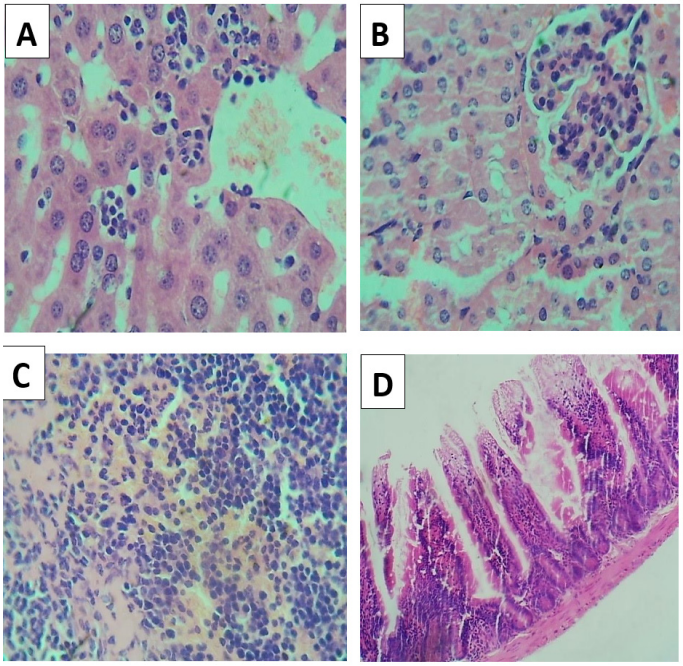


**Figure 4:** Histopathological changes in mice infected with *Klebsiella pneumoniae* (H and E stain, 400×): (A) liver, (B) kidney, (C) spleen, and (D) intestine. Section of liver (*K. pneumonia*) shows marked dilation with congestion of vein with marked peri vascular aggregation of leukocytes and sinusoidal infiltration of leukocytes & normal hepatocytes. H & E.400x

Tissues from *S. sonnei* infected mice demonstrated the most severe lesions. Liver sections demonstrated severe hepatitis with areas of necrosis and dense mononuclear infiltration extending through the sinusoids (Figure 6A). Renal tissues

demonstrated marked vacuolar degeneration of tubular epithelial cells (Figure 6B). The spleen demonstrated pronounced sinusoidal hemorrhage, congestion, and increased numbers of megakaryocytes along with splenic cord hyperplasia (Figure 6C). Intestinal tissues displayed moderate villous thickening, goblet cell hyperplasia, leukocyte infiltration, and intact crypts, indicating active but non-ulcerative inflammation (Figure 6D). Overall, these findings represent a clear gradient of pathogenicity, with *S. sonnei* inducing the most extensive tissue damage.

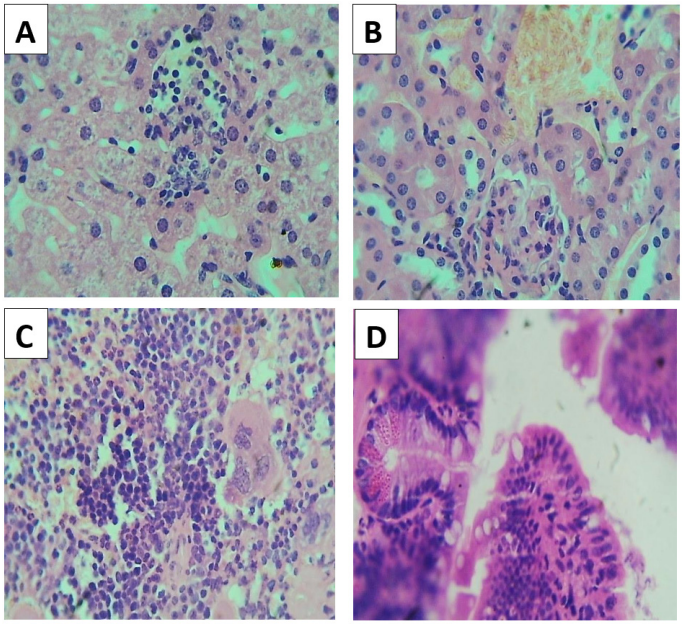


**Figure 5:** Histopathological changes in mice infected with *Escherichia coli* (H and E stain, 400×): (A) liver, (B) kidney, (C) spleen, and (D) intestine. Section of renal cortex (*K. pneumonia*) shows normal renal glomeruli and lining cells of renal tubules. H & E stain.400x.

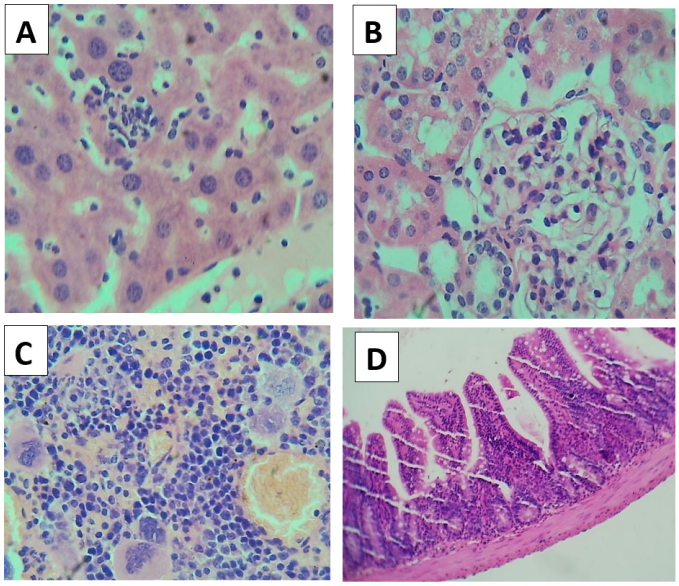


**Figure 6:** Histopathological changes in mice infected with *Shigella sonnei* (H and E stain, 400×): (A) liver, (B) kidney, (C) spleen, and (D) intestine.

## DISCUSSION

The present study demonstrates that *Klebsiella pneumoniae, Escherichia coli*, and *Shigella sonnei* isolated from diarrheic chickens in Baghdad exhibited heterogeneous pathogenic features upon testing with a murine infection model. The prevalence findings showed that *K. pneumoniae* (30%) and *E. coli* (25%) were the most predominant isolates, while *Shigella* spp. (12%) were less common. These findings are consistent with Iraqi studies, including Musa (2025), who reported hypervirulent *K. pneumoniae* strains with significant virulence gene profiles in Thi-Qar Province broiler chickens, and Al-Esawi *et al.* (2024); Li *et al.* (2024), who described *Enterobacteriaceae* from raw chicken meat collected from Iraqi markets with particular focus on the zoonotic potential of these bacteria. Also, (Alhassan and Abdul-Kareem, 2025; Cheng, 2024) demonstrated that poultry-derived *K. pneumoniae* and *E. coli* frequently multidrug resistance and virulence determinants, and Karmoker *et al.* (2023); Ramatla *et al.* (2025) reported the occurrence of multidrug-resistant *Shigella* in poultry, emphasizing their risk to food safety. Furthermore, the coupling of phenotypic identification and 16S rRNA gene sequencing in this study aligns with international recommendations (Yang *et al.*, 2024), offering diagnostic precision for the distinction of closely related *Enterobacteriaceae* species. In contrast, the phenotypic identification data obtained using the VITEK 2 Compact system were comparable with the molecular information obtained through 16S rRNA gene sequencing to ensure the accuracy of both tests. The only minor differences existed at the species level. *Klebsiella pneumoniae* and *Escherichia coli* were identified by the VITEK 2 system accurately using a high level of probability of 91%, which was similar to 99% similarity in their 16S rRNA gene sequence. On the other hand, *Shigella sonnei* exhibited 94% phenotypic identification probability but only 86% sequence homology with the GenBank reference strain. This may result from the intergeneric high genetic homology between *Shigella* and *E. coli*, which in most cases leads to the superimposition of biochemical profiles and makes phenotypic differentiation challenging. The molecular approach, particularly 16S rRNA sequencing, provided higher taxonomic resolution and unravelled the identification at the species level. These results indicate the complementarity of phenotypic and molecular methods: while VITEK 2 yields rapid, useful identification and antimicrobial profiling, 16S rRNA sequencing confirms genetic identification and resolves difficulties due to phenotypic similarity between members of the *Enterobacteriaceae* (Rudolph *et al.*, 2019; Chattaway *et al.*, 2017; Ragupathi *et al.*, 2017).

Experimental infection model confirmed dose-dependent pathogenicity. *E. coli* and *Shigella sonnei* at $6.0 \times 10^8$ CFU

caused depressed activity and reduced feed intake with little mortality, which shows that this dose is sufficient to establish infection with minimal lethality. This finding is like earlier experimental studies in which quantified infection doses were critical to measure virulence and host-pathogen interactions (Srinivasan *et al*., 2015). Histopathological lesions varied in severity between the three bacterial isolates. *Klebsiella pneumoniae* caused moderate hepatic congestion, perivascular leukocyte infiltration, and splenic hyperplasia, suggesting relatively moderate pathogenic effect. Renal tissues were largely normal, with intestinal sections showing villous thickening and enterocyte hyperplasia. *Escherichia coli* produced focal hepatocellular necrosis, sinusoidal dilation, and leukocyte infiltration. There was vascular congestion of the kidneys with no glomerular damage, and the spleen was unaffected. There was mild hyperplasia of intestinal villi, which indicated a more localized than systemic dissemination effect. Comparison with recent veterinary and molecular microbiology literature indicates that the prevalence patterns and disease outcomes observed here are generally as seen in other geographic locations, although also with regional variation in bacterial burden and virulence. Certain surveillance reports identified the common isolation of *Klebsiella pneumoniae* from poultry and retail meat with high genetic diversity and diverse virulence gene profiles, which are likely to be accountable for the dominance and the moderate pathogenicity of *K. pneumoniae* in our isolates (Mourão *et al*., 2024). Studies on chicken *Escherichia coli* in some countries also note high isolation rates and diverse clinical presentation ranging from focal enteritis to coli septicaemia supporting our observation of focal necrosis of the liver and limited renal involvement for the *E. coli* strains (Hasib *et al*., 2024).

Low- and middle-income setting reports also enhance frequent detection of multidrug-resistant *Enterobacteriaceae* along the poultry continuum, which may influence infection severity and results to experimental dose; this level of resistance and genomic heterogeneity could be contributing in part to inter-study variation in mortality and severity of lesions (Kahin *et al*., 2024). Finally, although *Shigella* spp. are less common in poultry than *E. coli* and *Klebsiella*, sporadic isolation of Shigella from poultry samples and retail meats is indicated by regional case reports and environmental sample surveys, which agrees with our finding of comparatively greater virulence for the isolate of *S. sonnei*; it appears that when present, *Shigella* has the ability to induce more tissue damage in experimental models (Hasib *et al*., 2024).

The most severe histopathological alterations were generated by *Shigella sonnei*, including severe hepatitis with necrosis, tubular degeneration in kidneys, splenic haemorrhage with hyperplasia, and villous thickening with goblet cell proliferation in intestines. These findings indicate vigorous pathogenicity and are consistent with the high virulence potential of *Shigella* spp. reported in literature (Karmoker *et al*., 2023). The graded nature of lesions most severe with *Shigella*, intermediate with *E. coli*, and least severe with *K. pneumoniae* illustrates the differential pathogenic strategies of these bacteria and supports previous data on their tissue tropism and mechanisms of virulence (Saidenberg *et al*., 2024; Bancroft and Gamble, 2013). These results are directly consistent with the goals set in the introduction of this study. The first objective to phenotypically and molecularly characterize *Klebsiella pneumoniae*, *Escherichia coli*, and *Shigella sonnei* isolated from diarrheic chickens was achieved by simultaneous VITEK 2 identification and 16S rRNA sequencing, which determined the genetic identity and cleared interspecies distinction. The second objective to investigate the pathogenicity of the isolates in a murine model of infection was achieved by determining the infective dose and correlating it with clinical manifestations and histopathological lesions. The documented gradient of pathogenicity, ranging from mild lesions in *K. pneumoniae* to widespread tissue damage in *S. sonnei,* meets the research goal of ascertaining relative virulence patterns among poultry-carried Enterobacteriaceae. Generally, the findings relate molecular identification with in-vivo pathogenic reactions, indicating that genetic variation among isolates is observed in the various histopathological effects, thus confirming the suggested integrative method set at the outset of the study. Similar integrative methods have been reported in veterinary and molecular microbiological research on the importance of combining phenotypic and genotypic data in explaining variation in virulence and zoonotic capability of *Enterobacteriaceae* from poultry (Ramatla *et al*., 2025; Musa, 2025; Mohammed *et al*., 2025; Yang *et al*., 2024; Saidenberg *et al*., 2024).

## CONCLUSION

The most common *Enterobacteriaceae* species isolated from diarrheic chickens in Baghdad were *Klebsiella pneumoniae*, followed by *Escherichia coli* and *Shigella sonnei.* The integration of phenotypic identification by the VITEK 2 system with confirmation at the molecular level by 16S rRNA sequencing ensured correct identification of the species and revealed interspecies variability in genetic similarity. Dose-dependent pathogenicity by experimental infection of a murine model was evident. *S.sonnei* showing utmost virulence and eliciting extensive hepatic, renal, splenic, and intestinal lesions whereas *K. pneumoniae* produced relatively less tissue alterations. The results emphasize differential pathogenic processes between poultry borne *Enterobacteriaceae* and their potential zoonotic impact. The study emphasizes the importance of

incorporating molecular diagnostics into in-vivo models to enhance our comprehension regarding the virulence of bacteria as well as guide public health policy, biosecurity strategies, and antimicrobial stewardship of the poultry industry in Iraq.

## ACKNOWLEDGEMENT

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## NOVELTY STATEMENT

This study is the first to be carried out in Baghdad, Iraq, to simultaneously isolate and molecularly characterize *Klebsiella pneumoniae*, *Escherichia coli*, and *Shigella sonnei* from chickens suffering from diarrhea and evaluates their pathogenic potential using a murine infection model. Unlike all the earlier studies, this study performs both microbial pathogenicity analysis and natural anti-inflammatory and antioxidant treatment, and hence it provides new leads to alternative treatments against *Enterobacteriaceae* infections in poultry and in experimental models.

## AUTHOR CONTRIBUTION

RJM conceptualized the study, led the experiments, and drafted the manuscript. IAA-S helped with conceptualizing and critically reviewed the manuscript. MSQ did experiments, data processing, and assisted in writing the results. HMAA-R participated in the histopathological analysis, interpretation of tissue findings, and critical review of final version of the manuscript. All authors have seen and approved the manuscript.

### Ethical approval

Ethical approval was acquired from the local animal care and use committee of the College of Veterinary Medicine, University of Baghdad (Approval Number P-G\436,19\2\2025).

### Generative AI and AI-assisted technology statement

Artificial intelligence tools were used only for grammar correction and sentnece editing. The authors affirm that all scientific content, data analysis, interpretations, and conclusions are solely their own.

### Conflict of interest

The authors have declared no conflict of interest.

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