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Isolation, identification and antibiotic sensitivity profiling of *Escherichia coli* and *Salmonella spp*. from duck: An issue of public health

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ABSTRACT: *Escherichia coli* and *Salmonella spp.* are involved in a variety of pathogenic processes in domestic animals and humans. The study aimed to isolate and identify *Escherichia coli (E. coli)* and *Salmonella spp*. bacteria, and ascertain the specific antibiotic resistance percentages. A total of 40 samples of duck liver and intestinal contents were collected and selected from different villages in Savar Upazila and tested for *E. coli* and *Salmonella spp*., with the isolates subjected to antibiotic sensitivity tests. Cultural characteristics, biochemical testing, and Gram's staining were used to isolate and identify bacterial genera/species. Each bacterium has a different prevalence rate. The highest prevalence rate was found in *E. coli* (52.5%), followed by *Salmonella spp.* (37.5%). Antibiotic sensitivity test by disc diffusion method or Kirby-Bauer test was performed against the five most commonly used antibiotics. Ciprofloxacin was the most sensitive to *E. coli* isolates (95.2 %), followed by gentamycin (80.9%), while amoxicillin (90.5%), followed by tetracycline and ampicillin (80.95%), were the most resistant. *Salmonella spp.* isolates were found to be the most susceptible to Ciprofloxacin (100%), followed by Gentamycin (86.67%). The highest resistant pattern of *Salmonella spp.* was shown against amoxicillin (100%), followed by ampicillin (80%) and tetracycline (60%), with an intermediate resistance 40%. According to the findings of this study, duck contains a resistance profile to *E. coli* and *Salmonella spp*. pathogens on both the duck liver and intestinal contents. *Salmonella spp* and the resistance profile of *E. coli* are dangerous bacteria that can spread to people by contact with them or through the food chain, raising major public health problems.

Keywords: Antibiotic sensitivity, *E. coli,* isolation, *Salmonella spp.*

INTRODUCTION

Duck is a large-billed, short-necked and relatively small waterfowl species that belongs to the family Anatidae. It is a species of poultry that provides the second-most poultry meat and eggs, just after chicken. The population of it has been estimated at 61.746 million, occupying the second position in poultry in Bangladesh according to the Department of Livestock Services (DLS, 2021). However, it can also be a potential source of pathogen transmission to other domestic fowls (Majumder *et al*., 2017).

Escherichia coli is characterised as a gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae (Tenaillon *et al.,* 2010). Some *E. coli* bacterium strains (such as the O157:H7 strain) can cause severe anaemia or renal failure, which can lead to death (Lowenfels A 2013). *E. coli* serogroups harbored different virulent factors responsible for diarrhea and hemorrhage

(Darwish, W. S., 2015). Enteropathogenic *E. coli* (EPEC) is a common cause of diarrhoea in people. EPEC infection of cultured intestinal epithelial cells induces attaching and effacing (A/E) lesions, alters intestinal ion transport, increases paracellular permeability, and stimulates inflammation (Savkovic *et al.,* 2005). Shiga toxin-producing *E. coli* (STEC) causes the hemolytic uraemic syndrome, which is produced by the release of Shiga toxins (Stxs) during intestinal infection and subsequent absorption into the circulation (Tran *et al.,* 2014). *E. coli* can get into the meat during processing, the possible contamination routes such as handling, slaughtering, and cross-contamination from equipment or surfaces (Yang *et al.,* 2017). If the infected meat is not cooked to 160°F (71°C), the bacteria can survive and infect when eating the meat (James *et al*., 2021). The use of a wider range of *E. coli* antisera in the laboratory is necessary to identify new pathogenic strains (Khoo *et al.,* 2010). This is the most common way people become infected with *E. coli*. Food that has raw meat can become infected as well.

Salmonella spp. are motile (exclude *S. pullorum* and *S. gallinarum),* gram-negative, rod-shaped, non-spore-forming, non-capsulated, facultative anaerobic bacteria that belong to the family Enterobacteriaceae (Agbaje *et al*., 2011). *Salmonella* is an important cause of foodborne diseases (Chen *et al.,* 2020). Food borne diseases continue to be a common and serious threat to public health all over the world (Bell and Kyriakides, 2002). *Salmonella* is primarily found in the intestines of humans and animals. Contaminated meats, primarily from avian and cattle sources, are the most likely source of human salmonellosis and thus the most serious meat-borne public health threat (Buncic *et al*. 2014; Lutful Kabir, 2010). *Salmonella* serotypes in ducks also indicate that ducks should be considered an important source of foodborne pathogens (Cha *et al*., 2013).

As a result, studying the susceptibility and resistance patterns of isolated organisms to various antibiotics is critical for the better treatment of animal diseases. In Bangladesh, duck contributes to a major part of its economy as where livestock contributes about 1.98% in GDP at constant price (DLS, 2021). The economic aspect of duck disease, as well as their mortality and morbidity due to bacterial infection, is of great concern to livestock owners and the government, but due to a lack of awareness and research, the presence of bacteria in ducks and the consequences of this on human health remain unknown. The aim of this study was achieved with the findings.

MATERIALS AND METHODS

Experimental design

There were two main steps to the entire study: Source selection, sample collection, isolation, identification, and characterisation of microorganisms based on colony shape, staining qualities, motility and biochemical parameters, and identification were all part of the first phase. The second step involved determining the current drug sensitivity and resistance pattern of 40 different microorganism isolates that had been taken from ducks.

Study duration

This study was conducted at the laboratory of the Department of Medicine and Public Health, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka 1207, during the period of January to June 2022.

Study area

Samples were collected from the selected duck farm at Savar Upazilla (Sub-district), Dhaka. Collected samples were then transported carefully with an ice box and examined in the Department of Medicine & Public Health laboratory, Sher-e-Bangla Agricultural University.

Sample size

There were a total of 40 duck samples, comprising intestinal contents (n = 20) and liver (n = 20) from 20 ducks (10 sick and 10 dead). The samples were collected from selected farms in Savar Upazilla. Selected whole bird samples were transported in an ice box to the Department of Medicine and Public Health Laboratory, Dhaka. Postmortem procedure was performed to collect the intestine and liver. The samples were selected randomly among the affected ducks.

Isolation and Identification of collected samples

The procedure outlined by Collins and Lynne (1976) was used to isolate and identify bacteria. *E. Coli* and *Salmonella spp.* organisms were isolated and identified by culturing them in different selective media and analysing the traits of the colonies.

The entire experimental design is carried out in two stages. On the first step, bacteria were isolated from duck liver and intestinal content, and then *E. coli* and *Salmonella spp.* were identified based on cultural and morphological characteristics. Motility test with hanging drop preparation and carbohydrate fermentation tests were also done to confirm the isolated organism as *E. coli.* and *Salmonella spp*. The second step included the study of the response of the isolated bacteria were tested against commercially available antibiotic discs.

Table 1. Morphological and cultural properties of *E. coli* isolated from duck.

Feature	Appearance
Nutrient agar	Smooth, circular, white to greyish white colonies were found.
Eosin Methylene Blue agar	Smooth, circular, black colour colonies with a metallic sheen were produced.
Mac-Conkey agar	Rose pink lactose-fermented colonies were formed.
Staining property	Gram-negative, pink coloured, small rod-shaped organisms arranged in single, pairs or short chains were observed.
Motility	The organisms were motile.

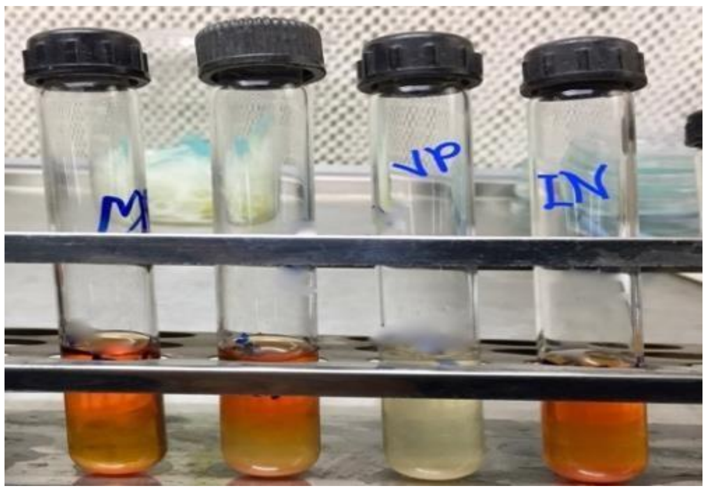

Figure 1. Indole positive, VP negative, and MR positive of *E. coli*.

Staining properties

Morphological identification of bacteria using Gram's staining methods. Merchant and Packer (1976) recommended using Gram's staining to identify the size, shape, and arrangement of bacteria. The motility test was carried out according to Cowan (1985) method to distinguish motile bacteria from non-motile bacteria.

Antimicrobial susceptibility test

The test to ascertain the drug sensitivity and resistance pattern, as well as to evaluate their potential for causing disease, was conducted using commercially available antimicrobial discs (OXOID Limited, Canada), as like *E. coli* isolated from ducks in Penang, Malaysia were examined for their susceptibility to eleven different antibiotics (Adzitey *et al*., 2013). The discs with their concentrations were Gentamycin (GM), Amoxicillin (AMX), Ampicillin (AMP) -10 (µg /disc), Tetracycline (TET) - 30 (µg /disc) and Ciprofloxacin (CIP) - 5 (µg /disc). In order to evaluate the sensitivity and resistance pattern of the chosen *E. Coli* and *Salmonella spp.* isolates from the intestinal contents and liver of the duck, the following antimicrobial drugs were utilised, along with their disc concentration. Through CLSI (The Clinical and Laboratory Standards Institute), 2020, the number of drugs for the Enterobacteriaceae family and their Zone Diameter Interpretive Standard (mm) were tallied.

RESULTS

The findings show how bacteria were isolated and identified from the liver and intestinal contents of ducks from various farms and homes in the Dhaka region. The study's results also display the isolates' sensitivity and resistance pattern to various medications, with the use of tables and graphs, and potential interpretations are provided under the following headings.

Identification of *E. coli* by biochemical test

Morphological and cultural properties of *E. coli* isolated from the duck are shown in Table 1. All the isolates of *E. coli* showed a positive reaction on Methyl red (MR) and Indole test by indicating red colour development after adding the respective reagents. On the other side, *E. coli*

Table 2. Biochemical reaction patterns of *E. coli.*

Fermentation properties with five basic sugars					Indole test	VP Test	MR Test	Citrate test
DX	ML	L	S	MN				
AG	AG	AG	A↓G↓	A G	+	−	+	−

Legends: DX = Dextrose; ML = Maltose; L =Lactose; S = Sucrose; MN = Mannitol; A = Acid production; G = Gas production; A↓ = Less acid production; G↓ = Less gas production; NF =No Fermentation; + = Positive reaction; - = Negative reaction.

Table 3. Morphological and cultural properties of *Salmonella spp.* isolated from the duck.

Feature	Appearance
SS agar	Opaque, translucent, colourless, smooth, round colonies were found
BG agar	Red-colored, pinkish-white colonies were shown
Staining property	Gram-negative, pink colour, small rod-shaped organisms arranged in single or paired was observed

Table 4. Biochemical reaction patterns of *Salmonella spp*.

Fermentation properties with five basic sugars					Indole Test	MR Test	VP Test	Citrate Test
DX	ML	L	S	MN				
AG	AG	NF	NF	A↓G	−	+	−	+

Legends: DX = Dextrose; ML = Maltose; L =Lactose; S = Sucrose; MN = Mannitol; A = Acid production; G = Gas production; A↓ = Less acid production; NF = No Fermentation; + = Positive reaction; - = Negative reaction.

showed no colour change on Voges-Proskauer and Citrate reduction test (Figure 1). And all the isolates of *E. coli* fermented the five basic sugars (dextrose, maltose, lactose, sucrose, and mannitol) and produced both acid and gas. The colour change of the sugar media from reddish to yellow showed acid production, while the accumulation of gas bubbles in the inverted Durham tube indicated gas production (Table 2)

Identification of *Salmonella spp.* by biochemical test

Morphological and cultural properties of *Salmonella spp.* isolated from the duck are shown in Table 3. *Salmonella spp.* fermented all the sugars and produces both acid and gas, except sucrose and lactose, as shown in Table 4. *Salmonella spp.* shows a positive reaction in the MR and Citrate reduction test; otherwise, it produces a negative reaction on the VP and Indole test (Figure 2).

Prevalence of *E. coli* bacteria on the duck

The prevalence of *E. coli* in duck intestinal content and liver is given in Table 5. Those bacteria are confirmed on different cultural and biochemical tests. Among the 40 samples, 21 were found to be positive for *E. coli*. The overall prevalence of *E. coli* on duck samples is 52.5%. The prevalence formula is calculated in percentage as follows:

$$\text{Prevalence} = \frac{\text{Number of existing cases of a disease or condition}}{\text{Total population at risk}} x100$$

Prevalence of *Salmonella spp.* bacteria on the Duck

Based on different cultural, biochemical, and staining procedures, *Salmonella spp.* are identified, and the prevalence rate is given in Table 6. Among the 40 samples, 15 samples were found to be positive for *Salmonella spp*. The overall prevalence of *Salmonella spp.* on duck samples is 37.5%.

Antibiotic sensitivity profiling of *E. coli*

A total of 21 *E. coli* isolates that were separated from duck samples were utilised in order to ascertain the pattern of antibiotic susceptibility. 19 of the 21 *E. coli* positive isolates have amoxicillin resistance, and 17 have ampicillin and tetracycline resistance. However, 20 samples are ciprofloxacin-sensitive and 14 are gentamycin-sensitive. Significant resistance to amoxicillin was observed in all isolated bacteria (90.47%), followed by tetracycline and amoxicillin together (80.95%). This analysis indicated that 66% of the samples were responsive to gentamycin and 95.24% to ciprofloxacin (Figure 3). This figure represents that 95.2% isolates were sensitive to ciprofloxacin and 80.9% sensitive to gentamycin, with 19.1% intermediate resistant. Amoxicillin was shown to be 90.5% resistant,

Figure 2. Indole negative, VP negative, and MR positive of *Salmonella spp.*

Table 5. Prevalence of *E. coli* bacteria on ducks.

Bird type	Sample type	Total sample	*E. coli*	Prevalence of *E. coli*
Sick	Liver	10	2	20%
Sick	Intestinal content	10	7	70%
Dead	Liver	10	3	30%
Dead	Intestinal content	10	9	90%

Table 6. Prevalence of *Salmonella spp.* bacteria on ducks.

Bird type	Sample type	Total sample	*Salmonella spp.*	Prevalence of *Salmonella spp.*
Sick	Liver	10	1	10%
Sick	Intestinal content	10	4	40%
Dead	Liver	10	3	30%
Dead	Intestinal content	10	7	70%

followed by both ampicillin and tetracycline (80.9%), with 19.1% intermediate resistant.

Antibiotic sensitivity profiling of *Salmonella spp.*

A total of 15 isolates of *Salmonella spp.* isolated from duck samples were further used to determine the antibiotic sensitivity pattern. Out of 15 *Salmonella spp.* positive isolates of all the 15 samples were resistant to amoxicillin, 12 to ampicillin, 9 to tetracycline, with 6 intermediate resistant. On the other hand, 15 samples were sensitive to ciprofloxacin and 13 were gentamycin. All isolated bacteria showed significant resistance to amoxicillin (100%), followed by ampicillin (80%), and tetracycline (60%). 100% sensitive to ciprofloxacin and 86.67% to gentamycin are found in this study (Figure 4). This figure represents that 100% isolates were sensitive to ciprofloxacin and 86.67% sensitive to gentamycin, with 13.33% intermediate resistant. Amoxicillin was shown to be 100% resistant, followed by ampicillin (80%) and tetracycline (60%), with 40% intermediate resistance.

DISCUSSION

The study was conducted to determine the bacteriological state of sick and dead ducks from different villages of

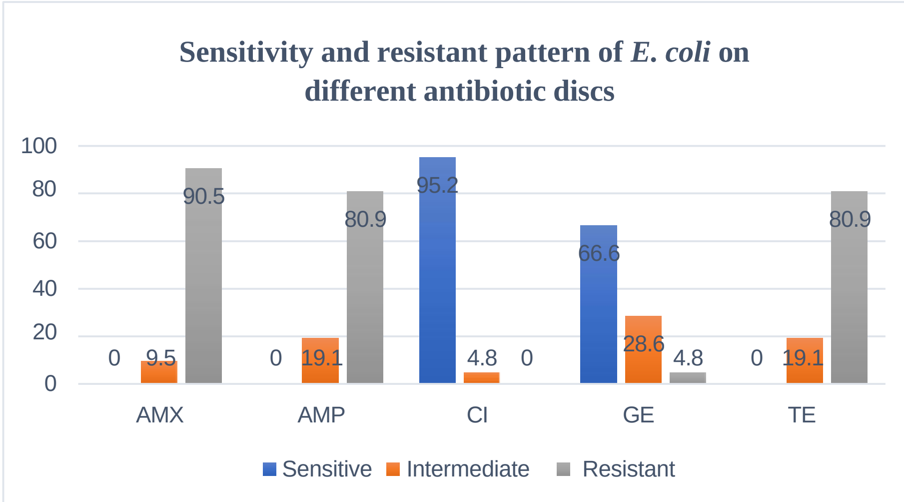

Figure 3. Antibiotic sensitivity pattern of *E. coli* on different antibiotic discs. **Legend:** AMX = Amoxicillin, AMP = Ampicillin, CI = Ciprofloxacin, GE = Gentamycin, TE = Tetracycline, X-axis = antibiotics, Y-axis = percentage of sensitivity.

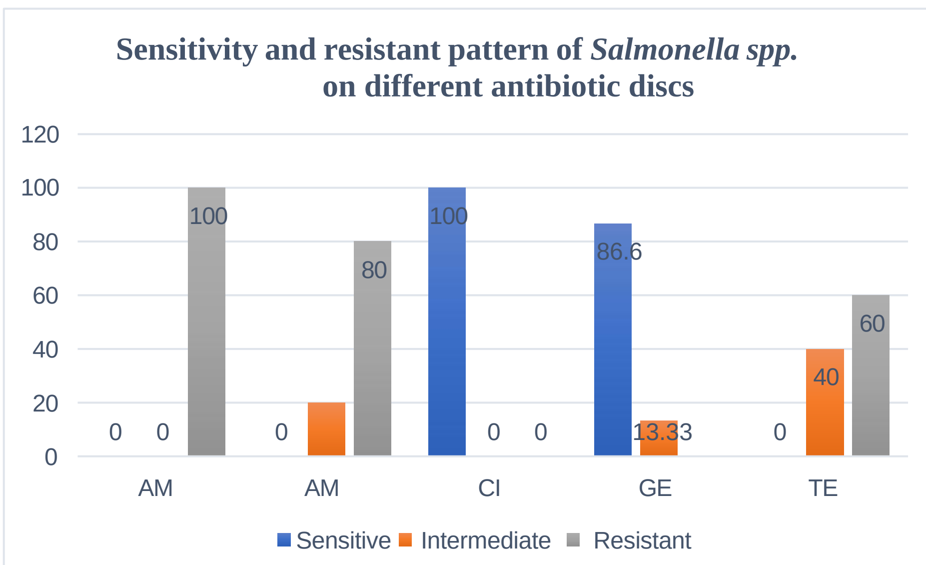

Figure 4. Antibiotic sensitivity pattern of *Salmonella spp.* **Legend:** AM = Amoxicillin, AM = Ampicillin, CI = Ciprofloxacin, GE = Gentamycin, TE = Tetracycline, X- axis = antibiotics, Y-axis = percentage of sensitivity.

Savar Upazila, as well as the isolation, identification, and characterisation of the bacterial flora present in duck liver and intestine, and the public health significance. Cultural examination, morphological studies, staining properties, and biochemical analyses were carried out in the laboratory to characterise the isolated bacteria. The antibiogram study was carried out on bacterial isolates to investigate their sensitivity and resistance profile against the most commonly used antibiotics on the market.

E. coli and *Salmonella spp*. were detected in duck liver and intestinal content in the current investigation, which was supported by Dey *et al.* (2016), Rahman *et al.* (2017), Eid *et al.* (2019) and Kim *et al*. (2016). In this work, the organism was cultured using several selective and enriched culture media simultaneously. The media utilised in this study were selected considering the experience of the past researcher who worked in various fields relevant to the present study by Eid *et al.* (2019), Kim *et al*. (2016)

and Dey *et al.* (2016).

In this study, colony characteristics of *E. coli* observed in EMB, MC, and NA were like the findings of Cheesbrough (2006), Burrows and Freeman (1985) and Buxton and Fraser (1977). The morphology of the isolated bacteria was Gram-negative short rods, grouped in single or paired and motile, as supported by various writers, including Britannica (2015), Cowan (1985) and Merchant and Packer (1977).

The *E. coli* isolates showed a complete fermentation of 5 basic sugars by producing both acid and gas, which were supported by Merchant and Packer (1977), Burrows and Freeman (1985) and Cheesbrough (2006). The isolates also revealed positive reaction in the MR test and Indole test, but negative reaction in the VP test (Cheesbrough, 2006; Buxton and Fraser, 1977).

Salmonella spp. colony features obtained in NA, SS and BG agar were comparable to those observed by Cheesbrough (2006), Burrows and Freeman (1985) and Buxton and Fraser (1977). In Gram's staining, the morphology of the isolated bacteria exhibited Gram-negative small rods arranged in single or paired and motile, which was supported by several authors (Cheesbrough, 2006; Burrows and Freeman, 1985); Merchant and Packer, 1977; Cowan, 1985).

Isolates of *Salmonella spp*. showed a complete fermentation of 5 basic sugars and production of both acid and gas, except sucrose and lactose, which were supported by OIE Manual (2000), Cheesbrough (2006) and Burrows and Freeman (1985). *Salmonella spp.* shows a positive reaction in the MR and Citrate reduction test; otherwise, it produces a negative reaction on the VP and Indole test. This was supported by Buxton and Fraser (1977) and Merchant and Packer (1967).

The total overall prevalence of *E. coli* in ducks is 52.5%. Dead duck intestinal content had the highest prevalence (90%), and sick duck liver had the lowest (20%) of *E. coli* prevalence. The findings were like those of Kim *et al.* (2016), who reported that the prevalence of *E. coli* was 91% in ducks. Another study of Eid *et al.* (2019) in Egypt found a 22.8% prevalence rate in the duck sample. This discrepancy could be attributed to differences in season or environmental variation in different study areas.

The overall prevalence of *Salmonella* in ducks is 37.5%, dead bird intestinal content having the greatest prevalence (70%) and sick bird liver having the lowest (10%). In another study, Rahman *et al.* (2017) found a slightly higher prevalence rate (39.1%) of *Salmonella spp.* in Dinajpur district. In a previous study, Kim *et al.* (2016) showed that the prevalence of *Salmonella spp.* was 20.75% in Korea, which was lower compared to Savar. This slight difference might be due to variation of season or environmental variation in different study areas, or sample handling and processing techniques.

In the present study, it was found that the *E. coli* isolated from the duck sample were sensitive to ciprofloxacin (95.2%), followed by gentamycin (66.6%). The results strengthen the earlier observations of Eid *et al.* (2019), who found them to be sensitive to ciprofloxacin and Gentamycin. Resistance of *E. coli* was observed against amoxicillin (90.5%), ampicillin (80.9%) and tetracycline (80.9%). The result was supported by Eid *et al.* (2019), who found resistance to ampicillin and amoxicillin.

In the current investigation, it was found that the *Salmonella spp.* isolated from the duck sample are sensitive to ciprofloxacin (100%), followed by gentamycin (86.67%). The results strengthen the earlier observations of Rahman *et al.* (2017) and Eid *et al.* (2019), who both found that ciprofloxacin and gentamycin are sensitive to *Salmonella spp*. Resistance of *Salmonella spp.* was observed against amoxicillin (100%), ampicillin (80%) and tetracycline (60%), with 40% intermediate resistance. The results strengthen the earlier observation of Eid *et al.* (2019), who found 71.5% ampicillin and amoxicillin resistance in the duck sample.

The presence of such characteristics in *E. coli* and *Salmonella spp.* isolates indicate that the organisms may have acquired resistance due to the indiscriminate use of antibiotics. The presence of isolates should be considered detrimental to health, and risk factors should be avoided. However, in the current investigation, ciprofloxacin and gentamycin were found to be the most efficient antibiotics for treating *E. coli* and *Salmonella spp.* infection. Still, ciprofloxacin and gentamycin are best for the treatment of both bacterial infections, but they may become resistant in the near future.

Conclusion

The study revealed that 21 out of 40 samples tested positive for *Salmonella* spp., and 15 samples tested positive for *Escherichia coli* (*E. coli*), corresponding to prevalence rates of 37.5% and 52.5%, respectively. The lowest prevalence of *Salmonella* spp. (10%) and *E. coli* (20%) was observed in liver samples from sick ducks, while the highest prevalence rates were found in the intestinal contents of dead ducks.

The use of antibiotics in livestock is often described as a significant contributor to the clinical problem of resistant diseases in human medicine. Five commonly used classes of antibiotics, readily available for field use, were evaluated for their effectiveness (antibiograms) against *Salmonella* spp. and *Escherichia coli* (*E. coli*) isolates. Ciprofloxacin showed high sensitivity against both *Salmonella* spp. (95.2%) and *E. coli* (100%), followed by gentamicin, which was effective against 66.6% of *Salmonella* spp. and 86.67% of *E. coli* isolates. In contrast, tetracycline, amoxicillin, and ampicillin were ineffective against all tested isolates. Based on the findings, *E. coli* and *Salmonella* spp. were more prevalent in the intestinal contents of dead birds compared to other sample types.

While most isolates exhibited varying resistance profiles, they were generally sensitive to ciprofloxacin and gentamicin, and resistant to amoxicillin and ampicillin.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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