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Isolation and identification of multidrug resistance bacterial agents implicated in duck enteritis with first record of *Salmonella enterica* subspecies arizonae in Egypt

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Abstract

Background: Bacterial infections causing digestive problems are among the most serious threats to Egypt's duck industry, owing to their effects on feed utilization and body weight gain.

Aim: As a result, the goal of this study was to identify bacterial pathogens causing enteritis in ducks as well as testing their antimicrobials resistance capabilities.

Methods: Forty-two duck flocks from different localities at four Egyptian Governorates (El-Sharkia, El-Gharbia, El-Dakahlia, and El-Qaliobia) have been subjected to clinical and postmortem examination as well as bacterial isolation and identification. The liver samples have been collected aseptically from freshly euthanized ducks for bacterial isolation followed by identification using conventional biochemical tests, VITEK 2 system, and confirmatory polymerase chain reaction (PCR) for detection of the uid A gene (beta-glucuronidase enzyme) of *Escherichia coli.* In addition, antimicrobial sensitivity testing for the isolates against different antimicrobials by the VITEK 2 system was used.

Results: Forty-six positive bacterial isolates were identified using conventional methods and the VITEK 2 system including *Staphylococcus* spp. (52.17%), *E. coli* (41.30%), and 2.17% for each of *Enterococcus casseli lavus,*, *Salmonella enterica subspecies arizonae,* and *Enterobacter cloacae*. PCR was positive for *E. coli* uid A gene at 556 bp. The antibiogram patterns of isolated pathogens from naturally infected ducks in our work demonstrated 87% multidrug resistance with varying results against different antimicrobial drugs tested. Such findings supported the fact of the upgrading multidrug resistance of Staphylococci and Enterobacteriacae.

Conclusion: The most prevalent bacterial pathogens associated with duck enteritis were Staphylococcus spp. and *E. coli* with the first report of *S. enterica subspecies arizonae* causing duck enteritis in Egypt.

Keywords: Duck enteritis, *Staphylococci*, *Salmonella*, *E. coli*, Antimicrobials.

Introduction

Bacterial infections in ducks have higher incidence rates compared with viral diseases. Mortality rates of bacterial infections have increased globally (Enany *et al.*, 2018). The studies have concentrated on determining the intestinal load of pathogenic bacteria such as *Staphylococci* spp., *Escherichia coli*, and *Salmonellae* spp. (Cao *et al.*, 2008). Ducks with bacterial infection experience diarrhea, lack of coordination, depression, dehydration, and a high mortality rate. In the poultry business, these illnesses result in great financial losses for various regions of the world (Brans and Gross, 1997). *Staphylococci* inhabit the skin and mucosal surface of the most critical organs of mammals and birds (El-Jakee *et al.*, 2008). In poultry, it produces considerable economic losses in a variety of ways, including septicemia, lower body weight, decreased egg production, and osteomyelitis, which result in lameness and carcass condemnation at slaughter (McNamee and Smyth, 2000 and Andreasen, 2008). *Escherichia coli* infects ducks of all ages, causing septicemia with a death rate of 10%–50%. Young ducklings are more commonly affected, and mortality rates in birds aged 4–9 weeks can approach 20%. Colibacillosis

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is manifested either as a systemic or localized form. Pericarditis is commonly seen in colisepticemia. The pericardial sac becomes cloudy and the epicardium becomes edematous and covered with a light-colored exudate (Saif *et al.*, 2010). Also, congested spleen, perihepatitis, airsacculitis, and enteritis have been recorded by Aggad *et al.* (2010). The localized form may be in the form of coliform omphalitis/yolk sac infection (Montagomery *et al.*, 1999), coliform cellulitis (Gomis *et al.*, 2000), swollen head syndrome (Van de Zande *et al.*, 2001), diarrheal disease (Saif *et al.*, 2010), or venereal colibacillosis (acute vaginitis) (Gerardian *et al.*, 2000).

Salmonellosis is a dangerous duck disease with clinical symptoms more common in very young ducklings and more prevalent in the immediate post-hatched period (Henry, 2000). The clinically affected ducklings showed depression, inappetence, huddling together, loss of weight, closed eyes, and a staggering gait appeared 72 hours post infection, which was followed by tremors, droopy wings, diarrhea, and feather pasting around the vent (Mondal *et al.*, 2008). Ducklings with salmonellosis have postmortem findings that range from no grossly evident lesions to a septicemic picture with congestion of the internal organs, including the liver, spleen, lungs, and kidneys. Typhlitis, pericarditis, and perihepatitis are also frequently seen (Lister and Barrow, 2008). Discrete necrotic lesions in the lungs, liver, and heart may be observed. Birds that survive the acute septicemic phase of the infection may have peritonitis and hemorrhagic enteritis (Pattison *et al.*, 2008). The VITEK 2 system is considered an efficient and dependable method for carrying out bacterial identification and testing for antibiotic susceptibility.

The polymerase chain reaction (PCR) is a very sensitive technique for identifying different pathogens in clinical samples. Numerous PCR assays have been created to detect and identify bacterial pathogens in ducks (Gomis *et al.*, 2003).

Since most bacterial illnesses in duck flocks cannot be totally prevented by vaccination, antibiotics are often recommended as a control measure. In the poultry industry, improper usage of antibiotics results in higher rates of resistance, leading to the growth of multidrug-resistant bacteria and subsequently raises concern (Ammar *et al.*, 2021).

This study aims to identify the bacterial pathogens incriminated in the duck enteritis problem using conventional biochemical tests, VITEK 2 system as well as PCR. Furthermore, the study assesses the antibiotic sensitivity of the identified pathogens.

Material and Methods

Examined birds

Forty-two duck flocks from four Egyptian governorates (El-Sharkia, El-Gharbia, El-Dakahlia, and El-Qaliobia) suffering from enteric disease were subjected to clinical and postmortem examination. The examined flocks were of different breeds (Pekin, Mallard, and Muscovy) including 32 flocks of young ages 5–45 days and 10 older flocks ages 8–57 weeks. The birds were subsequently transported to the Avian and Rabbit Medicine Department at Zagazig University's Faculty of Veterinary Medicine in Egypt. For bacterial isolation and identification, 42 liver samples were collected aseptically from freshly dead ducks.

Bacterial isolation and identification

A loopful of liver samples were inoculated onto five different media (nutrient agar, mannitol salt agar, MacConkey's agar, xylose lysine desoxycholate agar, and sheep blood agar) and then incubated for 24 hours at 37°C. Separate pure colonies were identified morphologically via using Gram's stain as well as biochemically using methods described by Quinn *et al.* (2002).

VITEK 2 compact analysis

VITEK 2 compact analysis was applied for phenotypical confirmation of bacterial isolates. Following the introduction of a uniform suspension of the unidentified organism into every single self-contained card, the device's inbuilt optics reads the cards after incubation. By comparing the results of the samples to the known species-specific reactions found in the VITEK 2 database, sophisticated colorimetry technology allowed for the identification of organisms and the testing of antibiotic sensitivity (Wallet *et al.*, 2005).

Conventional PCR for E. coli identification

Bacterial DNA extraction

DNA extraction by boiling was performed by mixing bacterial culture with 200 µl of distilled water then boiled for 10 minutes at 95°C in a heat block or dry water bath. The resultant solution was centrifuged using a cooling centrifuge at 4°C for 10 minutes then the supernatant was used as a template of DNA which was stored at −20°C until used.

Preparation of PCR master mix

The DreamTaq Green PCR Master Mix (2×) kit, code number K1082 (Thermo ScientificTM), was utilized. 12.5 µl of DreamTaq Green PCR Master Mix, 7.5 µl of PCR-grade water, 1 µl of each forward and reverse 20 pmol primer, and 3 µl of template DNA make up the total amount of 25 µl/reaction.

Thermal cycle conditions for the PCR assay used to identify *E. coli*

The temperature and timing conditions of the PCR for detecting the uid A gene were as follows: Denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 60 seconds, followed by final extension at 72°C for 7 minutes (Anbazhagan *et al.*, 2011).

Screening of PCR products

About 10 µl of the amplified PCR product was analyzed by electrophoresis on a 2% agarose gel stained with 0.5 µg of ethidium bromide/ml.

Electrophoresis was carried out in 1 × TAE buffer at 80 volts for 1 hour. Gels were visualized under an ultraviolet transilluminator (UVP, UK) and photographed (Lee *et al.*, 2012).

Data analysis

Data analysis and visualization were performed using R software (R Core Team, 2022; version 4.2.0). Using the "Complex heatmap" program, a heatmap of the isolates' antimicrobial resistance patterns was produced (Gu *et al.*, 2016).

Ethical approval

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Zagazig University.

Results

Clinical and postmortem findings

The examined ducks of different ages and breeds were suffering from severe diarrhea and general signs of illness expressed by off food, depression, ruffling feathers, and weakness. All flocks under investigation had diarrhea (watery, whitish, bloody, and greenish). In addition, 59.5% of the flocks had respiratory symptoms, 16.7% had stunted growth, and 16.7% had a swollen tongue and shorter beak. The postmortem findings include enteritis (100% of examined flocks of different ages), increased pericardial fluid, pericarditis, and perihepatitis (64% of examined flocks) (Fig. 1).

Bacterial isolation and identification using conventional methods

Out of 42 liver samples taken from ducks suffering enteritis revealed 46 isolates either in single or mixed occurrence. They were identified as *Staphylococcus* spp*, Enterococcus* spp*, E. coli, Salmonella* spp*, and Enterobacter* spp (Table 1).

Findings of bacterial identification using VITEK 2 analysis and conventional PCR

Positive isolates identification was based on morphology, biochemical characters (VITEK 2), and confirmatory PCR for *E. coli* revealing the presence of 24 isolates of *Staphylococcus* spp., 19 isolates of *E. coli,* one isolate of each *E. Casseliflavus*, *Salmonella* spp*,* and *Enterobacter cloacae complex*.

The *E. coli* isolates were successfully identified by the detection of the uidA gene (beta-glucuronidase enzyme) at the band size 556 bp (Table 1 and Fig. 2). Mixed infection was proved in 13 out of 42 flocks studied (30.95%) with two bacterial agents; only one flock was infected with *Staphylococcus* spp. and *Salmonella enterica subspecies arizona,* while 12 flocks got mixed infections with *Staphylococcus* spp. and *E. coli.*

Antimicrobial sensitivity

A representative 15 out of the 46 isolates exhibited resistance to multiple tested antimicrobial agents (Fig. 3). Antibiotic susceptibility of the *Staphylococcus* spp. isolates displayed high sensitivity to amikacin, cephradine, fosfomycin, vancomycin, tigecycline, linezolid, nitrofurantion, rifampicin, and quinupristin/dalfopristin, (100%); gentamycin, ciprofloxacin, moxifloxacin, trimethoprim/sulfamethoxazole, and doxycycline (66.7%) while isolates were highly resistant to penzylpenicillin, ampicillin, colistin, and spiramycin (100%); levofloxacin, erythromycin, oxacillin, clindamycin, tetracycline, streptomycin, and difloxacin (66.7%).

While *E. coli* isolates showed high sensitivity to amikacin, fosfomycin, ticarcillin/clavulanic acid, piperacillin/tazobactam, meropenem, and imipenem (100%); minocycline, doxycycline, and difloxacin (78%); tobramycin and ciprofloxacin (66.7%), while isolates were resistant to colistin, erythromycin, spiramycin, ampicillin, piperacillin, and ticarcillin (100%); cephradine, trimethoprim/sulfamethoxazole, and streptomycin (88.9%); ceftazidime, cefepime, and aztreonam (78%).

The antimicrobial sensitivity pattern of *E. Casseliflavus* isolate showed high sensitivity to amikacin, cephradine, fosfomycin, gentamycin, vancomycin, tigecycline, doxycycline, linezolid, oxacillin, and trimethoprim/sulfamethoxazole; moderately sensitive to streptomycin, clindamycin, moxifloxacin, nitrofurantion, rifampicin, and quinupristin/dalfopristin, and resistant to penzylpenicillin, ampicillin, colistin, and spiramycin, erythromycin, tetracycline, ciprofloxacin, levofloxacin, and difloxacin.

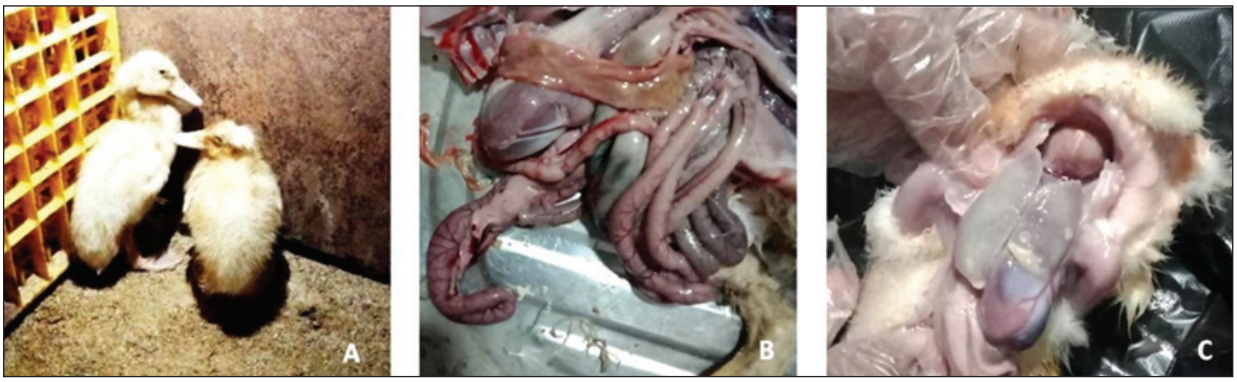

Figure 1. Clinical and postmortem lesions of ducklings, naturally infected with *E. coli*: a) Ruffled feathers and weakness; b) Closed and opened intestine displaying enteritis; c) Perihepatitis and pericarditis.

Table 1. Results of bacterial isolation and identification from duck flocks suffering from enteritis.

Flock No	Isolate No	Breed	Locality	Total No	Age/day	Mortality[a] percentage	Conventional identification		Identification using VITECK 2 / PCR
							Gram stain	Growth in mediums / biochemical tests	
1	–	Pekin	Sharkia	2,000	22	5	–	–	–
2	–	Pekin	Sharkia	4,000	5	9	–	–	–
3	–	Pekin	Qaliobia	4,000	14	15	–	–	–
4	–	Muscovy	Qaliobia	3,000	14	8	–	–	–
5	1	Muscovy	Sharkia	2,000	46	9	GP Cocci	*Enterococcus* spp	*E. Casseliflavus*
6	2	Muscovy	Sharkia	2,000	21	7	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
7	3	Muscovy	Sharkia	3,500	41	6	GN Bacilli	*E. coli*	*E. coli*
8	–	Muscovy	Sharkia	1,500	25	13	–	-	-
9	–	Pekin	Sharkia	3,000	38	2	–	-	-
10	4	Muscovy	Sharkia	2,500	35	9	GP cocci, Grapes like	*Staphylococcus* spp.	*S. aureus*
11	5	Pekin	Dakahlia	4,000	70	7	GP cocci, Grapes like	*Staphylococcus* spp.	*S. lentus*
12	6	Muscovy	Dakahlia	18,000	76	2	GN Bacilli	*E. coli*	*E. coli*
13	7	Muscovy	Gharbia	3,500	400	12	GN Bacilli	*E. coli*	*E. coli*
14	–	Pekin	Sharkia	4,000	38	8	-	-	-
15	8	Muscovy	Dakahlia	3,200	45	14	GP cocci, Grapes like	*Staphylococcus* spp.	*S. aureus*
16	9	Muscovy	Dakahlia	2,500	350	11	GN Bacilli	*E. coli*	*E. coli*
17	10	Pekin	Gharbia	2,400	180	9	GP cocci, Grapes like	*Staphylococcus*	*S. aureus*
	11						GN Bacilli	*E. coli*	*E. coli*
18	12	Muscovy	Gharbia	5,000	62	5	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
19	13	Muscovy	Gharbia	3,600	180	12	GN Bacilli	*E. coli*	*E. coli*
20	14	Muscovy	Sharkia	1,000	12	75	GN Bacilli	*Enterobacter spp*	*E. cloacae complex*
21	-	Pekin	Sharkia	4,000	14	10	-	-	-
22	15	Muscovy	Sharkia	1,500	13	40	GN Bacilli	*E. coli*	*E. coli*
23	16	Pekin	Qaliobia	3,000	48	10	GP cocci, Grapes like	*Staphylococcus Spp.*	*Staphylococcus spp.*
24	17	Pekin	Sharkia	3,000	15	15	GP cocci, Grapes like	*Staphylococcus Spp.*	*Staphylococcus spp.*
	18						GN Bacilli	*E. coli*	*E. coli*
25	19	Mullard	Sharkia	15,000	14	30	GP cocci, Grapes like	*Staphylococcus* spp.	*S. aureus*

Flock No	Isolate No	Breed	Locality	Total No	Age/day	Mortality[a] percentage	Conventional identification		Identification using VITECK 2 / PCR
							Gram stain	Growth in mediums / biochemical tests	
26	20	Muscovy	Sharkia	2,000	9	27	GP cocci, Grapes like	*Staphylococcus* spp.	*S. aureus*
	21						GN Bacilli	*Salmonella* spp.	*S. enterica subspecies arizonae*
27	22	Pekin	Qaliobia	3,500	12	12	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
	23						GN Bacilli	*E. coli*	*E. coli*
28	24	Pekin	Sharkia	1,500	12	18	GN Bacilli	*E. coli*	*E. coli*
29	25	Mullard	Sharkia	8,000	10	25	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
	26						GN Bacilli	*E. coli*	*E. coli*
30	27	Pekin	Sharkia	20	30	10	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
	28						GN Bacilli	*E. coli*	*E. coli*
31	29	Muscovy	Qaliobia	100	17	40	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
	30						GN Bacilli	*E. coli*	*E. coli*
32	31	Pekin	Sharkia	4,000	45	10	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
	32						GN Bacilli	*E. coli*	*E. coli*
33	33	Pekin	Sharkia	1,500	18	8	GP cocci, Grapes like	*Staphylococcus* spp.	*Staphylococcus* spp.
	34						GN Bacilli	*E. coli*	*E. coli*
34	35	Pekin	Sharkia	2,000	25	12	GP cocci, Grapes like	*Staphylococcus Spp*	*Staphylococcus Spp*
	36						GN Bacilli	*E. coli*	*E. coli*
35	-	Pekin	Sharkia	2,000	18	35	-	-	-
36	37	Pekin	Sharkia	3,000	21	5	GP cocci, Grapes like	*Staphylococcus Spp*	*Staphylococcus Spp.*
	38						GN Bacilli	*E. coli*	*E. coli*
37	39	Muscovy	Sharkia	500	18	40	GP cocci, Grapes like	*Staphylococcus Spp*	*S. lentus*
38	40	Pekin	Sharkia	300	28	7	GP cocci, Grapes like	*Staphylococcus Spp*	*Staphylococcus Spp*
	41						GN Bacilli	*E. coli*	*E. coli*
39	42	Muscovy	Sharkia	1,500	13	60	GP cocci, Grapes like	*Staphylococcus Spp*	*Staphylococcus Spp*
40	43	Muscovy	Gharbia	4,000	65	7	GP cocci, Grapes like	*Staphylococcus Spp*	*Staphylococcus Spp*
41	44	Muscovy	Qaliobia	1,000	18	30	GP cocci, Grapes like	*Staphylococcus Spp*	*S. scirui*
42	45	Pekin	Sharkia	1,400	42	15	GP cocci, Grapes like	*Staphylococcus Spp*	*Staphylococcus Spp*
	46						GN Bacilli	*E. coli*	*E. coli*

The cumulative mortalities in infected flocks were recorded over 5 days from the start of the symptoms. GP (Gram-positive bacteria) and GN (Gram-negative bacteria).

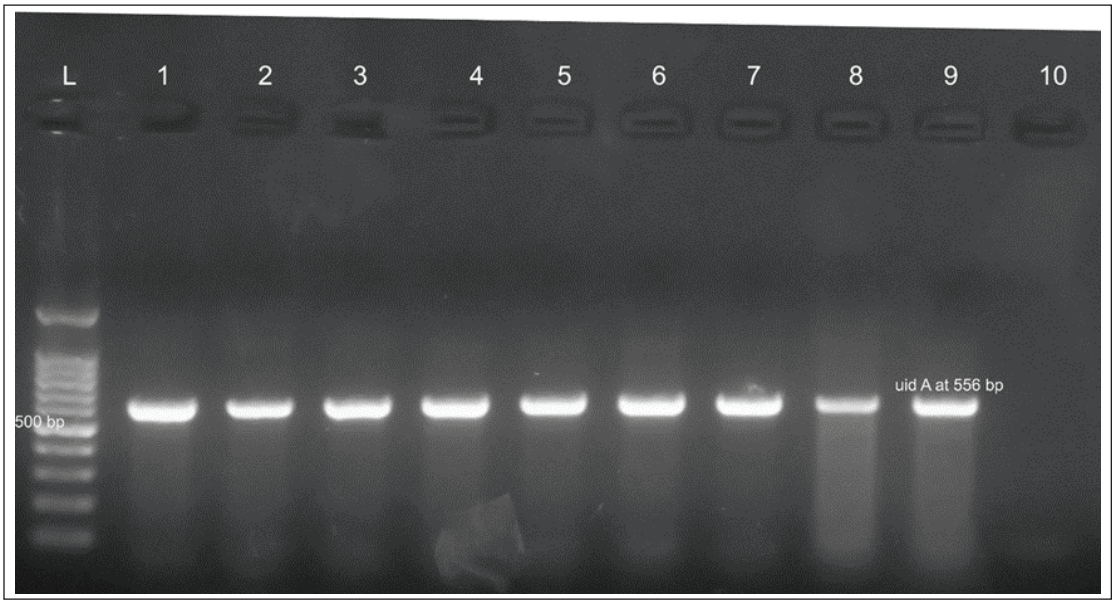

Figure 2. Agarose gel electrophoresis of the PCR products of *E. coli* :Lanes L: 100 bp ladder, 1: 13, 2: 18, 3: 23, 4: 28, 5: 30, 6: 32, 7: 34, 8: 36, and 9: positive control uid A at 556 bp. 10: negative control.

The *E. cloacae complex* isolate showed high sensitivity to amikacin, fosfomycin, difloxacin, piperacillin/tazobactam, meropenem, and imipenem; moderately sensitive to ticarcillin/clavulanic acid and tobramycin, and resistant to ampicillin, piperacillin, ticarcillin, cephradine, ceftazidime, cefepime, aztreonam, gentamycin, streptomycin, erythromycin, spiramycin, ciprofloxacin, levofloxacin, minocycline, doxycycline trimethoprim/sulfamethoxazole.

Interestingly *S. enterica subspecies arizonae* isolate showed high sensitivity to amikacin, gentamycin, ciprofloxacin, difloxacin, levofloxacin, minocycline, doxycycline, fosfomycin, piperacillin, piperacillin/tazobactam, meropenem, imipenem, ticarcillin, ticarcillin/clavulanic acid, tobramycin, ceftazidime, cefepime, aztreonam, and trimethoprim/sulfamethoxazole, and resistant to cephradine, colistin, ampicillin, streptomycin, erythromycin and spiramycin. Results of antimicrobial resistance testing show an increase in the number of isolates causing duck enteritis that is multidrug resistant, with 87% of the tested isolates having resistance to five or more antimicrobials (Table 2).

Discussion

Enteritis is a devastating disease for the poultry performance particularly in ducklings which subtracts from the economic outcome of this poultry sector. Consequently, the bacterial agents causing duck enteritis among 42 flocks from four Egyptian governorates (El-Sharkia, El-Gharbia, El-Dakahlia, and El-Qliobia) were investigated. The birds were subjected to clinical examination and bacterial and molecular identification as well as antimicrobial testing using VITEK 2.

The frequently observed clinical signs among the examined flocks were watery, whitish, bloody, and greenish diarrhea. The obtained results were analogs to that previously recorded by Ibrahim, (2003) who infected 14-day-old ducklings intranasal with 7.5×10^6 cfu *E. coli* O86:K61 experimentally and noticed whitish and greenish diarrhea 48 hours post infection. The clinically affected ducklings showed diarrhea with pasting of feathers around the vent which is comparable with Barrow *et al.* (1999) findings that isolated and identified *Salmonella typhimurium* from ducklings during the first 2 weeks of life in the United Kingdom. Most of the flocks under examination had respiratory distress, labored breathing, and gasping. The obtained results were parallel to those previously stated by Abd El-Samie *et al.* (2019) who observed respiratory symptoms in 40 diseased ducks of different ages (1–8 weeks old) in Sharkia governorate, Egypt, between June and September 2018 affected by colibacillosis.

The necropsy revealed enteritis in the ducks under investigation. Similarly, Abd El Tawab *et al.* (2015) observed enteritis in ducks infected with *S. Inganda*, *S. Infantis,* and *S. Larochelle* in the Egyptian governorates of Dakahlia and Damietta.

In addition, in this study, increased pericardial fluid, pericarditis, and serofibrinous perihepatitis were noted as necropsy findings and these results were agreed with Aggad *et al.* (2010) who isolated *E. coli* from 1 to 8-week-old chickens in western Algeria. The results were also consistent with those reported previously by Abd El-Samie *et al.* (2019), who noted air sacculitis, pericarditis, perihepatitis, and peritonitis in young diseased ducks suffering from colibacillosis in Sharkia governorate, Egypt, between June and September 2018.

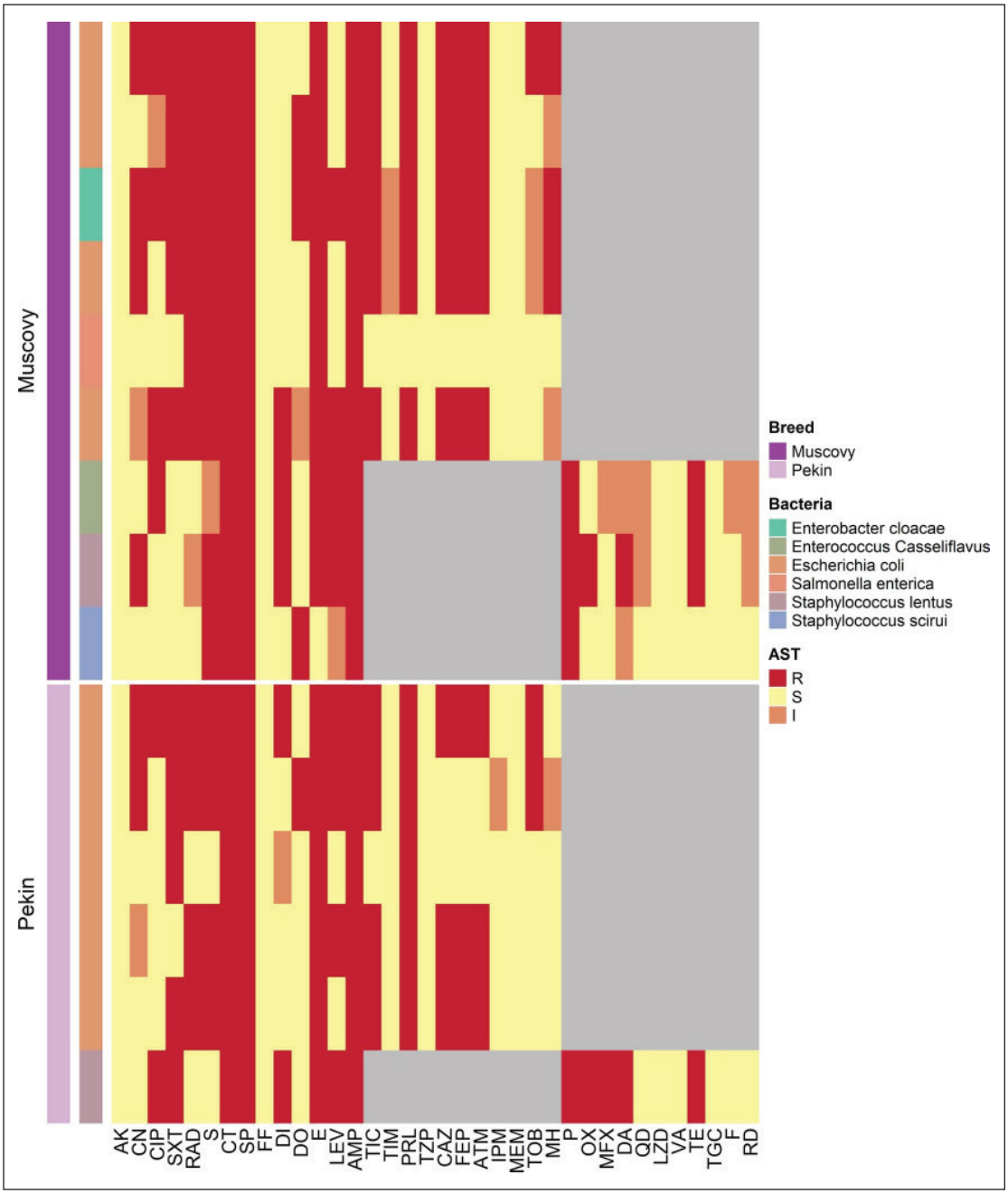

Figure 3. Heatmap representation of isolates source and antimicrobial resistance patterns.

Bacterial isolation, bacterial identification utilizing (VITEK 2), and confirmatory PCR were carried out to evaluate the bacterial etiology of enteritis in the examined ducks.

In this study, the examined livers yielded 46 bacterial isolates identified as follows *Staphylococcus* spp (52.17%), *E. coli* (41.30%), *and the least percentage of* 2.17% for *E. casseliflavus* (*S. enterica subspecies arizonae* and *E. cloacae complex*). These results were obtained by Bisgaard (1981) who isolated a higher percentage of *Staphylococcus* species than *E. coli* from ducks (Mallard, Muscovy, and white Pekin) at the age of 55 days. Also, partially agreed with Safwat *et al.* (1986) who isolated *Salmonella*, *E. coli*, *Enterobacter species,* and *Pasteurella multocida* from the internal organs of 150 balady ducks (4 weeks old) at a percentage of 19%, 15%, 13.3%, and 5%, respectively. The inability to isolate *Pasteurella* in our study may be due to medicated or vaccinated flocks. The second highest percentage of isolated bacteria in this study was *E. coli (*41.30%). This result was agreed with Asway and Abd-El latif (2010) who isolated *E. coli* from the

Table 2. Resistant proportions of 15 isolates from ducks with enteritis.

Antimicrobial[a]	Gram-Negative isolates (*n* = 11)	Antimicrobial[a]	Gram-Positive isolates (*n* = 4)
	No. (%) of resistant		No. (%) of resistant
TIC	9 (81.8)	P	4 (100)
TIM	0 (0.0)	AMP	4 (100)
PRL	10 (90.9)	OX	2 (50)
TZP	0 (0.0)	CN	1 (25)
CAZ	8 (72.7)	CIP	2 (50)
FEP	8 (72.7)	LEV	3 (75)
ATM	8 (72.7)	MFX	1 (25)
IPM	0 (0.0)	E	3 (75)
MEM	0 (0.0)	DA	2 (50)
AK	0 (0.0)	QD	0 (0.0)
CN	5 (45.5)	LZD	0 (0.0)
TOB	3 (27.3)	VA	0 (0.0)
CIP	4 (36.4)	TE	3 (75)
MH	3 (27.3)	TGC	0 (0.0)
SXT	9 (81.8)	F	0 (0.0)
RAD	10 (90.9)	RD	0 (0.0)
S	10 (90.9)	SXT	1 (25)
CT	11 (100)	AK	0 (0.0)
SP	11 (100)	RAD	0 (0.0)
FF	0 (0.0)	S	2 (50)
DI	2 (18.2)	CT	4 (100)
DO	3 (27.3)	SP	4 (100)
E	11 (100)	FF	0 (0.0)
LEV	5 (45.5)	DI	3 (75)
AMP	11 (100)	DO	1 (25)

Ticarcillin (TIC), Ticarcillin/ clavulanic (TIM), Piperacillin (PRL), Piperacillin / tazobactam (TZP), Ceftazidime (CAZ), Cefepime (FFP), Aztreonam (ATM), Imipenem (IPM), Meropenem (MEM), Tobramycin (TOB), Minocycline (MH), Benzylpenicillin (P), Ampicillin (AMP), Oxacillin (OX), Gentamycin (CN), Ciprofloxacin (CIP), Levofloxacin (LEV), Moxifloxacin (MOX), Erythromycin (E), Clindamycin (DA), Quinupistin / Dalfopristin (Q/D), Linezolid (LZD), Vancomycin (VA), Tigecycline (TGC), Tetracycline (TE), Nitrofurantoin (F), Rifampicin (RD), Trimethoprim /sulfamethoxazole (SXD), Amikacin (AK), Cephradine (RAD), Streptomycin (S), Colistin (CT), Spiramycin (SP), Fosfomycin (FF), Difloxacin (DI) and Doxycycline (DO).

internal organs of ducks with a higher percentage in the liver (42.5%).

Infections with two bacterial pathogens were detected in 30.95%. Whereas 12 flocks had mixed infections with *Staphylococcus* spp. and *E. coli*, just one flock had *Staphylococcus* spp. and *S. enterica subspecies arizonae* infection. These findings are similar to those obtained by Asway and Abd-El latif (2010), who studied bacterial enteritis in 120 freshly dead ducks and 40 fecal samples from clinically diseased ducks of various ages (1–30 days) obtained from private farms in the Dakahlia Governorate and recorded mixed infections of *E. coli*, *Staphylococcus aureus,* and *Salmonella.* However, negative results from bacterial isolation testing, enteritis was found in four of the duck flocks (1–4) under investigation. This could be related to various factors such as enteric viruses, as explored by Abd El-Ghany (2021) who investigated the common emerging viral infections affecting ducks in Egypt, parasites, or poor hygiene and sanitary conditions.

In our study, different species of *Staphylococcus* were isolated including *S. aureus*, *Staphylococcus lentus,*

and *Staphylococcus scirui*. The identification of both later new species of *Staphylococci* from cases of enteritis in our study may be due to using one of the most sensitive techniques for bacterial identification (VITEK 2 automated system). These results are similar to that obtained by Marek *et al.* (2016) who isolated *Staphylococcus species* including *S. lentus* and *S. scirui* from heart, liver, tarsal joints, and bone marrow from poultry flocks (broilers, laying hens, breeding hens, turkeys, ducks, and geese). The bird in the aforementioned study had increased mortality, inflammation of the skin and s/c tissue (dermatitis and cellulitis), lameness, arthritis, decreased weight gain, omphalitis, and yolk sac infections. This finding emphasizes the elevated significance of both staphylococcus species as disease-causing agents in poultry flocks.

Salmonella enterica subspecies arizonae was isolated from 9 days duckling in this study. To the best of our knowledge, this is considered the first record of *S. arizonae* isolation from ducks suffering enteritis in Egypt. Previously Abd El Tawab *et al.* (2015) isolated *S. Inganda*, *S. Infantis,* and *S. Larochelle* from 104 ducks from flocks scattered in two Egyptian governorates (Dakahlia and Damietta).

Conventional techniques that we used in this study for isolation and identification recognized the genus of the pathogenic bacteria, whereas VITEK 2 identified the bacterium to the species level. Furthermore, the VITEK 2 system is a simpler approach to implement than the traditional method (Carroll and Weinstein, 2007). VITEK 2 is quickly becoming a common approach for laboratory microbiology (Wallet *et al.*, 2005), and we used it in this study for bacterial identification and antibiotic susceptibility testing.

PCR was able to confirm VITEK 2 automated system results in the case of *E. coli* isolates by the detection of the uidA gene (beta-glucuronidase enzyme) at the band size 556 bp which agreed with Anbazhagan *et al.* (2011) who employed the uidA *E. coli* O177-specific gene sequence as the target of a single-plex PCR experiment.

The antibiogram patterns of isolated pathogens from naturally infected ducks in our work demonstrated varying results against different antimicrobial drugs tested, indicating the rise of drug- and multidrug-resistance of *Staphylococci*, *E. coli*, *Enterococcus,* and *Enterobacter. Staphylococci* isolates were extremely resistant penzylpenicillin, ampicillin, colistin, spiramycin (100%), and levofloxacin, erythromycin, oxacillin, clindamycin, tetracycline, streptomycin and difloxacin (66.7%). This result partially matched with Awad *et al.* (2023) who examined the prevalence and antimicrobial resistance of *S. aureus* isolated from 500 broilers and ducks in the Egyptian governorates of El-Dakahlia and El-Sharkia. The authors found that chicken *S. aureus* isolates had higher resistance rates to trimethoprim/ sulfamethoxazole (84.2%) and oxytetracycline (73.7%) while duck *S. aureus* isolates were resistant to oxacillin (87.5%), followed by trimethoprim/ sulfamethoxazole (50%).

Conversely, enterobateriacae isolates were extremely resistant to colistin, erythromycin, spiramycin, ampicillin, piperacillin, and ticarcillin (100%); cephradine, trimethoprim/sulfamethoxazole, and streptomycin (88.9%); ceftazidime, cefepime, aztreonam (78%), and less resistant to gentamicin and levofloxacin (45%). This finding was consistent with that of Bushen *et al.* (2021), who reported that the enterobateriacae isolates found in 140 fresh chicken dropping samples collected in Southwest Ethiopia from April to June of 2018 were highly resistant to ampicillin (91.7%) and trimethoprim-sulfamethoxazole (70.8%) and less resistant to gentamicin (41.7%). In addition, during 7 weeks of duck breeding in Slovakia, Hleba *et al.* (2011) noted resistance of Enterobacteriaceae to tetracycline (32.43%), streptomycin, and ampicillin on the same level of 8.10%, and chloramphenicol (5.40%).

Antibiotic susceptibility of the *Staphylococcus* spp. isolates revealed high sensitivity to amikacin, cephradine, fosfomycin, vancomycin, tigecycline, linezolid, nitrofurantion, rifampicin, and quinupristin/ dalfopristin, (100%); gentamycin, ciprofloxacin, moxifloxacin, trimethoprim/sulfamethoxazole, and doxycycline (66.7%) and levofloxacin, difloxacin, streptomycin, oxacillin, and tetracycline (33.3%). These results were similar to Nabil (2010) who stated that *Staphylococcus* isolates were sensitive to ciprofloxacin, norofloxacin, streptomycin, and gentamycin.

The *E. coli* isolates were highly sensitive to amikacin, fosfomycin, ticarcillin/clavulanic acid, piperacillin/ tazobactam, meropenem, and imipenem (100%); minocycline, doxycycline, and difloxacin (78%); tobramycin and ciprofloxacin (66.7%). These findings partially matched to those of Cambrea (2014), who noted that *E. coli* isolates were highly sensitive to amikacin (85.7%), cephalosporins (80%), ceftazidime (79.5%), ceftriaxone (75%), trimethoprim/sulfamethoxazole (38.4%), and tetracycline (29.5%), as well as Adam *et al.* (2022) who noted that all *E. coli* isolates were highly sensitive to imipenem (100%).

Salmonella enterica subspecies arizonae isolate was highly sensitive to amikacin, gentamycin, ciprofloxacin, difloxacin, levofloxacin, minocycline, doxycycline, fosfomycin, piperacillin, piperacillin/tazobactam, meropenem, imipenem, ticarcillin, ticarcillin/ clavulanic acid, tobramycin, ceftazidime, cefepime, aztreonam, and trimethoprim/sulfamethoxazole. These findings agreed with Lebdah *et al.* (2017), who found that Salmonella isolates were susceptible to amikacin (80.9%); ciprofloxacin (76.2%); norofloxacin (71.2%); and cefotaxime (52.4%). In addition, Ćwiek *et al.* (2020) reported that *Salmonella* isolates were susceptible to gentamicin, tazobactam, cefotaxime, meropenem, ciprofloxacin, azithromycin, tigecycline, and trimethoprim.

Conclusion

Because enteric pathogens are recognized as one of the most serious concerns confronting the duck business, various studies to understand such sickness situations are required. In Egypt, the most common bacterial pathogens causing duck enteritis were *Staphylococcus* spp. and *E. coli*. To our knowledge, the isolation of *S. lentus*, *S. scirui, E. casseliflavus*, *S. enterica subspecies arizonae,* and *E. cloacae complex* was first recorded in Egypt from duck enteritis. Eighty percent of the duck enteritis isolates were multidrug resistant to five or more antimicrobials. This underlines the ways in which duck pathogens may serve as a source of antimicrobial resistance traits and may spread to humans and other birds via the food chain and environment.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Authors' contributions

AH, MH, and EM designed this study, collected samples, and epidemiological data, performed the bacterial identification and molecular diagnosis, and wrote the manuscript. IE performed statistical analysis. AE and MS revised and commented on the manuscript.

Data availability

All data supporting the findings of this study are available within the manuscript. Any extra data needed are available from the corresponding authors upon reasonable request.

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