Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 53, Number 2, p:165-182 June 2025
DOI: 10.21608/zvjz.2025.348075.1276

RESEARCH ARTICLE

Identification of Bacterial Agents Implicated in Mortalities in Turkey Flocks in Sharkia Governorate during 2021-2024

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ARTICLE INFO

Article History:
Received: 15 January 2025
Accepted: 10 March 2025
Published online: June 2025

Key words:
Bacteria, Identification, Turkeys, Mortality, Antimicrobials.

ABSTRACT

There are several bacterial agents causing mortalities in turkey flocks and have a negative impact on the Egyptian turkey industry. Routine isolation, identification, and surveillance of these pathogens are essential tools for disease monitoring. The research work was conducted for identification of bacterial agents involved in mortalities in turkey flocks in Sharkia governorate during 2021-2024. A total of 126 samples (liver, heart, and lung) were collected from 42 birds (freshly dead or live diseased) representing 10 turkey flocks of various breeds and from different localities, ranging in age from 20 to 100 days and suffering from diarrhea, respiratory distress, arthritis, and a mortality rate ranging from 2-8%. All collected morbid turkeys were subjected to clinical and/ postmortem, and bacteriological examinations. Bacterial isolation on different media and identification by traditional biochemical tests were performed. E. coli was recognized in six flocks (60%), Klebsiella spp. in two flocks (20%), Salmonella spp. in four flocks (40%), Pseudomonas spp. in two flocks (20%), Enterococcus spp. in one flock (10%), Staphylococcus spp. in one flock (10%), and mixed infections in six flocks (60%). An in vitro antibiogram test was performed to select antibiotics of choice. Most of the tested isolates were sensitive to difloxacin, amikacin, and apramycin. All the tested isolates were multidrug resistant (MDR) (100%) due to resistance to 3 or more antimicrobials. It could be concluded that turkey mortality in Sharkia Governorate is primarily caused by E. coli, Salmonella, Pseudomonas, Klebsiella, Staphylococcus, and Enterococcus. The liver is the most suitable organ for isolation. Difloxacin, amikacin, and apramycin are effective against these bacterial isolates. Monitoring antibiotic use is crucial for control.

Introduction

Turkey industry in Egypt is considered a major source of animal protein, ranking second to chicken [1]. Turkey industry faces a significant economic challenge due to different causes of mortalities, which may be viral, bacterial, and others [2]. There are several bacterial agents that cause massive economic losses in turkeys, such as *E. coli, Salmonella spp., Pseudomonas spp., Klebsiella spp., Staphylococcus spp., Enterococcus spp.*, and others [3]. Colibacillosis is one of the main contributing factors to global economic losses in the turkey industry [4]. Although *E. coli* is normally present in gastrointestinal tract [5], only certain strains known as Avian pathogenic *E. coli* APEC have virulence factors and are able to induce airsacculitis, perihepatitis, pericarditis, and septicemia in turkeys [6, 7].

Turkey industry is frequently challenged by Salmonella, which colonizes the intestinal tract and induces whitish diarrhea, respiratory signs, arthritis, and mortality [8]. *Staphylococcus* infection is one of the septicemic diseases in turkey flocks, which causes osteomyelitis, septicemia, and mortality [9]**.** Other bacterial diseases causing economic losses in turkey industry include *Pseudomonas*, *Klebsiella*, *Enterococcus*, and others [10-12]. Since most research over the past three years in Egypt has focused on identifying the viral causes of death in turkeys [13, 14]. We assumed it was essential to investigate the reasons behind bacterial mortality. The aim of this study is to identify the bacterial agents implicated in mortalities in turkey flocks in Sharkia governorate during 2021-2024 and to select the antibiotic of choice against the identified isolates using antibiogram.

Material and Methods

Ethics Declaration

The study was approved by Institutional Animal Care and Use Committee of Zagazig University with approval number ZU-IACUC/2/F/37/2025 and was carried out in agreement with the approved guidelines.

Flock history and samples collection

During the period from 2021 to 2024 in Sharkia Governorate, a total of 126 samples, including liver, heart, and lung, were collected from 42 birds, either freshly dead or live diseased, representing 10 turkey flocks of various breeds (Optima, Grade Maker, Big 6, and Converter) and from different localities, ranging in age from 20 to 100 days. The selected flocks suffered from respiratory signs, whitish and greenish diarrhea, arthritis, and mortalities ranging from 2-8%. The collected morbid turkeys were submitted to the laboratory of the department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt, for clinical, postmortem, and bacteriological examinations**.**

Data of the examined flocks, including total number, age, breed, locality, medication, vaccination, morbidity, and mortality percentages are summarized in Table 1.

Bacterial isolation and identification

Immediately after collection, the samples were inoculated into a nutrient broth and incubated at 37°C overnight. After enrichment in nutrient broth, an inoculum was streaked on solid media (HiMedia Laboratories Private Limited, Wagle Industrial Area, India) including nutrient agar (Cat No. M001-500G) (a general medium that promotes the growth of various non-fastidious organisms),

MacConkey agar (Cat No. MH081-500G) (for isolation of gram-negative bacteria such as *E. coli, Salmonella spp., Klebsiella spp., and Pseudomonas spp.*, and differentiation of lactose fermenting from non-fermenting gram-negative bacteria), blood agar (Cat No. M073-500G) (an enriched medium that promotes the growth of fastidious bacteria such as *Staphylococcus spp. and Enterococcus spp.* and differentiates bacteria based on their hemolytic characteristics), and Eosin Methylene Blue agar (Cat No. M317-500G) (EMB, a selective and differential media for *E. coli* identification). Different media were incubated at 37°C for 24-72 hours. Colony morphology (shape, size, surface texture, edge and elevation, color, and opacity) developed after 24-72 hours of incubation in different media was carefully studied and recorded.

Isolates showing characteristic colony morphology on nutrient agar, blood agar, EMB, and MacConkey agar were subjected to Gram staining, examined under a microscope using oil immersion 100X lens, and then subjected to biochemical tests such as catalase, coagulase, oxidase, indole, methyl red, Voges Proskauer, and citrate. Pure culture of the isolated organisms was preserved in sterilized 80% glycerin and utilized as stock culture. An equal volume of 80% glycerin and bacterial culture was combined, sealed with paraffin wax, and kept at -80 °C for future use [15-18].

Table 1. Descriptive data of the examined turkey flocks

Flock no.	Total no.	Season	Age in days	Locality	Breed	No. of birds examined	Clinical signs	Morbidity (%)	Mortality (%) *	Medication (Age in days)	Vaccination (Age in days)	
1	300	Summer	45	Met Gaber	Grade maker	5	Respiratory signs and whitish diarrhea	40	4	Tylosin+ Neomycin (1)	Vitapest (3)	ND+H5 (5)
											Clone (30)	
2	500	Winter	33	Met Gaber	B six	3	whitish diarrhea	15	2	Diflobiotic+ Robadiar (5)	ND+H5 (1)	Vitapest (5)
											LaSota (25)	
3	600	Autumn	80	Saadia	Converter	6	Arthritis and whitish diarrhea	45	6	Tylosin+ Colistin (30)	ND+H5 (3)	Clone (5)
											Avinew (30)	LaSota (55)
											Cholera (65)	
4	900	Summer	48	Met Abo Ali	Converter	5	Greenish diarrhea	40	5	Immulant+ AD3E (10)	ND+H9+H5 (1)	Vitapest (5)
											Clone (30)	
5	1000	Summer	50	Met Gaber	Converter	4	Respiratory signs and whitish diarrhea	50	8	Diflobiotic + Toxinil (21)	ND+H5 (3)	Avinew (7)
											Clone (30)	
6	200	Spring	43	Met Abo Ali	Grade maker	3	Arthritis and greenish diarrhea	10	3	Fosfomycin+ D tox (15)	ND+ H9 (1)	Clone (5)
											LaSota (30)	
7	800	Summer	55	Hefna	Converter	6	Respiratory signs and greenish diarrhea	30	5	Miarom +Tylosin (7)	ND+H5+H9 (1)	Avinew (5)
											Clone (30)	
8	400	Winter	100	Met Gaber	B six	2	Respiratory signs and arthritis	40	6	Vitalyte+ Colistin (1)	Avinew (3)	AI-H9 (5)
											AI-H5 (12)	LaSota (30)
											Clone (60)	Cholera (70)
9	5000	Summer	90	Hefna	Optima	5	Greenish and whitish diarrhea	15	3	Doxycycline+ Tylosin (5)	ND+H9 (3)	Clone (5)
											AI-H5 (7)	Clone (35)
											Avinew (65)	Cholera (80)
10	400	Winter	20	Belbies	Converter	3	Respiratory signs and whitish diarrhea	50	7	Lincospectin+ Colistin (15)	Clone (7)	ND+H5 (1)

*****The mortality rate was recorded within three days from the start of the disease, (-): Age in days.

Antibiotic sensitivity test

An antimicrobial sensitivity test for our bacterial isolates against ten antimicrobial antibiotics [representing different antibiotic groups (Oxoid Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom) (florfenicol FFC 30, colistin sulfate CT 25, difloxacin INN 5, doxycycline DOX 30, erythromycin E 15, fosfomycin FOS 200, apramycin APR 15, amikacin AMK 30, penicillin P 10, and trimethoprim-sulfamethoxazole SXT 25)] was performed by disc diffusion method. Three pure colonies from each isolate were transferred using sterile loop to 5 ml sterile 0.9% physiological saline tube. The turbidity was adjusted to match 0.5 McFarland standard tube using adequate light. Using a sterile cotton swab, the surface of the plate was completely swabbed with continuous rotation to create a uniform layer of bacteria. The antimicrobial discs were arranged on the inoculated plate, pressed, and distributed evenly. The plates were inverted and then incubated at 37°C for 24-72 hours [19]. The zone of inhibition was measured, recorded, and interpreted according to the Clinical Laboratory Standard Institute [20]. Bacterial isolates are considered MDR when they become resistant to at least one agent in three or more antimicrobials of different groups [21].

Statistical Analysis

GraphPad Prism version 8 for Windows, GraphPad Software, La Jolla, California, USA, www.graphpad.com, was used for the determination of the isolation rate of different bacterial agents from different organs and the analysis of antibiotic resistance patterns. The results with $P < 0.05$ were considered statistically significant.

Results

Clinical and postmortem findings

General signs, including ruffled feathers, decreased feed and water intake, and depression, were recorded among all the diseased flocks, while specific signs, including respiratory symptoms (coughing, sneezing, rale, and ocular and nasal discharge), white diarrhea, and greenish diarrhea were also recorded in most of the flocks. Whitish diarrhea was recorded in 6 flocks (60%), respiratory signs in 5 flocks (50%), greenish diarrhea in 4 flocks (40%), and arthritis in 3 flocks (30%). Furthermore, variable mortalities were recorded among the affected flocks, ranging from 2 to 8%.

Some of the examined turkey flocks showed fibrinous pericarditis, fibrinous air sacculitis, and fibrinous perihepatitis, which was recorded in 5 flocks (50%); septicemic lesions in the form of congested subcutaneous tissues (S/C), trachea, and lungs were recorded in 4 flocks (40%); enteritis was recorded in 9 flocks (90%); renal nephrosis was recorded in 5 flocks (50%); and hepatic necrosis was recorded in 2 flocks (20%). The clinical and postmortem findings of infected turkeys are demonstrated in Figure 1 as well as their incidence percentages in Figure 2.

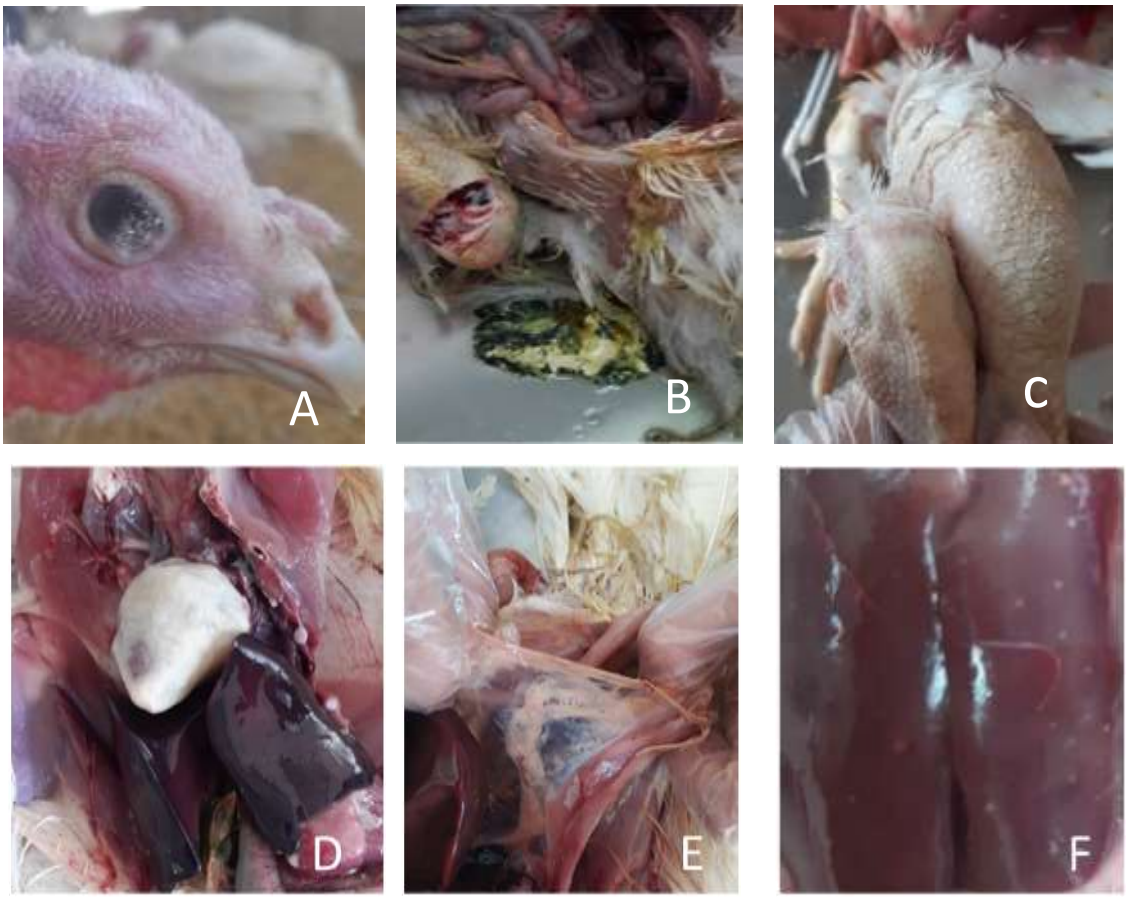
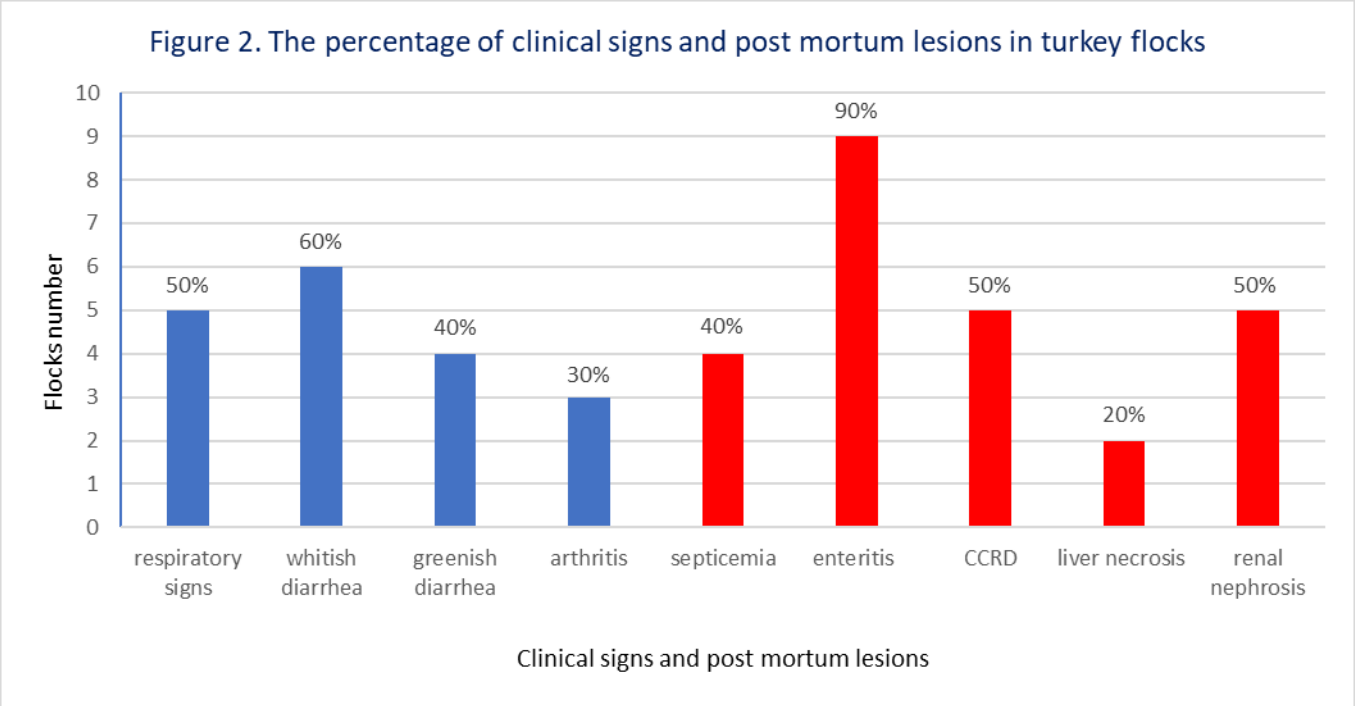


Figure 1. Clinical and postmortem findings of infected turkeys: A:100-day-old turkey showing ocular discharge, B: 58-day-old turkey showing greenish and whitish diarrhea, C: 43-day-old turkey showing arthritis, D: 45-day-old turkey showing fibrinous pericarditis, E: air sacculitis, and F: 33-day-old turkey showing liver necrosis.



Bacterial isolation and identification

Colony appearance

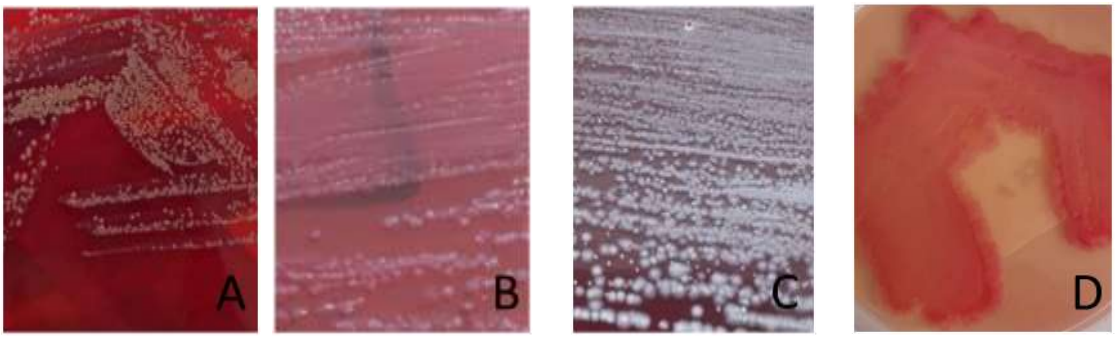
Escherichia coli colonies presented as pink colonies on MacConkey agar and green metallic sheen on EMB. *Klebsiella spp.* colonies appeared as pink mucoid colonies on MacConkey agar. *Salmonella spp.* colonies were pale colonies on MacConkey. *Enterococcus spp.* colonies were gray colonies with γ-hemolysis on blood agar. *Staphylococcus spp.* colonies appeared as yellow colonies with γ-hemolysis on blood agar (Figure 3).

Biochemical reactions

Staphylococcus spp., Escherichia coli, Salmonella spp., and *Klebsiella spp.* colonies were positive for catalase and negative for oxidase tests. *Pseudomonas spp.* was positive for oxidase, while other bacterial species were negative. *Staphylococcus aureus* was positive for coagulase, while other bacterial species were negative.

Microscopical examination

Staphylococcus spp. appeared as violet grape-like shaped cocci. *Enterococcus spp.* appeared as violet cocci arranged in chains, while *Salmonella spp*., *Pseudomonas spp.*, *Klebsiella spp.*, and *Escherichia coli* appeared as red medium-sized bacilli (Figure 4).



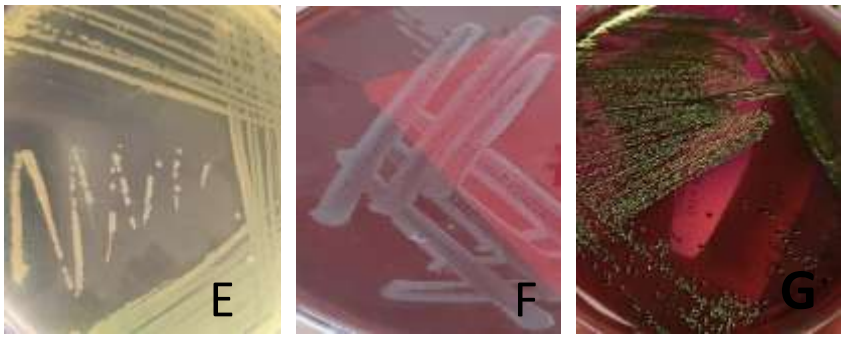


Figure 3. Bacterial characterization using culture methods. Suspected *Staphylococcus* isolated on blood agar showing golden yellow colonies with γ hemolysis (A). Suspected *E. coli* isolated on MacConkey agar showing pink colonies (B). Suspected *Salmonella* isolated on MacConkey showing pale colonies (C). Suspected *Klebsiella* isolated on MacConkey showing pink mucoid colonies (D). Suspected *pseudomonas* isolated on nutrient agar showing green colonies (E). Suspected *enterococcus* isolated on blood agar showing gray colonies with γ-hemolysis (F). Suspected *E. coli* isolated on EMB showing green metallic sheen colonies (G).

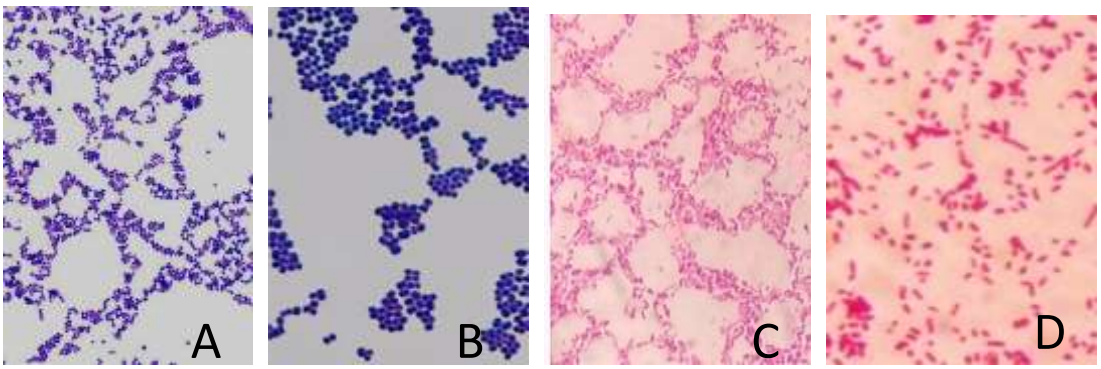


Figure 4. Bacterial characterization using Gram staining. Suspected *Enterococcus* showed Gram-positive cocci arranged in chains (A). Suspected *Staphylococcus* showed Gram-positive cocci arranged in grapes (B). Suspected *Salmonella* showed Gram-negative bacilli (C). Suspected *E. coli* showed Gram-negative bacilli (D).

The highest percentage of *E. coli*, *Salmonella spp.*, and *Staphylococcus spp.* isolation was from the liver, while the highest percentage of *Pseudomonas spp.* and *Klebsiella spp.* isolation was from the lung and the highest percentage of *enterococcus spp.* isolation was from the heart. The Prevalence of bacterial agents isolated from different organs is illustrated in Table 2.

Table 2. The Prevalence of bacterial agents isolated from different organs of the examined turkey poults

Bacterial species	Organs (42 each)					
	Liver		Heart		Lung	
	No.	%	No.	%	No.	%
E. coli	18	42.9	15	35.7	13	31
Salmonella	11	26	5	12	8	19
Klebsiella	4	9.5	3	7.1	6	14
Enterococcus	2	4.8	4	9.5	1	2.4
Pseudomonas	3	7.1	2	4.8	5	12
staphylococcus	4	9.5	3	7.1	1	2.4
Negative results for bacterial growth	0	0	10	23.8	8	19

Without Significant association between isolated bacteria and organ isolate with p = 0.5765, Chi-square, df = 6.635, 8 based on Chi-square test

In this study, out of 10 flocks, *E. coli* was identified in 6/10 flocks (60%), *Salmonella* spp. in 4 flocks (40%), *Klebsiella* spp. in 2 flocks (20%), *Pseudomonas* spp. in 2 flocks (20%), *Staphylococcus* spp. in 1 flock (10%), and *Enterococcus* spp. in 1 flock (10%). Mixed infections were recorded in six flocks (60%). Mixed infections of *E. coli* with each of *Salmonella*, *Klebsiella*, and *Pseudomonas* were recorded in 1 flock, and mixed infections of *Salmonella* with each of *Klebsiella*, *Pseudomonas*, and *Enterococcus* were recorded in 1 flock. Percentages of bacterial agents isolated from different organs and its overall prevalence among turkey flocks are illustrated in Figure 5. Culture and phenotypic bacterial identification are illustrated in Table 3.

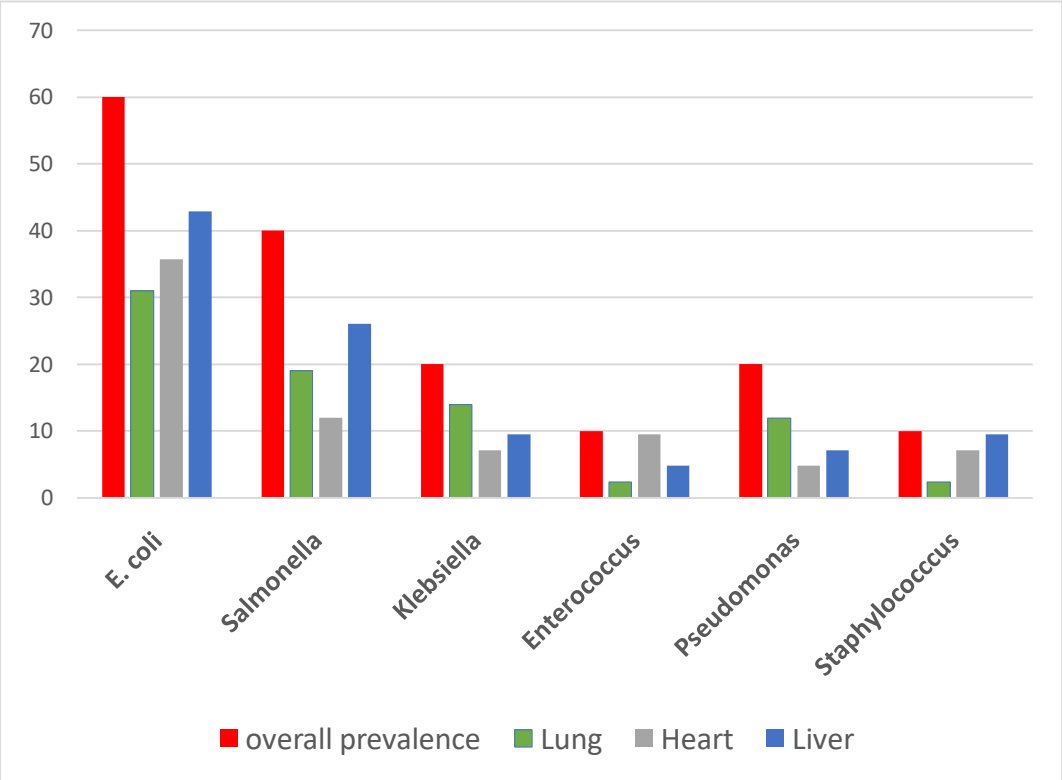


Figure 5: Percentages of bacterial agents isolated from different organs and its overall prevalence among turkey flocks

Table 3. Culture and phenotypic bacterial identification isolated from turkey poults.

Flock No.	Isolate reference No.	Growth on different media			Biochemical test							Gram stain	Suspected bacteria
		Nutrient agar	MacConkey (color of colonies)	Blood agar (hemolysis)	Indole	Methyl Red	Voges Proskauer	Citrate	Catalase	Coagulase	Oxidase		
1	1	White colonies	Pink	γ	+	+	-	-	+	-	-	GNB	*E. coli*
2	2	White colonies	Pale	γ	-	+	-	+	+	-	-	GNB	*Salmonella*
	3	White colonies	Pink	γ	+	+	-	-	+	-	-	GNB	*E. coli*
3	4	White colonies	----	γ	-	-	+	-	-	-	-	GPC/ chain	*Enterococcus*
	5	White colonies	Pale	γ	-	+	-	+	+	-	-	GNB	*Salmonella*
4	6	White colonies	Pink	γ	+	+	-	-	+	-	-	GNB	*E. coli*
5	7	Mucoid colonies	Pink	γ	-	-	+	+	+	-	-	GNB	*Klebsiella*
	8	White colonies	Pale	γ	-	+	-	+	+	-	-	GNB	*Salmonella*
6	9	Yellow colonies	-----	γ	-	+	+	+	+	+	-	GPC/grapes	*Staphyloc*

													occus
7	10	White colonies	Pink	γ	+	+	-	-	+	-	-	GNB	*E. coli*
	11	mucoid colonies	Pink	γ	-	-	+	+	+	-	-	GNB	*Klebsiella*
8	12	Green colonies	Pale	B	-	-	-	+	+	-	+	GNB	*Pseudomonas*
	13	White colonies	Pale	γ	-	+	-	+	+	-	-	GNB	*Salmonella*
9	14	White colonies	Pink	γ	+	+	-	-	+	-	-	GNB	*E. coli*
10	15	White colonies	Pink	γ	+	+	-	-	+	-	-	GNB	*E. coli*
	16	Green colonies	Pale	B	-	-	-	+	+	-	+	GNB	*Pseudomonas*

GPC, gram positive cocci; GNB; gram negative bacilli

In our study, we identified sixteen isolates: 6/16 *E. coli* isolates (37.5%), 4/16 *Salmonella* isolates (25%), 2/16 *Pseudomonas* isolates (12.5%), 1/16 *Enterococcus* isolates (6.25%), 2/16 *Klebsiella* isolates (12.5%), and 1/16 *Staphylococcus* isolates (6.25%). The percentage of identified isolates infecting the diseased turkey flocks is illustrated in figure 6.

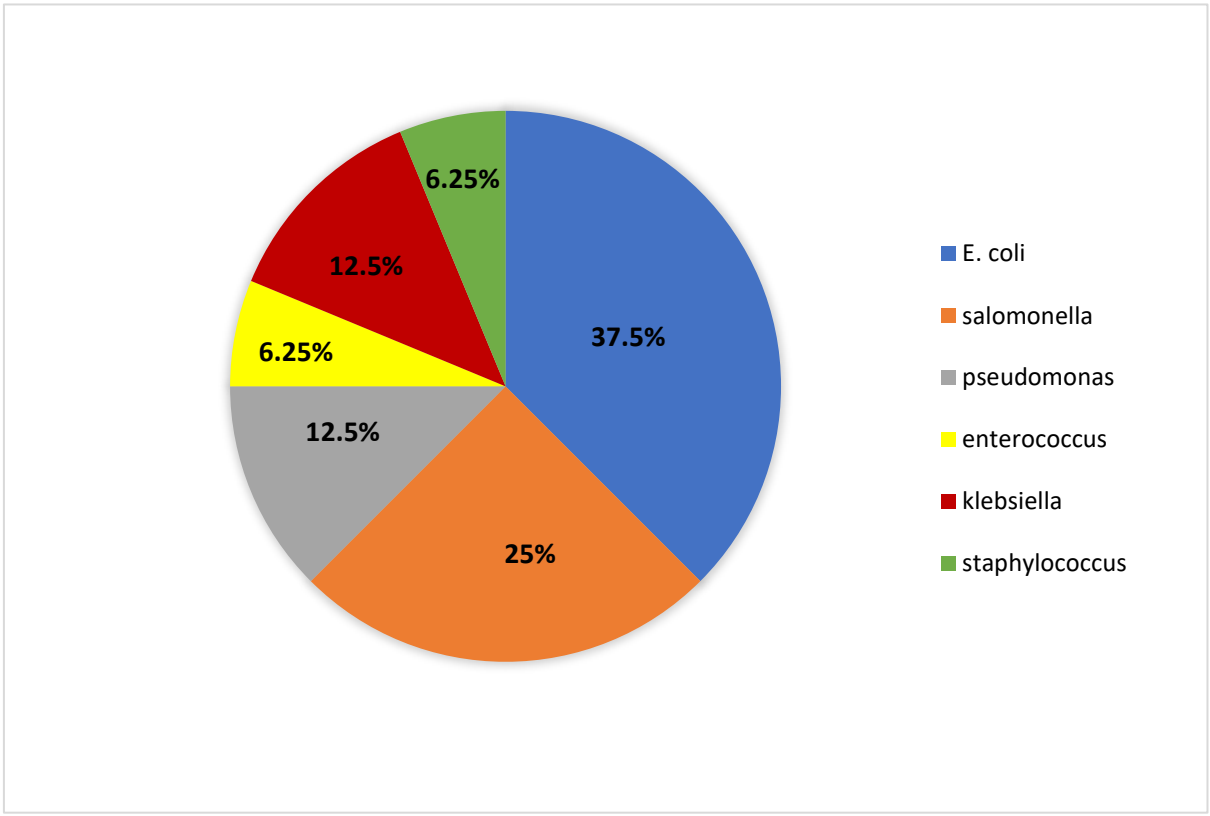


Figure 6**:** Percentage of identified bacterial isolates infecting the diseased turkeys per total number of the isolates

Antibiotic sensitivity test

Based on antibiogram, the isolates were categorized into 3 groups: sensitive, intermediate, and resistant. Most of the tested isolates were sensitive to difloxacin, amikacin, and apramycin. While most of the tested isolates were resistant to penicillin, erythromycin, and Trimethoprim-sulfamethoxazole. Overall, 100% of the tested isolates were MDR due to resistance to 3 or more antimicrobials of different groups. The results of the antibiotic sensitivity test are illustrated in Table 4.

Table 4. The results of the antibiotic sensitivity test versus bacterial isolates of turkey poults

Isolates reference no.	Tested microorganism	Antibiotic sensitivity pattern (Diameter of inhibition zone)										Number of antibiotics to which the isolate is resistant
		Florfenicol	Erythromycin	Colistin sulfate	Difloxacin	Doxycycline	Fosfomycin	Apramycin	Trimethoprim-sulfamethoxazole	Amikacin	Penicillin	
1	*E. coli*	R (0)	R (0)	R (5)	S (25)	R (5)	I (15)	S (25)	R (4)	S (22)	R (0)	6
2	*Salmonella*	S (20)	R (0)	S (18)	S (24)	I (12)	S (17)	S (19)	R (9)	S (23)	R (0)	3
3	*E. coli*	R (4)	R (0)	I (9)	S (28)	R (0)	I (13)	S (20)	R (0)	S (20)	R (0)	5
4	*Enterococcus*	R (0)	R (8)	R (0)	S (23)	R (7)	S (26)	S (22)	R (0)	S (21)	S (18)	5
5	*Salmonella*	S (25)	R (0)	I (10)	S (22)	R (8)	S (20)	S (20)	I (13)	S (25)	R (0)	3
6	*E. coli*	R (0)	R (0)	R (0)	S (26)	R (0)	R (10)	S (22)	I (11)	S (19)	R (0)	6
7	*Klebsiella*	S (20)	R (0)	S (15)	S (28)	I (11)	R (5)	S (18)	R (4)	S (19)	R (0)	4
8	*Salmonella*	S (22)	R (0)	S (17)	S (25)	I (12)	S (18)	S (21)	R (7)	S (23)	R (0)	3
9	*Staphylococcus*	R (0)	R (7)	R (0)	S (28)	R (4)	I (25)	S (24)	R (6)	S (25)	R (5)	6
10	*E. coli*	R (0)	R (0)	R (6)	S (27)	R (0)	I (15)	S (24)	R (0)	S (25)	R (0)	6
11	*Klebsiella*	S (22)	R (0)	S (16)	S (26)	I (11)	R (0)	S (20)	R (5)	S (20)	R (0)	4
12	*Pseudomonas*	R (0)	R (0)	R (5)	S (27)	R (0)	R (0)	I (14)	R (0)	S (18)	R (0)	7
13	*Salmonella*	S (23)	R (0)	S (19)	S (23)	I (11)	S (18)	S (20)	R (5)	S (22)	R (0)	3
14	*E. coli*	R (0)	R (0)	I (9)	S (30)	R (5)	I (13)	S (22)	R (4)	S (23)	R (0)	5
15	*E. coli*	R (5)	R (0)	R (0)	S (25)	R (0)	I (15)	S (26)	R (5)	S (27)	R (0)	6
16	*Pseudomonas*	R (0)	R (0)	R (0)	S (25)	R (0)	R (0)	S (20)	R (0)	S (20)	R (0)	7

S: sensitive, I: intermediate, R: resistant

Significant association between isolated bacteria and antimicrobial disc used with $p < 0.0001$, Chi-square, df = 449.5, 81 based on Chi-square test

Discussion

Turkey industry in Egypt is frequently affected by a wide range of bacterial agents. Routine isolation, identification, and monitoring of field avian pathogens are among the most important tools for disease prevention and control. So, this study was performed for identification of

bacterial agents that may be involved in turkey mortalities in Sharkia Governorate during 2021-2024. The clinical symptoms of morbid turkeys in our study include respiratory signs recorded in 5 flocks (50%); and that agrees with Giovanardi *et al.* [6], who recorded the respiratory signs in the form of rhinitis, sinusitis, and conjunctivitis in 4-week turkey flocks infected with APEC in Italy, and agrees with Al-baqir *et al*. [23], who recorded the respiratory signs in the form of nasal and ocular discharge, gasping, and head swelling in turkey flocks infected with *Mycoplasma gallisepticum* in Egypt during 2019-2022.

Whitish diarrhea was recorded in 6 flocks (60%), which was consistent with Moura-Alvarez *et al.* [24], who recorded whitish diarrhea in 22 turkey flocks ranging in age from 10 to 104 days infected with Turkey coronavirus (TCoV) and *Salmonella* spp. in Brazil. Greenish diarrhea recorded in the current study in 4 flocks (40%) and that could be attributed to Mor *et al.* [25], who recorded greenish diarrhea in 8-16-week-old turkeys infected with reovirus, adenovirus, and *E. coli* in Minnesota. Arthritis was recorded in 3 flocks (30%), and that could be attributed to Landman and Feberwee [26], who recorded arthritis in 14-19-week-old turkeys infected with *Mycoplasma synoviae* in the Netherlands.

On necropsy, some of the examined turkey flocks showed septicemic lesions in the form of congested subcutaneous tissues (S/C), tracheitis, and congested lung, which were recorded in 4 flocks (40%), fibrinous pericarditis, fibrinous air sacculitis, and fibrinous perihepatitis, which were recorded in 5 flocks (50%), and that could be attributed to Saumya *et al.* [27], who recorded septicemia, fibrinous pericarditis, and air sacculitis in 3-week-old turkeys infected with *Streptococcus gallolyticus* in Pennsylvania. Necrotic foci, variable in size and distribution, were recorded in the liver in 2 flocks (20%), which agrees with Hauck *et al.* [28], who recorded hepatic necrosis with multifocal coalescing foci in 2-15-week-old turkeys infected with histomoniasis in California. Enteritis was recorded in 9 flocks (90%), which agrees with Lojkić *et al.* [29], who recorded enteritis in turkeys ranging in the age from 10 days to 6 weeks infected with turkey coronavirus and astrovirus-2 in Croatia. Renal nephrosis was recorded in 5 flocks (50%), which agrees with Shehata and Hafez [30], who recorded renal swelling in turkey breeder flocks infected with avian influenza ranging in age from 67 to 79 weeks old with a mortality rate from 3.3 to 4.5% in Carolina.

E. coli was the most common identified pathogen in this study, which was identified from 6 flocks (60%). *E. coli* was isolated from the liver (42.9%), heart (35.7%), and lung (31%), with the highest percentage of *E. coli* isolation from the liver. The previous result concurs with Hussein *et al.* [14], who isolated *E. coli* from 14 turkey flocks in Egypt with a prevalence of 100%. *Salmonella spp.* was identified in 4 flocks (40%). *Salmonella spp.* was isolated from the liver (26%), heart (12%), and lung (19%), with a higher percentage of isolated bacteria from the liver, which indicates the importance of liver samples for isolation of *E. coli* and *salmonella* from infected turkeys. The previous results agree with Iseri and Erol [31], who isolated *salmonella spp*. from turkey flocks in Ankara.

Pseudomonas spp. was identified in 2 flocks (20%). *Pseudomonas spp.* was isolated from the liver (9.5%), heart (7.1%), and lung (14%), with a higher percentage of isolated bacteria from the lung. The previous results agree with Marouf *et al.* [11]), who isolated *Pseudomonas* from turkey flocks that suffered from high mortalities in the first 3 weeks of rearing in Egypt. *Klebsiella*

spp. was identified in 2 flocks (20%). *Klebsiella spp.* was isolated from the liver (7.1%), heart (4.8%), and lung (12%), with a higher percentage of isolated bacteria from the lung. The previous results agree with Eid and Samir [12], who isolated *Klebsiella* from turkey flocks that suffered from respiratory manifestations, with a history of treatment failure in Egypt.

Staphylococcus spp. was identified in one flock (10%). *Staphylococcus spp.* was isolated from the liver (9.5%), heart (7.1%), and lung (2.4%), with a higher percentage of isolated bacteria from the liver. The previous results agree with Moawad *et al.* [32], who isolated *staphylococcus* from 12 turkey flocks in Egypt ranging in age from 6 to 365 days.

Enterococcus spp. was identified in one flock (10%). *Enterococcus spp*. was isolated from the liver (4.8%), heart (9.5%), and lung (2.4%), with a higher percentage of isolated bacteria from the heart. The previous results agree with Alzahrani *et al.* [10], who isolated enterococcus spp. from nine turkey flocks in Poland in 2015.

Several bacterial agents have been implicated in turkey mortality in Sharkia province. *E. coli* is the most prevalent one, followed by *Salmonella, Pseudomonas, Klebsiella, Staphylococcus,* and *Enterococcus*, respectively. The liver is the best organ for isolation of *E. coli, Salmonella,* and *Staphylococcus*, while the lung is the best organ for isolation of *Pseudomonas* and *Klebsiella*, and the heart is the best organ for isolation of *Enterococcus*. Mixed infection with two bacterial agents plays a role in higher mortality in turkey flocks in comparison with other flocks infected by single bacterial agent. Flock No. 10. infected with *E. coli* and Pseudomonas showed a higher mortality rate (7%) than flock No. 9 infected with *E. coli* alone, which showed a lower mortality rate (3%). While flock no. 5, infected with Klebsiella and Salmonella, showed a higher mortality rate (8%) than flock no. 1, infected with *E. coli* alone, which showed a lower mortality rate (4%). These different bacterial agents have a negative impact on the Egyptian turkey industry due to mortality, downgraded carcass, increased condemnation rate at the abattoir, retarded growth, decreased egg production, and high cost of medication [1].

Based on antibiogram, antibiotic of choice must be selected to control these bacteria. In this study, *E. coli* isolates were sensitive to difloxacin, apramycin, and amikacin, while resistant to penicillin, doxycycline, florfenicol, colistin sulfate, fosfomycin, trimethoprim-sulfamethoxazole, and erythromycin, which agrees with Gosling *et al.* [33], who stated that *E. coli* isolated from turkeys were sensitive to apramycin and amikacin, while resistant to ampicillin, tetracycline, and sulfonamides. In general, antimicrobial resistance among pathogenic *E. coli* strains of avian origin is evolving. The high degree of resistance to *E. coli* identified in this study might be attributed to the widespread and unregulated use of antibiotics in turkey farms. Such overuse may lead to the creation of a pool of antibiotic-resistance genes, resulting in the selection of greater numbers of resistant *E. coli* colonies, which concurs with Samy *et al.* [34], who reported that most of the *E. coli* isolates from poultry in Egypt expressed multidrug resistance due to acquiring resistance genes such as blaTEM and tetA.

Salmonella spp. was sensitive to difloxacin, fosfomycin, florfenicol, amikacin, and apramycin, while resistant to penicillin, erythromycin, trimethoprim-sulfamethoxazole, and doxycycline, which is in agreement with Jahantigh *et al.* [35], who reported that *salmonella* isolates from turkeys were resistant to tetracycline (86.5%) and sulfonamides

(67.6%) while sensitive to ciprofloxacin (83.8%), streptomycin (40.6%), and chloramphenicol (51.4%).

Pseudomonas isolates were sensitive to difloxacin and amikacin, moderately sensitive to apramycin, while resistant to penicillin, florfenicol, colistin sulfate, trimethoprim-sulfamethoxazole, fosfomycin, doxycycline and erythromycin, which agrees with Shirazi *et al.* [36], who reported that *pseudomonas* isolates from turkeys were resistant to ampicillin, doxycycline, florfenicol, erythromycin, and sulfonamide + trimethoprim but sensitive to amikacin, difloxacin, and lincospectin.

Klebsiella isolates were sensitive to difloxacin, apramycin, colistin sulphate, florfenicol, and amikacin; moderately sensitive to doxycycline; and resistant to erythromycin, penicillin, sulphathiazole–trimethoprim, and fosfomycin, which agreed with Kowalczyk *et al.* [37], who reported that *Klebsiella* isolates from turkeys were resistant to amoxicillin (100%) but sensitive to colistin (92.9%), neomycin (90.14%), and florfenicol (88.56%).

Staphylococcus isolates were sensitive to apramycin, difloxacin, and amikacin; moderately sensitive to fosfomycin; and resistant to florfenicol, erythromycin, penicillin, colistin sulfate, doxycycline, and trimethoprim-sulfamethoxazole, which is in accordance with Argudín *et al.* [38], who reported that *staphylococcus* isolates from turkeys were resistant to penicillin (100%), tetracycline (100%), and streptomycin (6.5%) but sensitive to apramycin.

Enterococcus isolates were sensitive to apramycin, penicillin, fosfomycin, difloxacin, and amikacin, while resistant to florfenicol, erythromycin, colistin sulfate, doxycycline, and trimethoprim-sulfamethoxazole, which is in accordance with Woźniak-Biel *et al.* [39], who reported that *E. faecalis* isolates from turkeys were resistant to erythromycin (70.73%) and tetracycline (92.68%) but sensitive to ampicillin (78.05%), amoxycillin (78.05%), and gentamycin (100%).

All the tested isolates were multidrug resistant (MDR) (100%) due to resistance to 3 or more antimicrobials. Pathogenic bacteria can adapt and evolve to resist even modern antibiotics due to the unawareness and carelessness of the use of antibiotics. Resistance to these antibiotics is problematic since it may limit treatment options, result in extended illness, and raise the risk of morbidity and death [22]. Both *E. coli* and *Pseudomonas spp.* were resistant to the colistin, which threatens public health due to colistin importance in the treatment of Gram-negative infections in humans. So, significant prevalence of antibiotic resistance among bacterial isolates incriminated in turkey mortality requires epidemiological surveillance of these isolates' susceptibility for the optimum selection of the best antibiotics and prevention of the spread of resistant bacteria.

Conclusion

Several bacterial agents have been implicated in turkey mortality in Sharkia Governorate. *E. coli* is the most predominant one, followed by *Salmonella, Pseudomonas, Klebsiella, Staphylococcus*, and *Enterococcus*. The liver is the most suitable organ for isolation of *E. coli, Salmonella,* and *Staphylococcus*. Difloxacin, amikacin, and apramycin are effective against all bacterial isolates in our study. All the tested isolates were multidrug resistant to three or more antimicrobials.

Therefore, monitoring the use of antibiotics is necessary to control their resistance, and surveying more flocks is also essential to identify more different pathogens and study other infectious and managemental problems. Moreover,

prospective studies must be applied for detection and sequencing resistant genes to create a comprehensive resistance map.

Conflict of interest

None of the authors have no, conflict of interest.

Acknowledgement

The authors are thankful to Dr. Tamer Mahmoud Abdul Latif, Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, for his help in the statistical analysis.

Refrences

[1] Hafez, H. and Shehata, A. (2021): Turkey production and health: Current challenges. Ger J Vet Res, 1(1): 3-14 .

[2] Hafez, H.M. and El-Adawy, H. (2019): Some current factors and problems that influence turkey production and health. EC Vet Sci, 4: 140-7.

[3] Hafez, H.M. and Shehata, A.A. (2024): Turkey Diseases and Disorders Volume 1: Bacterial and Fungal Infectious Diseases (Vol. 1). Springer Nature.

[4] Hafez, H.M. and Hauck, R. (2005): Genetic selection in Turkeys and broilers and their impact on health conditions .

[5] Albazaz, R. and Bal, E.B. (2014): Microflora of digestive tract in poultry. KSÜ Doğa Bil. Derg, 17(1): 39-42 .

[6] Giovanardi, D.; Lupini, C.; Pesente, P.; Rossi, G.; Ortali, G. and Catelli, E. (2013): Characterization and antimicrobial resistance analysis of avian pathogenic Escherichia coli isolated from Italian turkey flocks. Poult Sci, 92(10): 2661-7 .

[7] Nolan, L.K.; Barnes, H.J.; Vaillancourt, J.P.; Abdul-Aziz, T. and Logue, C.M. (2013): Colibacillosis. Diseases of poultry: 751-805.

[8] Nayak, R.; Kenney, P.; Keswani, J. and Ritz, C. (2003): Isolation and characterisation of Salmonella in a turkey production facility. Br Poult Sci, 44(2): 192-202 .

[9] Alfonso, M. and Barnes, H.J. (2006): Neonatal osteomyelitis associated with Staphylococcus aureus in turkey poults. Avian Dis, 50(1): 148-51 .

[10] Alzahrani, O.M.; Fayez, M.; Alswat, A.S.; Alkafafy, M.; Mahmoud, S.F.; Al-Marri, T.; Almuslem, A.; Ashfaq, H. and Yusuf, S. (2022): Antimicrobial resistance, biofilm formation, and virulence genes in Enterococcus species from small backyard chicken flocks. J Antibiot, 11(3): 380 .

[11] Marouf, S.; Li, X.; Salem, H.M.; Ahmed, Z.S.; Nader, S.M.; Shaalan, M.; Awad, F.H.; Zhou, H. and Cheang, T. (2023). Molecular detection of multidrug-resistant Pseudomonas aeruginosa of different avian sources with pathogenicity testing and in vitro evaluation of antibacterial efficacy of silver nanoparticles against multidrug-resistant P. aeruginosa. Poult Sci, 102(10): 102995.

[12] Eid, S. and Samir, A.H. (2019): Extended-spectrum beta-lactamase and Class 1 integrons in multidrug-resistant Escherichia coli isolated from turkeys. Vet World, 12(7): 1167 .

[13] Abozaid, K.G. and Abdel-Moneim, A.S. (2022). Epidemiological surveillance of Newcastle disease virus in Egypt—A 6-year cohort study. Trop. Anim. Health Prod, 54(4): 243.

[14] Hussein, E.G.S.; Naem, N.A.E.S.; Mosaad, Z.; Elsebaey, H. Sh.; Elhaddad, G. Y. and Mahana, O. (2024). Genetic analysis on some microbial causes of mortalities in turkeys in Behira, Egypt, EJAH, 4(2): 39-58.

[15] Patra, J.K.; Das, G.; Das, S.K.; Thatoi, H.; Patra, J.K.; Das, G.; Das, S.K. and Thatoi, H. (2020). Isolation, culture, and biochemical characterization of microbes. A Practical Guide to Environmental Biotechnology: 83-133.

[16] Packer, R. and Merchant I. (1967). Veterinary bacteriology and virology. Lowa State University Press.

[17] Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z. A., and Yaseen, I. (2020). A mini-review on commonly used biochemical tests for identification of bacteria. IJRP, 54(1): 1-7

[18] Cruickshank, R. (1975). Medical Microbiology: A Guide to Diagnosis and Control of Infection. Edinburgh and London, UK: E and S Livingston Ltd, 888

[19] Lalitha, M. (2004). Manual on antimicrobial susceptibility testing. Performance standards for antimicrobial testing: Twelfth Informational Supplement, 56238: 454-456

[20] Clinical and Laboratory Standards Institute. (2016): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-10th ed. M07-A11. Clinical and Laboratory Standards Institute, Wayne, PA.

[21] Basak, S.; Singh, P. and Rajurkar, M. (2016). Multidrug resistant and extensively drug resistant bacteria: a study. J Pathog, 2016(1): 4065603.

[22] Frieri, M.; Kumar, K. and Boutin, A. (2017). Antibiotic resistance. Journal of infection and public health, 10(4): 369-378.

[23] Al-Baqir, A.; Hassanin, O.; Al-Rasheed, M.; Ahmed, M.S.; Mohamed, M. H.; El Sayed, M.S.; Megahed, M.; El-Demerdash, A.; Hashem, Y. and Eid, A. (2023). Mycoplasmosis in poultry: An evaluation of diagnostic schemes and molecular analysis of Egyptian Mycoplasma gallisepticum Strains. Pathog, 12(9), 1131.

[24] Moura-Alvarez, J.; Nuñez, L.F.; Astolfi-Ferreira, C.S.; Knöbl, T.; Chacón, J.L.; Moreno, A.M.; Jones, R.C. and Ferreira, A.J.P. (2014). Detection of enteric pathogens in Turkey flocks affected with severe enteritis, in Brazil. Trop Anim Health Prod, 46, 1051-1058 .

[25] Mor, S.K.; Kumar, R.; Sobhy, N.M.; Singh, A.; Kakrudi, N.; Marusak, R.A.; Goyal, S.M. and Porter, R.E. (2020). Enteric viruses associated with mid-growth turkey enteritis. Avian Dis, 64(4): 471-477.

[26] Landman, W. and Feberwee, A. (2012). Longitudinal field study on the occurrence of Mycoplasma synoviae in Dutch turkey flocks with lameness and experimental induction of the condition. Avian Pathol, 41(2): 141-149 .

[27] Saumya, D.; Wijetunge ,S.; Dunn, P.; Wallner-Pendleton, E.; Lintner, V.; Matthews, T.; Pierre, T. and Kariyawasam, S. (2014). Acute septicemia caused by Streptococcus gallolyticus subsp. pasteurianus in turkey poults. Avian Dis, 58(2): 318-322 .

[28] Hauck, R.; Stoute, S.; Chin, R.; Sentíes-Cué, C. and Shivaprasad, H. (2018). Retrospective study of histomoniasis (blackhead) in California turkey flocks, 2000–2014. Avian Dis, 62(1): 94-100 .

[29] Lojkić, I.; Biđin, M.; Biđin, Z. and Mikec, M. (2010). Viral agents associated with poult enteritis in Croatian commercial turkey flocks. Acta Veterinaria Brno, 79(1): 91-98 .

[30] Shehata, A.A. and Hafez, H. M. (2024). Diagnosis of Turkey Diseases. In Turkey Diseases and Disorders Volume 2: Infectious and Nutritional Diseases, Diagnostics and Control Strategies (pp. 259-279). Springer .

[31] Iseri, O. and Erol, I. (2010). Incidence and antibiotic resistance of Salmonella spp. in ground turkey meat. Br Poult Sci, 51(1): 60-66 .

[32] Moawad, A.A.; Hotzel, H.; Awad, O.; Roesler, U.; Hafez, H.M.; Tomaso, H.; Neubauer, H., and El-Adawy, H. (2019). Evolution of antibiotic resistance of coagulase-negative staphylococci

isolated from healthy turkeys in Egypt: First report of linezolid resistance. Microorganisms, 7(10), 476 .

[33] Gosling, R.; Clouting, C.; Randall, L.; Horton, R. and Davies, R . (2012). Ciprofloxacin resistance in E. coli isolated from turkeys in Great Britain. Avian Pathol, 41(1), 83-89 .

[34] Samy, A.; Mansour, A.S.; Khalaf, D.D. and Khairy, E.A. (2022). Development of multidrug-resistant Escherichia coli in some Egyptian veterinary farms. Vet World, 15(2): 488 .

[35] Jahantigh, M.; Jafari, S.M.; Rashki, A. and Salari, S. (2015). Prevalence and Antibiotic Resistance of Salmonella spp. in Turkey. Open J Med Microbiol, 5(03), 113 .

[36] Shirazi, M.H.N.; Majd, N.S. and Khodayari, M. (2023). Molecular and biochemical characterization of Pseudomonas aeruginosa isolated from a Turkey flock. JPSAD, 1(2): 42-46 .

[37] Kowalczyk, J.; Czokajło, I.; Gańko, M.; Śmiałek, M. and Koncicki, A. (2022). Identification and Antimicrobial Resistance in Klebsiella spp. Isolates from Turkeys in Poland between 2019 and 2022. Anim, 12(22): 3157.

[38] Argudín, M.A.; Cariou, N.; Salandre, O.; Le Guennec, J.; Nemeghaire, S. and Butaye, P. (2013). Genotyping and antimicrobial resistance of Staphylococcus aureus isolates from diseased turkeys. Avian Pathol, 42(6): 572-580 .

[39] Woźniak-Biel, A.; Bugla-Płoskońska, G.; Burdzy, J.; Korzekwa, K.; Ploch, S. and Wieliczko, A. (2019): Antimicrobial resistance and biofilm formation in Enterococcus spp. isolated from humans and turkeys in Poland. MDR, 25(2): 277-86 .

الملخص العربي

التعرف على المسببات البكتيرية المسببة للنفوق في قطعان الرومى فى محافظه الشرقيه خلال الفتره 2021-2024

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يهدف هذا العمل إلى عزل البكتريا المسببة للنفوق في قطعان الرومي والتعرف عليها من خلال الاختبارات المعملية مع عمل إختبار الحساسية لبعض المعزولات. في هذه الدراسه تم تجميع عدد 126 عينة من 42 طائر من 10 قطعان مختلفة من الرومي. تم العزل على 4 ميديات مختلفة (أجارالماكونكي وأجار الدم وأجار المغذيات وأجار الميثيلين الأزرق الأيوزيني) وتشكيل النتائج المبدئية من شكل وحجم ولون المستعمرات وتم إجراء صبغة الجرام والإختبارات الكيميائية الأخرى. وكانت النتائج 6 معزولات من الميكروب القولونى و4 معزولات من السالمونيلا ومعزولتان من كلا من السيدوموناس والكلبسيلا ومزولة واحدة من كلا من الميكروب العنقودي المكور والمكورات المعوية. وجاءت نسبه الإصابة بالميكروبات المعزولة كالآتي: الميكروب القولوني 16/6 (37.5%) والسالمونيلا 16/4 (25%) والسيدوموناس 16/2 (12.5%) والكلبسيلا 16/2 (12.5%) والميكرروب العنقودي المكور 16/1 (6.25%) والمكورات المعوية 1 /16 (6.25%). تم إجراء اختبار حساسيه لعدد 10 معزولات ووجد أن أغلب المعزولات كانت حساسة الأبراميسين والاميكاسين والدايفلوكساسين كما وجد أن كل المعزولات كانت مقاومة لثلاثه أو أكتر من المضادات الحيويه من المجموعات المختلفة. يمكن الاستنتاج أن المسببات البكتيرية للنفوق فى الديوك الرومية في محافظة الشرقية يرجع بشكل رئيسي إلى الإشريكية القولونية والسالمونيلا والسيدوموناس والكلبسيلا والمكورات العنقودية والمكورات المعوية. الكبد هو العضو الأنسب للعزل. الديفلوكساسين والأميكاسين والأبراميسين فعالة ضد هذه العزلات البكتيرية. مراقبة إستخدام المضادات الحيوية أمر بالغ الأهمية للسيطرة.