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Disinfectant and Multidrug-resistant Gram-negative Bacteria in Chicks

Mahmoud Ezzat[1], Mohamed Rady[2], Wael M. Elfeil[3], Mohamed AbduFadel[1], Reham M. El-Tarabili[1*]

[1]Department of Bacteriology, Immunology, and Mycology, Faculty of Veterinary Medicine, Suez Canal University, 41522, Ismailia, Egypt.

[2]National Laboratory for Veterinary Quality Control on Poultry Production, Agriculture Research center, Cairo, Egypt.

[3]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, 41522, Ismailia, Egypt.

***Correspondence**
Corresponding author: Reham M. El-Tarabili
E-mail address: Riham_tarabily@vet.suez.edu.eg

Abstract

In recent years there has been a dramatic development for multidrug-resistant and disinfectant-resistant bacteria in poultry farms, to investigate the current prevalence and losses associated with these phenomena, Samples from chicks were taken from 3 poultry stations A, B, and C beside broiler hatcheries (newly hatched chicks). Newly hatched chicks were monitored for Clinical signs, postmortem examination, and performance and mortality rate for one week old. All newly hatched chicks showed the appearance of gasped chicks with low vitality and the rates of gasping increased at the farm level with increased mortality from 8.8 %- 15.5% in the first week. Postmortem examination revealed the presence of nephritis with urate deposition in the ureter, air vasculitis with the appearance of a nodule, a large gall bladder, and a cecum with a greenish color. *E. coli* was recorded with a high percentage 57.4% followed by *S.* Typhimurium 8.2% and *P. aeruginosa* 4.1%. Clostin showed impressive results for treating isolated bacteria from chicks, while erythromycin, spiramycin, lincomycin, oxytetracycline, bacitracin, streptomycin, followed by ampicillin, doxycycline, and gentamycin showed high resistance among isolated bacteria. Alarmingly, 88.9% (8/9) of the *E. coli* strains were XDR to different classes. In contrast, 11.1% (1/9) of the isolated *E. coli* strains were multi-drug resistant. One isolate of *S.* Typhimurium was PDR and another isolate was MDR. All of the *P. aeruginosa* strains were XDR to different classes. The previous results showed the risk of infection comes from the improper disinfection of the hatchery and how it is associated with losses in poultry farms and there is a need for the prevalence of the disinfection genes in hatchery bacteria.

KEYWORDS
Pseudomonas, *E. coli*, *Salmonella*, Disinfectant resistant, XDR, PDR

INTRODUCTION

Good poultry performance depends on healthy chicks. Day-old chicks can be affected by many issues, some of which are challenging to identify. Management practices matter from the breeder's farm to the hatchery, transportation, and farm reception. Chicks can start well with optimized processes and preventive measures (Lima *et al*., 2019). Many hatchery infections and day-old chick-quality pathogens caused omphalitis, gasping, poor performance, high chick mortality, poor food conversion rate (FCR), and immunological suppression, resulting in poor vaccine response (Shahjada *et al*., 2017).

E. coli, *Salmonella*, *Proteus*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Staphylococcus*, *Streptococcus*, *Clostridium, Bacillus cereus*, and *Enterococcus* have been isolated from infected birds' yolk sacs. *Salmonella* infection causes huge financial losses in chickens due to treatment expenses, low growth, and high mortality (Algammal *et al*., 2023b). *P. aeruginosa* can infect many tissues opportunistically. Chickens acquire *P. aeruginosa* by skin wounds, egg dipping, and injection needles (Algammal *et al*., 2023a).

Omphalitis is a significant contributor to death rates among recently hatched chicks. The occurrence is attributed to unhygienic equipment within the hatchery facility (Rahman *et al*., 2007). According to Kahn *et al*. (2008), the afflicted chick exhibits signs of depression, such as a drooping head and a tendency to huddle near the heat source. Omphalitis, also known as umbilical inflammation, is a prevalent factor contributing to mortality in chicks within the initial week of their life. Both *E. coli* and Enterococcus faecalis have been recognized as the predominant bacterial infections linked to death within the first week (Olsen *et al*., 2012). The primary source of infection is widely recognized to be fecal contamination of eggs. Nevertheless, it is worth noting that bacteria can also migrate from the chick's gastrointestinal tract or bloodstream (Olsen *et al*., 2012).

Colibacillosis is an avian infectious disease that is attributed to the presence of *Escherichia coli*, a bacterium known to act as either a primary or secondary pathogen (Barnes *et al*., 2008). Colibacillosis is a prominent factor contributing to the mortality and morbidity rates observed in chickens and turkeys, leading to substantial financial repercussions within the poultry sector. This translates into considerable economic losses amounting to millions of dollars annually across various regions of the global poultry industry (Barnes *et al*., 2008).

Antimicrobials have been used for decades to treat poultry colibacillosis and other infections. *E. coli*, like many other bacteria, can develop antibiotic resistance under antibiotic pressure (OIE, 2016). When antibiotics are overused prophylactically or underdosed, resistance develops (Quinn *et al*., 2011). The abil-

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ity of bacteria to change their outer membrane composition, produce enzymes that degrade antimicrobials, or alter their metabolism creates antibiotic resistance. Bacteria can also transfer antibiotic-resistance genes to other bacteria that infect animals or humans, making certain bacteria resistant and threatening their health. Genetic mutations or horizontal transfer of plasmids between two *E. coli* cells can make it resistant (Algammal *et al*., 2020). Antibiotic resistance in *E. coli* strains is a growing area of study since it reflects the significant variation in antibiotic use around the world (Ventola, 2015). Several studies have reported *Salmonella* serovar MDR patterns to aminoglycosides, tetracyclines, and penicillins (Algammal *et al*., 2023b). *P. aeruginosa*'s antibiotic resistance is due to acquired and innate mechanisms, including inadequate outer membrane permeability, antibiotic resistance genes, and active efflux pumps (Algammal *et al*., 2023a).

This study aimed to check the possible microbiological causes of gasping, mortality, and their effect on broiler poultry performance, to study the source of infection, and to aid in the control of the source of infection. To know the main germs responsible for this problem; Application of antimicrobial sensitivity testing against isolates of bacteria to detect the multidrug-resistant strains and disinfectant resistance strains.

MATERIALS AND METHODS

Ethics Statement

All institutional and national bird care and use requirements were followed and authorized by the Faculty of Veterinary Medicine, Suez Canal University Institutional Animal Care and Use Committee (IACUC).

Samples collection and processing

A total of 195 chicks were selected clinically healthy from private farms (source B and source C) and hatcheries (source A, source B, and source C) and transferred alive and dead to the laboratory under aseptic conditions to be examined, as shown in Table 1.

Table 1. Numbers and areas of collected samples.

Hatchery and farm	location	No. of the examined sample
Hatchery	Source A	50 chicks
Hatchery	Source B	60 chicks
Hatchery	Source C	80 chicks
Farm	Source B	30 chicks
Farm	Source C	30 chicks

Isolation and identification of bacteria

The chicks were diagnosed postmortem. To test for bacteria, yolk sacs and visceral organs (liver, lung, and brain) were aseptically collected. Loopfuls from the yolk sac and viscera have been grown on selective and differential media to isolate bacteria. All media were incubated at 37°C for 24–48 hours. To enrich *Salmonella*, they were cultured in Rappaport Vassiliadis broth at 37°C for 24 hours before being inoculated on XLD (Xylose Lysine Deoxycholate agar). For *E. coli* inoculated on Eosin methylene blue (EMB), For *P. aeruginosa* inoculated on cetrimide media.All media and agar were made per the manufacturer's instructions (DIFCO, UK). Colonies were evaluated for cultural and morphological features on growth media after 24–48 hours of incubation at 37°C. Gram staining. Pure colonies underwent indole, Voges Proskauer, methyl red, citrate utilization, and glucose, lactose, arabinose, lactose, sorbitol, sucrose, and mannitol fermentation assays for species identification (Bergy *et al*., 1994).

Serological typing

The *Salmonella* isolates were serologically identified using diagnostic polyvalent and monovalent *Salmonella* "O" and "H" antisera (Sifin Diagnostics, Gmbh, Berlin, Germany) according to Kauffmann and Das Kauffmann (2001).

Antibiogram

Using a Kirby-Bauer disk diffusion assay, nine selective *E. coli* strains, six selective *Pseudomonas aeruginosa* strains, and two selective *Salmonella* strains were tested for antimicrobial susceptibility technique according to the standards and interpretive criteria described by CLSI (2020). Twelve antimicrobials were used: ampicillin (AMP,10ug), cefradine (CED,30 µg); streptomycin (S 30 ug), neomycin (NEO,30µg); gentamycin (GEN,10 ug); doxycycline (DO,30 µg), oxytetracycline (OTC,30 µg); lincomycin (LIN,10 µg); erythromycin(E,15 µg); spiramycin (SPM,100 µg); clostin (CT,10 µg); Bacitracin (BA,10 µg). According to Magiorakos *et al*. (2012), the isolated strains were classified into MDR and XDR. Furthermore, the MAR index was evaluated according to Krumperman (1983).

Disinfectant

Virocid was purchased from cid linesleper (Belgium - Europe), Verkon-S and TH4 were purchased from LANXESS Cologne Germany-Europe), Sinerges was purchased from Neogen (USA / Canada), and Shift was purchased from Evans Vanodine (Lancashire England).

Preparation of tested disinfectants

Tested disinfectants

TH^4+®: a combination of 4 quaternary ammonium, glutaraldehyde, and 2 terpene derivatives
Virocid® : a combination of quaternary ammonium, glutaraldehyde, and isopropanol
Shift®: a heavy-duty detergent liquid for power washing.
Sinerges®: Quaternary ammonium/ glutaraldehyde combination active chemistry
Virkon-S®: Contains potassium peroxymonosulfate, sodium dodecylbenzenesulfonate, sulfamic acid and inorganic buffers.

USP-filtered water at pH 5-7 from sterilized tap water was used to dilute tested disinfectants.

The antibacterial effectiveness test

Five commercial disinfectants were prepared following production procedures and supplier guidelines .The final dilution of the test solution uses USP pure water at pH 5.0–7.0. To account for dilution errors and variability during biocidal agent manufacture, disinfectants were diluted to 90% of the working concentration and a specific volume of settled microbial culture was used during the test. To assess the effectiveness of five commercial disinfectants against *E. coli*, *P. aeruginosa*, and *S.* Typhimurium at

a titer of 3×106/cm2, they were applied in vitro at 5% concentrations for 60 minutes on contaminated areas (Linton *et al.*, 1987).

RESULTS

Clinical findings of naturally infected chicks

Chicks were collected from different hatcheries and farms. Diseased chicks showed a decrease in FCR, loss of balance, gasping, and respiratory distress, in addition to the affected chicks increasing in mortality, the Appearance of gasped chicks with low vitality and the rates of gasping increased at the farm level with increased mortality from 3.4%- 15% at the first week. Post mortem exhibited air sacs turbidity with the appearance of nodules, Extended caecum with gases extended gall blader-two ceci filled with green material, and Nephritis with urate, as shown in Figures 1,2,3.

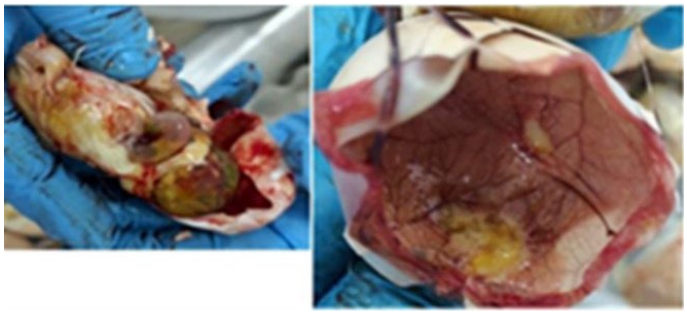

Fig. 1. Turbidity in air sac with nodules.

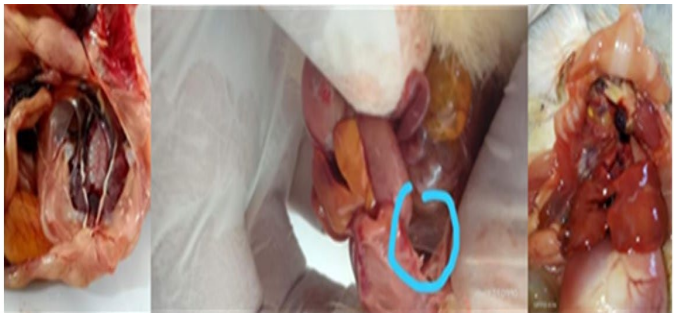

Fig. 2. Extended cecum with gases.

Bacterial isolation

The prevalence of *E. coli* among the examined chick viscera was 57.4% (112/195), *Salmonella* Typhimurium 8.2% (16/195), and *P. aeruginosa* 4.1% (8/195), as illustrated in Figure 4. Concerning the distribution of *E. coli* among different organs, the

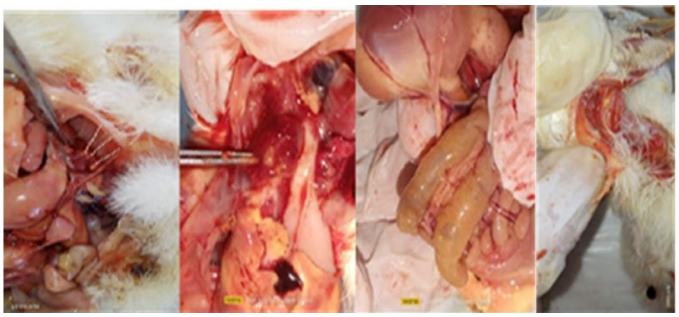

Fig. 3. Nephritis with urate.

highest prevalence was observed in the lung (34.8%, 39/112), yolk sac(30.4%,34/112), liver(26.8%,30/112), and brain (8.4%, 9/112). For *Salmonella* Typhimurium 16 strains were only detected in the yolk sac with a percentage 8.2%. For *P. aeruginosa*, the highest prevalence was observed in the yolk sac (62.5%, 5/8), lung (25%, 2/8), liver (12.5%,1/8), and not detected in the brain, as illustrated in Table 2. A significant difference in bacteria among different organs. A significant difference in prevalence among E .coli and *S.* Typhimurium except *P. aeruginosa*.

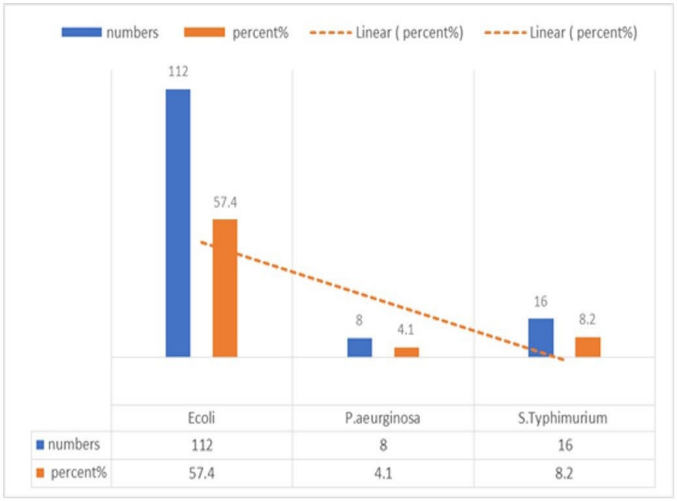

Fig. 4. Prevalence of isolated bacteria from chicks.

Antimicrobial sensitivity testing of isolated bacteria among chicks.

The antimicrobial resistance results of the retrieved *E. coli* isolates exhibited that the recovered strains were resistant against erythromycin (100%), sipramycin (100%), lincomycin (100%), oxytetracycline (100%), bactricin (100%), and streptomycin (100%), followed by ampicillin (88.9%), doxycycline (88.9%), and gentamycin (77.8%). Moreover, all recovered isolates were sensitive to clostin (100%). The antimicrobial resistance results of the retrieved *S.* Typhimurium isolates exhibited that the recovered strains were resistant against erythromycin (100%), spiramycin (100%), linco-

Table 2. Prevalence of isolated bacteria among different organs.

Bacteria	Liver		Yolk sac		Lung		Brain		Chi-square P value
	No.	%	No.	%	No.	%	No.	%	
E. coli	30	26.8	34	30.4	39	34.8	9	8.4	18.64 0.00
S. Typhimurium	0	0	16	8.2	0	0	0	0	48 P>0.001
P. aeruginosa	1	12.5	4	50	3	37.5	0	0	5 0.1718^{NS}
Chi-square	56.19		35.33		52.13		18		
P value	P>0.001		P>0.001		P>0.001		0.00		

mycin (100%), oxytetracycline (100%), bacitracin (100%), ampicillin (100%), doxycycline (100%). Moreover, all recovered isolates were sensitive to clostin (100%). The antimicrobial resistance results of the retrieved *P. aeruginosa* isolates exhibited that the recovered strains were resistant against erythromycin (100%), sipramycin (100%), lincomycin (100%), oxytetracycline (100%), bactricin (100%), and followed by ampicillin (100%), doxycycline (100%), then streptomycin (83.3%), and gentamycin (83.3%). Moreover, recovered isolates were sensitive to clostin (66.7%) ,as shown in Table 1.

Phenotypic patterns of resistance of the isolated strains

Alarmingly, 88.9% (8/9) of the *E. coli* strains were XDR to different classes. In contrast, 11.1% (1/9) of the isolated *E. coli* strains were multi-drug resistant. One isolate of *S.* Typhimurium was PDR

Table 3. Antibiotic susceptibility results of isolated bacteria.

Antimicrobial class	Antimicrobial agent	*E. coli*		*S.* Typhimurium		*P. aeruginosa*	
		R	S	R	S	R	S
Penicillins	Ampicillin	8(88.9)	1(11.1)	2(100%)	0(0%)	6(100%)	0(0%)
Cephalosporins	Cefradine	5(55.6%)	4(44.4%)	1(50%)	1(50%)	3(50%)	3(50%)
Aminoglycosides	Gentamycin	7(77.8%)	2(22.2%)	1(50%)	1(50%)	5(83.3%)	1(16.7%)
	Streptomycin	9(100%)	0(0%)	1(50%)	1(50%)	5(83.3%)	1(16.7%)
	Neomycin	3(33.3%)	6(66.7%)	1(50%)	1(50%)	4(66.7%)	2(33.3%)
Macrolides	Erythromycin	9(100%)	0(0%)	2(100%)	0(0%)	6(100%)	0(0%)
	Spiramycin	9(100%)	0(0%)	2(100%)	0(0%)	6(100%)	0(0%)
Tetracycline	Doxycycline	8(88.9)	1(11.1)	2(100%)	0(0%)	6(100%)	0(0%)
	Oxytetracycline	9(100%)	0(0%)	2(100%)	0(0%)	6(100%)	0(0%)
Lincosamides	Lincomycin	9(100%)	0(0%)	2(100%)	0(0%)	6(100%)	0(0%)
Polymyxin	Clostin	0(0%)	9(100%)	0(0%)	2(100%)	2(33.3%)	4(66.7%)
Polypeptides	Bactricin	9(100%)	0(0%)	2(100%)	0(0%)	6(100%)	0(0%)

Table 4. Patterns of phenotypic resistance of the isolated bacteria (*E. coli*, n = 9; *S.* Typhimurium, n=2;*P. aeruginosa*, n=6).

No. of Isolates		Source	Type	Patterns of resistant	MARI
NO.	%				
			E. coli		
3	33.40%	Yolk sac Brain Lung	XDR	9 Antimicrobial agents/ 6 Classes: AMP, DO, OTC, E, SPM, S, GEN, LIN, and BA	0.75
2	22.20%	Lung Liver	XDR	11 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, NEO, GEN, LIN, CED and BA	0.92
1	11.10%	Brain	MDR	8 Antimicrobial agents/ 5 Classes: DO, OTC, E, SPM, S, GEN, LIN and BA	0.67
1	11.10%	Lung	XDR	10 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, NEO, LIN, CED and BA	0.83
1	11.10%	Liver	XDR	9 Antimicrobial agents/ 7 Classes: AMP, OTC, E, SPM, S, GEN, LIN, CED and BA	0.75
1	11.10%	Lung	XDR	9 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, LIN, CED and BA	0.75
			Salmonella Typhimurium		
1	50%	Yolk sac	XDR	11 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, GEN, NEO, LIN, CED and BA	0.92
1	50%	Yolk sac	MDR	7 Antimicrobial agents/ 5 Classes: AMP, DO, OTC, E, SPM, LIN, and BA	0.58
			Pseudomonas aeruginosa		
1	16.70%	Yolk sac	XDR	11 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, GEN, NEO, LIN, CT and BA	0.92
1	16.70%	Yolk sac	XDR	9 Antimicrobial agents/ 6 Classes: AMP, DO, OTC, E, SPM, S, GEN, LIN, and BA	0.75
1	16.70%	Lung	XDR	8 Antimicrobial agents/ 6 Classes: AMP, DO, OTC, E, SPM, GEN, LIN, and BA	0.67
1	16.70%	Lung	PDR	12 Antimicrobial agents/ 8 Classes: AMP, DO, OTC, E, SPM, S, GEN, NEO, LIN, CT, CED and BA	1
1	16.70%	Lung	XDR	9 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, LIN, CED and BA	0.75
1	16.70%	Liver	XDR	10 Antimicrobial agents/ 7 Classes: AMP, DO, OTC, E, SPM, S, GEN, LIN, CED and BA	0.83

and another isolate was MDR. All of the *P. aeruginosa* strains were XDR to different classes, as shown in Table 4. The multiple antibiotic resistance (MAR) index values ranged from 0.67-1 indicating high-risk contamination (Table 4).

Disinfectant-resistant testing of isolated bacteria among chicks

The results of disinfectant resistance of *E. coli* isolated from chicks showed that all isolates showed 100% resistance to shift but were sensitive to other examined disinfectant agents. The results of disinfectant resistance of *S.* Typhimurium isolated from chicks showed that all isolates showed 100% resistance to shift but were sensitive to other examined disinfectant agents. The results of disinfectant resistance of *P. aeruginosa* isolated from chicks showed that all isolates showed 100% resistance to all examined disinfectant agents.

DISCUSSION

Poultry meat and its derivatives are widely consumed in Egypt, as well as globally, due to its significant popularity as a food source. It is not surprising that this food item is well regarded for its palatability, nutritional value, and affordability, as it serves as a commendable and cost-effective protein source that is both delectable and easily digestible (Samaha *et al*., 2012). The current work aimed to check the possible microbiological causes of losses in the first week of age and their effect on broiler poultry cycle performance, to study the source of infection and to aid in the control of the source of infection also to know and understand the main germs responsible for this problem. Isolation Percentage of microorganisms, where *E. coli* 57.4%; *Salmonella* Typhimurium 8.2% and *Pseudomonas aeruginosa* 4.1%

In the present study, the clinical findings during the infection of *E. coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa* were shown to be poor FCR, balance, increased gasping and respiratory distress, in addition to chicks showed increased mortality, Appearance of gasped chicks with low vitality; Air sacs turbidity with the appearance of nodules, distended caecum with gases and nephritis with urates, the obtained results were nearly similar to those obtained at one-day old chick in previous records (Mohamed., 2004; Olsen *et al*., 2012). In the present study, mortality and FCR during the cycle with infection of *E. coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa* were shown in farms and hatcheries these results were obtained with minor changes (Shukla and Mishra, 2015).

Regarding conventional methods for the identification of bacteria recovered from naturally infected chicks, the morphological, cultural, and biochemical reactions, the organisms were identified as *E. coli*, *Salmonella*, and *P. aeruginosa* which coincided with the scheme presented in Bergey's Manual of Systemic Bacteriology (Holt *et al*.,1985; Quinn *et al*.,2011).

For almost 50 years, antibiotics have been used in animal production as growth promoters and therapeutics (Al Sattar *et al*., 2003). Most of these drugs were widely used due to their efficacy and cost (Joerger, 2003). Thus, abuse and overuse of these antimicrobials established AMR-carrying microbial reservoirs in livestock, including poultry. Some animal antimicrobials are also used in people, hence AMR transmission threatens the efficient treatment of major bacterial infections in humans, leading to higher medical expenses, extended hospital stays, and higher death (Abreu *et al*., 2023). The antimicrobial resistance results of the retrieved *E. coli* isolates exhibited that the recovered strains were resistant against erythromycin(100%), sipramycin(100%), lincomycin (100%), oxytetracycline(100%), bactricin(100%), and streptomycin (100%), followed by ampicillin(88.9%), doxycycline(88.9%), and gentamycin(77.8%) which coincides with Roy *et al.* (2006) who stated that the high resistance of *E. coli* to multiple drugs with 100% resistance seen against Ampicillin/Cloxacillin,and Gentamycin (61.3%). The results of the retrieved *S.* Typhimurium isolates exhibited that the recovered strains were resistant against erythromycin(100%), spiramycin (100%), lincomycin(100%), oxytetracycline(100%), bacitracin (100%), ampicillin(100%), doxycycline(100%) which coincides with Lapierre *et al*. (2020). Retrieved *P. aeruginosa* isolates exhibited that the recovered strains were resistant against erythromycin(100%), sipramycin(100%), lincomycin(100%), oxytetracycline(100%), bactricin(100%), and followed by ampicillin(100%), doxycycline(100%), then streptomycin (83.3%), and gentamycin(83.3%) nearly agreed with reported by Kousar *et al*. (2021). Moreover, recovered isolates were sensitive to clostin(66.7%) agreeing with those discussed previously by Sans-Serramitjana *et al*. (2016).

Thus, explain in partial, why chicks do not respond to the commonly used medication in the first week of age and reflect in common losses in broiler's cycle in his agreed with previous records investigated the unresponsiveness of disease broiler with bacteria to commonly used medication in the poultry industry in recent years (Samaha *et al*., 2012). The existence of MDR and XDR gram-negative bacteria is a public health problem. Uncontrolled antibacterial usage in poultry.

CONCLUSION

The determination of the antibiotic of choice and the screening of newly emerging MDR, XDR, and PDR strains depends on the regular implementation of disinfectant effectiveness testing and antimicrobial susceptibility testing. Clostin exhibits a hopeful in-vitro antimicrobial activity against isolated bacteria in chickens.

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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