Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Molecular Characterization of antimicrobial resistant *Escherichia coli* and *Salmonella* species isolated from retail chicken with control trial using organic acids in vitro.

Safaa M. Shabana[*] and Shereen A. Yassin[**]

[*]Department of Bacteriology, [**]Department of Food Hygiene, Animal Health Research Institute, Kafr-Elsheikh branch, Agriculture Research Center (ARC), Egypt.

Received in 4/12/2023
Received in revised from 20/12/2023
Accepted in 17/1/2024

Keywords:

Retail chicken
E. coli
Salmonella
antimicrobial resistance genes
organic acids.

ABSTRACT

In total, 100 samples of chicken meat, including breast and thigh portions, as well as giblets, were collected and examined to assess the overall prevalence of *E. coli* and *Salmonella* spp. The findings proved that out of 100 samples investigated, 12 samples (12%) tested were positive for *E. coli*, and 3 samples (3%) for *Salmonella enterica.* Twelve *E. coli* isolates were serotyped into (O44:H18), (O159), (O2:H6), (O26:H11), (O121:H7), (O91:H21), (O78) and (O128:H2). The *Salmonella enterica* isolates were identified as one *Salmonella enterica* serovar Alachua was isolated from gizzard samples and two *Salmonella enterica* serovar Havana were isolated from breast meat samples. Antibiotic resistance profile of *E.coli* isolates to amoxicillin clavulinic acid, tetracycline, trimethoprim–sulfamethoxazole,cefotaxim, gentamycin, sterptomycin ,enrofloxacin, cefoperazone and fosfomycin were 100%, 100%, 58.3%, 41.7%, 41.7%, 41.7%, 33.3%, 25% and 25%, respectively. On the other hand, *Salmonella enterica* serovars were resistant to amoxicillin clavulinic acid, tetracycline, gentamycin, enrofloxacin, trimethoprim-sulfamethoxazole and fosfomycin. Concerning *bla*TEM, *tet*A, *sul*1 antimicrobial resistance genes analysis in 9 isolates(6 *E. coli* and 3 *Salmonella*) indicated that *bla*TEM and *tet*A resistance genes were detected in all 9 (100%) isolates while *sul*1gene was identified in *E.coli* and *Salmonella* isolates with percentage of 66.7% and 100%, respectively. An investigation was conducted for the purpose of determining the antimicrobial activity of acetic and lactic acid (0.5%,1% and 2% concentrations) against antimicrobial resistant *Escherichia coli* and *Salmonella enterica* serovars obtained in this study, demonstrated that all concentrations of the two applied organic acids were able to inhibit all the examined isolates. The results of our study confirmed that acetic acid and lactic acid could be effective in reducing antimicrobial-resistant food-borne pathogens, offering a promising strategy to mitigate the transmission risk of these pathogens in chicken processing plants.

Corresponding author: Safaa M. Shabana, Department of Bacteriology, Kafr-Elsheikh branch, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt..
E-mail: sofy.shabana@yahoo.com
DOI: 10.21608/ejah.2024.336965

INTRODUCTION

The chief causes of foodborne diseases were the pathogenic and food spoilage microorganisms which led to decline of food quality and numerous economic loss annually **(Over et al. 2009**). These microorganisms had been recognized as the leading factor contributing to both mortality and morbidity in the human population **(Tan et al. 2022)**. The contamination with these microorganisms could occur at several stages along the food chain, such as on-farm production, processing, distribution, retail marketing and during handling or preparation **(Akil & Ahmad, 2019).**

Escherichia coli and *Salmonella* serovars were the major pathogens of Enterobacteriaceae responsible for food borne diseases. Poultry meat considered as the most animal origin foods that related to cases of non-typhoidal *Salmonella* (NTS) disease and some contaminated vegetables **(Antunes et al. 2016).**

Poultry meat hold a crucial place in diets, particularly in developing countries. Its popularity stems from being a relatively easily producing protein source and a cost-effective in comparison to other meat products **(Musaba and Mseteka, 2014)**. Nevertheless, the substantial demand for poultry meat led to a significant load on producers who must constantly encounter the increasing market request (**Ahuja and Sen, 2007).** To address these challenges, producers frequently employ strategies such as utilization of antibiotics in prevention the poultry diseases, aiming to enhancing the growth **(Apata, 2009)**.

Antimicrobial resistance (AMR) has become one of the main concerns for global public health today, given that antibiotics were consider as one of the most commonly recommended drugs classes in human medicine. This issue posed significant challenges to effective disease treatment and control. Nevertheless, antibiotics were extensively employed not only for the treatment of various infections in humans but also as therapeutic agents for a broad spectrum of infections in animals. The antibiotics utilization in poultry and other livestock for disease treatment or as antimicrobial growth promoters (AMGPs) was related to the increasing of antibiotic-resistant microorganisms, which could potentially contaminate meat products **(Van den Bogaard and Stobberingh, 2000).** This widespread using of antimicrobial agents had been strongly linked to the emergence of bacterial antimicrobial resistance **(WHO, 2011).**

The development of antibiotic resistance in bacteria was also an emerging public health hazard which resulted from the compromised efficiency in the infectious diseases treatment **) Helmy et al. 2017)**. Moreover, among the bacterial enter pathogens causing food borne diseases, *E. coli* and *Salmonella* sp. were major contributors to the millions of annual cases, occasionally resulting in fatal outcomes **(Muonga et al. 2020).** *Salmonella* had demonstrated resistance to individual antibiotics like ampicillin and chloramphenicol, with documented cases of multiple drug resistance (MDR) reported globally **(Raji et al. 2021).**

The progression of antimicrobial resistance of *Salmonella enteric* and *E.coli* isolates was the significance concerns in Egypt, which mostly were resistant to sulphonamides, penicillin, tetracycline and cephalosporins **(Moawad et al. 2017).**

The Federal Drug Administration (FDA) has recognized organic acids as safe substances (GRAS) classification. Moreover, these acids have been approved as food additives by FDA, the European Commission, and (FAO/WHO). They had been proved as effective sanitizers to control the contamination of bacteria and elimination of foodborne pathogens during insufficient producing and processing **(Wang et al. 2013)** , cost-effectiveness, and their simplicity.

Researchers had recommended that new plans must be established to control and inhibition growth of foodborne pathogens by organic acids. The organic acids such as citric , acetic, lactic, propionic, formic, and butyric acids were effective against major pathogens included *Enterococcus faecium, Pseudomonas aeruginosa, Campylobacter, Staphylococcus aureus, Shiga toxin-producing Escherichia coli O157 (O157 STECs), Salmonella* which identi-

fied by the European Food Safety Authority in 2015as the major foodborne pathogens **(Beier, 2021)**.

This study highlights the characterization of *Escherichia coli* and *Salmonella* spp. resistant to antibiotics isolated from retail chicken meat and chicken giblets. Additionally, the study examined the antimicrobial properties of certain organic acids, including acetic and lactic acids, and their ability to inhibit the growth of these bacteria in vitro.

MATERIALS and METHODS:

Sample collection:

One hundred random samples of chicken meat were gathered, comprising 35 samples of chicken breast, 35 samples of chicken thigh, and 30 samples of chicken giblets, including liver and gizzard. These samples were obtained from various markets which found in Kafr El-Sheikh Governorate, Egypt and aseptically transported to Kafr El Sheikh Animal Health Research Institute lab. to examined bacteriologically.

Preparation of samples homogenate (ISO 6887-2: 2003):

A chicken meat sample weighing twenty-five grams was combined with sterile buffered peptone (225 ml) and thoroughly blendedby using a sterile blender for a period of 1-1.5 minutes. The resulting prepared samples underwent isolation techniques for both *Escherichia coli* and *Salmonella*.

Bacteriological isolation and identification of *Escherichia coli* (Islam et al. 2014):

The homogenized sample was cultured on MacConkey agar then incubated at 37°C for 24 hours. Subsequently, Suspected colonies of *E. coli* were streaked onto eosin methylene blue (EMB) agar and after that incubation at 37°C for an additional 24 hours. Finally, colonies displaying a distinctive green metallic sheen underwent morphological and biochemical identification, following the procedures outlined by **Quinn et al. (2013).**

Bacteriological isolation and identification of *Salmonella* (Quinn et al. 2002):

The mixtures of samples were incubated for 18 hours at 37 ºC. Subsequently,0.1 ml of the mixture was inoculated into Rappaport-Vassiliadis broth (10 ml), vortexed and left to incubate for 24 hours at 37°C. From each incubated tube about 3 mm loopful was streaked on (XLD) agar and incubated for 24 hours at 35° C. Pink colonies with or without black center were the typical colonies of *Salmonella.* After streaking one colony onto the nutrient agar, it was incubated for 24 hours at 37°C. It was then kept at 4 ºC until it was biochemically identified in accordance with the method outlined by **(Hammack et al. 2001).**

Serological identification of *Escherichia coli* and *Salmonella*:

The *Escherichia coli* and *Salmonella* isolates, which were biochemically confirmed, underwent serological identification according to **Kok et al. (1996)** and **(Kauffman, 1974).** respectively by a standard slide and tube agglutination test using commercial polyvalent and monovalent O and H antisera (SIFIN. 13088 Berlin, Germany. Berliner Allee 317-321) at Serological unit, Animal Health Research Institute, Dokki, Giza, Egypt

Antimicrobial susceptibility test:

The Kirby-Bauer disc diffusion method was used to detect the antimicrobial sensitivity phenotypes of *E. coli* and *Salmonella* isolates, in accordance with **Finegold and Martin (1982).**

Antimicrobial discs of Amoxicillin–clavulanic acid (AMC), 30µg; Cefotaxime (CTX), 30 µg; Cefoperazone (CEP),75µg; Gentamycin (CN), 10µg; Streptomycin (S), 25 µg; Enrofloxacin(ENR), 5µg; Trimethoprim-sulfamethoxazole (SXT) 25 µg; Fosfomycin (FO) 200µg; Levofloxacin (LV), 5 µg and Tetracyclin (TE), 30 µg were used (Oxoid). Bacterial suspension was prepared according to **National Committee for Clinical Laboratory Standards, (2003)** and visually comparing its turbidity to the 0.5 MacFarland standards. Interpretation as resistant, moderately susceptible or susceptible according to the Clinical and

Laboratory Standards Institute **(CLSI, 2021).**

Detection of antimicrobial resistance genes:

Extraction of DNA.

Extraction process occur from samples through using QIAamp DNA Mini kit (Qiagen, Germany, GmbH), according to the manufacturer's recommended protocol.

Oligonucleotide Primers.

Table.1 lists the primers that were used for detection of (*tet*A) to Tetracycline resistance gene, (*bla*TEM) to β-lactams resistance gene and (*sul*1) to Trimethoprim-sulfa methoxazole resistance gene.

PCR amplification.

Primers were used in a 25- µl reaction including 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primerat a concentration of 20 pmol, 5.5 µl of water, and 5 µl of DNA template. Reaction was carried out by an Applied biosystem 2720 thermal cycler.

PCR Products Analysis:

PCR products were dissociated by electrophoresis on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature, employing a gradient of 5V/cm. For gel analysis, 20 µl from the products was loaded into every gel slot. Ageneruler 100 bp ladder (Fermentas, Germany) was employed to ascertain the fragment sizes. Finally, gel was then photographed via a gel documentation system (Alpha Innotech, Biometra) and data was conducting through software of the computer.

Table .1 Primers utilized in sequences of target genes, sizes of amplicon and cycling circumstances

Target	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
*Tet*A(A)	GGTTCACTCGAACGACGTCA	570	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Randall *et al.* 2004
	CTGTCCGACAAGTTGCATGA							
*bla*TEM	ATCAGCAATAAACCAGC	516	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Colom *et al*., 2003
	CCCCGAAGAACGTTTTC							
*Sul*1	CGGCGTGGGCTACCTGAACG	433	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Ibekwe *et al*., 2011
	GCCGATCGCGTGAAGTTCCG							

Antibacterial activity of organic acids against *E.coli* and *Salmonella* isolates:

Organic acids preparation:

Acetic acid glacial 96% (Adwic) and Lactic acid 80% (Henan Jindan lactic acid technology Co., Ltd) were purchased and prepared with sterile distilled water to reach (0.5 , 1.0 and 2.0% concentration).

Agar well diffusion test:

The effectiveness of organic acids in inhibiting bacterial growth was assessed using the

agar well diffusion method against isolated strains from both *E. coli* and *Salmonella*, as described by **Geoprincy et al. (2012).** In summary, isolated bacteria were cultured at 37 ºC overnight in nutrient broth. Then, in sterile normal saline a standard inoculum toe very strain was made and compared with a 0.5 McFarland standard solution (approximately 1 × 10^8 CFU/mL). The suspension of bacteria was uniformly distributed across the Muller Hinton agar plates by a sterile swab. Wells (6 mm) were created using a sterile cork borer on the agar. Various concentrations (0.5%, 1.0%, and 2.0%) of acetic and lactic acids were added to each well. Plates were then incubated for 24 hours at 37°C. Inhibition zone surrounding the well was measured in mm and contrasted with the control well.

RESULTS:

The results tabulated in table (2) indicated that 12 (12%) *E. coli isolates* were obtained from 100 samples of retail chicken meat and giblets. The positive *E. coli isolates* percentages from chicken breast, chicken thigh and chicken giblets were 11.4% (4/35), 8.6% (3/35) and 16.7% (5/30), respectively. However, *Salmonella* incidence was detected in 3% (3/100) from the total samples and found in 5.7% (2/35), 0% (0/35) and 3.3% (1/30) from examined chicken breast, chicken thigh and chicken giblets, respectively.

Table 2. Occurrence of *E.coli* and *Salmonella* isolated from retail chicken samples:

Samples	Samples No.	Positive samples			
		E. coli		*Salmonella*	
		No.	%	No.	%
Breast meat	35	4 (4/35)	11.4%	2 (2/35)	5.7%
Thigh meat	35	3 (3/35)	8.6%	-	-
giblets	30	5 (5/30)	16.7%	1 (1/30)	3.3%
Total	100	12 (12/100)	12%	3 (3/100)	3%

+Serological identification of all the positive *E. coli* isolates was recorded in Table (3). It was recorded that the identified strains of *E. coli* from examined retail chicken meat were **(O44:H18)** by 8.33%, **(O159)** by 8.33%, **(O2:H6)** by 25%, (**O26:H11)** by 8.33%, **(O121:H7)** by 8.33% , **(O91:H21)** by 16.67, **(O78)** by 16.67, and **(O128:H2)** by 8.33% isolated from chicken breast, thigh, liver and gizzard. While, *Salmonella* serotypes are *S. enterica* serovar Alachua 1(33.3%) isolated from gizzard and *S. enterica* serovar Havana 2 (66.7%) isolated from chicken breast.

Table 3. Serotyping of *E.coli and Salmonella* isolated from retail chicken samples:

NO.	Isolates	Samples source	Antigenic structure and serotype	Prevalence of serotype	
				No.	%
1	*E.coli*	Breast	(O44:H18)	(1 out of 12)	8.33%
2	*E.coli*	Breast	(O159)	(1 out of 12)	8.33%
3	*E.coli*	Breast, thigh, liver	(O2:H6)	(3 out of 12)	25%
4	*E.coli*	Breast	(O26:H11)	(1 out of 12)	8.33%
5	*E.coli*	Liver	(O121:H7)	(1 out of 12)	8.33%
6	*E.coli*	Thigh, Liver	(O91:H21)	(2 out of 12)	16.67%
7	*E.coli*	Liver , Gizzard	(O78)	(2 out of 12)	16.67%
8	*E.coli*	Thigh	(O128:H2)	(1 out of 12)	8.33%
9	*Salmonella enterica* serovar Alachua	Gizzard	35:Zu Z 23:-	(1 out of 3)	33.3%
10	*Salmonella enterica* serovar Havana	Breast	1,13,23:f,g,{s},-	(2 out of 3)	66.7%

The results demonstrated in table (4) revealed that all *E. coli* serovars were resistant to amoxicillin clavulinic acid (AMC) and tetracycline (TE) with percentage of (100%) followed by trimethoprim–sulfamethoxazole (SXT) with percentage of (58.3%), cefotaxim (CTX), gentamycin (CN), streptomycin (S) with percentage of (41.7%), enrofloxacin (ENR) and levofloxacin (LV) with percentage of (33.3%) then cefoperazone (CEP) and fosfomycin (FO) with percentage of (25%).Among 12 *E. coli* isolates 9 isolates with percentage of(75%) are showed phenotypic multidrug resistant (MDR) against three or more antimicrobial classes.

On the other hand, *Salmonella enterica* serovar Alachua was only showed phenotypic resistant to amoxicillin clavulinic (AMC), tetracyclin (TE), gentamycin (CN), enrofloxacin (ENR), trimethoprim-sulfamethoxazole (SXT) and fosfomycin (FO) but is intermediate to cefotaxim (CTX), levofloxacin (LV), streptomycin(S) and is susceptible to cefoperazone (CEP) . Two *Salmonella enterica* serovar Havana were resistant to amoxicillin clavulinic acid (AMC) and trimethoprim–sulfamethoxazole (SXT), and were susceptible to cefoperazone (CEP), cefotaxim (CTX), tetracyclin (TE), gentamycin (CN), streptomycin (S) enrofloxacin (ENR), levofloxacin (LV), trimethoprim-sulfamethoxazole (SXT) and fosfomycin (FO). Based on that, *Salmonella enterica* serovar Alachua was only showed phenotypic multidrug resistant (MDR) to at least three different antimicrobial classes with

Table 4. Incidence of phenotypic antimicrobial resistance of *E.coli and Salmonella* serovars

Antimicrobial drugs	*E.coli* serovars						*Salmonella* serovars					
	Sensitive		Intermediate		Resistant		Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxicillin clavulinic acid	0	0%	0	0%	12	100%	0	0%	0	0%	3	100%
Cefotaxim	7	58.3%	0	0%	5	41.7%	2	66.7%	1	33.3%	0	0%
Cefoperazone	8	66.7%	1	8.3%	3	25%	3	100%	0	0%		
Gentamycin	7	58.3%	0	0%	5	41.7%	2	66.7%	0	0%	1	33.3%
Streptomycin	4	33.3%	3	25%	5	41.7%	2	66.7%	1	33.3%	0	0%
Tetracycline	0	0%	0	0%	12	100%	0	0%	0	0%	3	100%
Fosfomycin	8	66.7%	1	8.3%	3	25%	2	66.7%	0	0%	1	33.3%
Enrofloxacin	8	66.7%	1	8.3%	3	25%	2	66.7%	0	0%	1	33.3%
Levofloxacin	8	66.7%	0	0%	4	33.3%	2	66.7%	1	33.3%	0	0%
Trimethoprim-sulfamethoxazole	4	33.3%	1	8.3%	7	58.3%	0	0%	0	0%	3	100%

Table 5. Antimicrobial resistance pattern and multiple antibiotic resistance index (MAR) of *E. coli* and *Salmonella* serovars:

Serotypes	Antimicrobial disc										Resistance pattern	*MAR Index	**MDR isolates	
	AMC	CTX	CEP	TE	CN	S	ENR	LE	SXT	FO			NO.	(%)
E.coli O44:H18	R	R	R	R	S	R	R	R	R	R	AMC,CTX,CEP,TE,S, ENR,LE,SXT,FO	0.9	+	(9 out of 12)
E.coli O159	R	R	I	R	R	R	S	S	R	R	AMC,CTX,TE,CN,S, SXT,FO	0.7	+	(75%)
E.coli O2:H6	R	S	S	R	S	I	S	S	R	S	AMC,TE,SXT	0.3	+	
E.coli O2:H6	R	S	S	R	S	S	S	S	S	S	AMC,TE	0.2	-	
E.coli O2:H6	R	R	R	R	R	R	S	S	R	I	AMC,CTX,CEP,TE, CN, S,SXT	0.7	+	
E.coli O26:H11	R	S	S	R	R	S	S	S	R	S	AMC,TE,CN,SXT	0.4	+	
E.coli O121:H7	R	S	S	R	S	I	R	R	S	S	AMC,TE, ENR, LE	0.4	+	
E.coli O91:H21	R	S	S	R	S	I	S	S	S	S	AMC, TE	0.2	-	
E.coli O91:H21	R	R	S	R	S	R	I	R	R	S	AMC, CTX, TE, S, LE, SXT	0.6	+	
E.coli O78	R	S	S	R	R	S	S	S	R	S	AMC, TE, CN, SXT	0.4	+	
E.coli O78	R	R	R	R	R	R	R	R	I	R	AMC, CTX, CEP, TE, CN,S, ENR, LE, FO	0.9	+	
E.coli O128:H2	R	S	S	R	S	S	S	S	S	S	AMC,TE	0.2	-	
Salmonella enterica serovar Alachua	R	I	S	R	R	I	R	I	R	R	AMC,TE, CN, ENR, SXT, FO	0.6	+	(1 out of 3)
Salmonella enterica serovar Havana	R	S	S	S	S	S	S	S	R	S	AMC,SXT	0.2	-	(33.3 %)
Salmonella enterica serovar Havana	R	S	S	S	S	S	S	S	R	S	AMC, SXT	0.2	-	

*MAR Index (Multiple antibiotic resistance Index) = the number of antibiotics to which the isolates were resistant/the total number of antibiotics tested.
**MDR: Multidrug resistance to at least three different antimicrobial classes.

The results tabulated in table (6) reveal detection of *bla* TEM, *tet*A, *sul*1 resistance genes in six *E.coli* isolates randomly selected from 12 isolates and three *Salmonella* isolates isolated from retail chicken meat and giblets. *E. coli* resistance coding genes (*bla*TEM, *tet*A) detect percentage of with 100% (6/6) but (*sul*1) gene detected with percentage of 66.7% (4/6). Moreover *Salmonella enterica* resistance coding genes (*bla* TEM, *tet*A, *sul*1) detect with percentage of 100% (3/3) for each gene, **Figure 1,2,3.**

Table 6. The antimicrobial resistance encoding genes detection in *E.coli* and *Salmonella enterica* serovars using PCR technique:

Isolates	No. of isolates	Antimicrobial resistance genes					
		*bla*TEM		*Tet*A		*sul*1	
E.coli		No.	%	No.	%	No.	%
	6	6 (6/6)	100	6 (6/6)	100	4 (4/6)	66.7
Salmonella	3	3 (3/3)	100	3 (3/3)	100	3 (3/3)	100

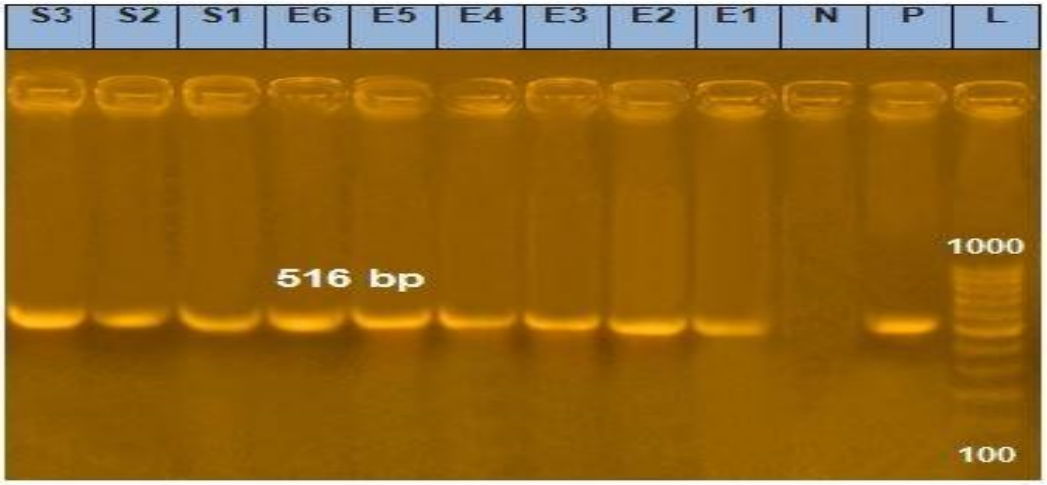

Figure 1: PCR with amplification of *bla*TEM gene at 516 bp. Lanes E1 to E6 showing amplification of *E.coli* while lanes S1 to S3 showing amplification of *Salmonella enterica* serovars."P" lane of positive control, "N": Negative control.

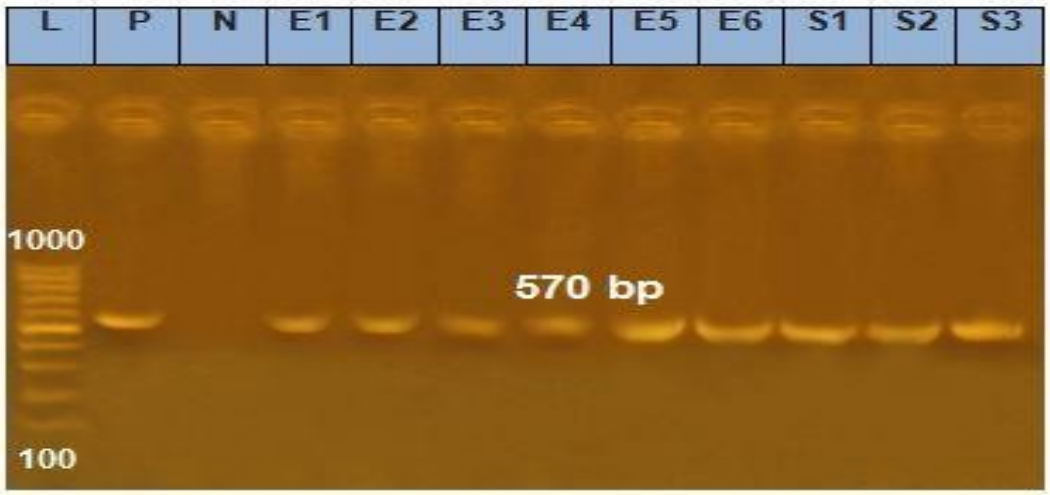

Figure 2: PCR with amplification of *tet*A gene at 570 bp. Lanes E1 to E6 showing amplification of *E.coli* while lanes S1 to S3 showing amplification of *Salmonella enterica* serovars. "P" lane of positive control, "N": Negative control.

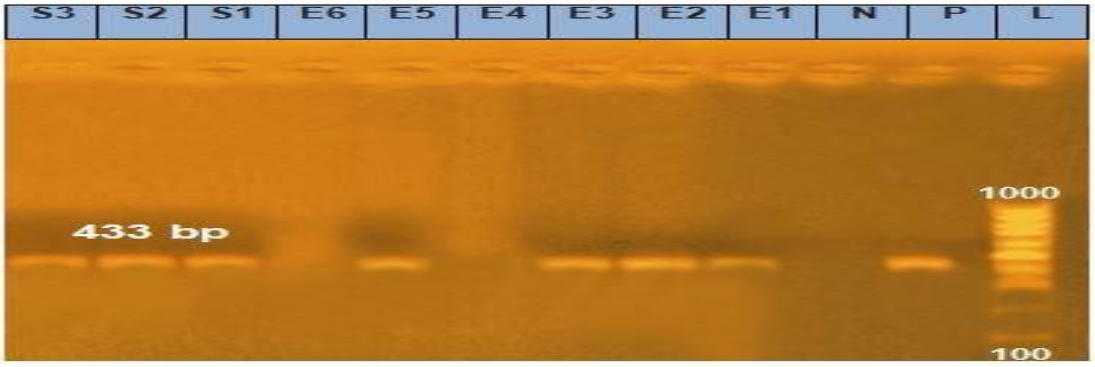

Figure 3: PCR with amplification of *Sul* 1 gene at 433 bp. Lanes E1 to E6 showing amplification of *E.coli* while lanes S1 to S3 showing amplification of *Salmonella enterica* serovars."P" lane of positive control, "N": Negative control.

Organic acids consider as safe compounds for the environment and are generally used as preservatives in food. These organic acids have an antimicrobial activity.

The results presented in table (7) showed variation of the inhibition zone of agar well diffusion method of two organic acids (acetic and lactic acids) in concentrations 0.5%, 1% and 2% for each acid against eight *E. coli* serovars and two *Salmonella* serovars which were resistant to different antimicrobial classes. The inhibitory zones of acetic acid against isolates of *E .coli* at concentrations (0.5%) was with the range of (10 to 17 mm), at concentrations (1%) gave inhibitory zone range (12 to 20 mm), and at concentrations (2%) with the range of (16 to 22 mm), while inhibitory zone of lactic acid against *E .coli* isolates at concentrations (0.5%) was with the range of (9 to 18 mm), at concentrations (1%) zone diameter ranging from (10 to 19 mm) and at concentrations (2%) zone range was (13 to 21 mm). Likewise ,the inhibitory zone of acetic acid against *Salmonella* serovars was at range (11 to 15 mm) at concentrations of 0.5%, (13 to 15 mm) at concentrations of 1% and (17 to 22 mm) at concentrations of 2%. Also, lactic acid exhibits an inhibitory zone spanning to the range(9 to 12 mm) at concentrations of 0.5%, (12 to 15 mm) at concentrations of 1% and (15 to 19 mm) at concentrations of 2%.

Table 7. The antimicrobial activity of acetic acid and lactic acid against *E. coli* and *Salmonella enterica* serovars:

Serotypes	Acetic acid			Lactic acid		
	0.5%	1%	2%	0.5%	1%	2%
E.coli (O44:H18)	17 mm	20 mm	22 mm	9 mm	11 mm	13 mm
E.coli (O159)	11 mm	14 mm	20 mm	16 mm	17 mm	20 mm
E.coli (O2:H6)	11 mm	15 mm	20 mm	17 mm	17 mm	19 mm
E.coli (O26:H11)	10 mm	12 mm	16 mm	9 mm	10 mm	14 mm
E.coli (O121:H7)	11 mm	13 mm	18 mm	12 mm	14 mm	16 mm
E.coli (O91:H21)	12 mm	16 mm	19 mm	11 mm	12 mm	16 mm
E.coli (O78)	15 mm	16 mm	19 mm	10 mm	12 mm	15 mm
E.coli (O128:H2)	10 mm	18 mm	22 mm	18 mm	19 mm	21 mm
Inhibitory zonerange	(10- 17 mm)	(12 - 20 mm)	(16 - 22 mm)	(9 - 18 mm)	(10 - 19 mm)	(13- 21 mm)
Salmonella enterica serovar Alachua	15 mm	15 mm	22 mm	12 mm	15 mm	19 mm
Salmonella enterica serovar Havana	11 mm	13 mm	17 mm	9 mm	12 mm	15 mm
Inhibitory zonerange	(11 - 15 mm)	(13 - 15 mm)	(17 -22 mm)	(9-12mm)	(12-15mm)	(15-19mm)

DISCUSSION:

E.coli and *Salmonella* serovars are the major pathogens of *Enterobacteriaceae* family that cause food-borne infections. In many countries, salmonellosis is a major foodborne illness that can be caused by *Salmonella* spp., whereas *E. coli* can cause a wide range of illnesses, including pneumonia, respiratory disorders, diarrhoea, and urinary tract infections **(Elumba et al. 2018).**

Results for *E.coli* incidence were closely agree with the recorded result by **Moawad et al. (2017)** and **Ukut et al. (2010)** who determined prevalence of *E. coli* species in retail chicken were 11.7% and 11.1%, respectively. Higher prevalence of *E.coli* were recorded by **Zelpina & Rizaldi (2023)** and **Adeyanju & Ishola (2014)** who detected *E.coli* in fresh chicken meat by 20% and 43.4%, respectively. Low prevalence of *E.coli* 10% and 4.6% were recorded by **Hossam, (2012)** and **Lee et al. (2009),** respectively. *E. coli* is a sign of fecal contamination, which can happen when food is poorly prepared or during evisceration **(Kim and Yim, 2016).** The results were not acceptable with EOS for chicken carcasses (free *E. coli*) when compared to those obtained from **EOS 1651, (2005).**

Our findings regarding the prevalence of *Salmonella* in retail chicken closely matched those approved by **Khaled et al. (2015), Hashem et al. (2022)** and **Oscar, (2013)** who found the incidence of *Salmonella* spp. in retail chicken were 3.3% , 2.7% and 3%, respectively. While, lower rate of *Salmonella* prevalence was noted by **Shekhar et al. (2013), Guran et al. (2017)** and **Mpundu et al. (2019)** at 0.94%, 2% and 1.5%, respectively. Higher frequency detected by **Moawad et al. (2017) who** confirmed *Salmonella* in chicken meat by 8.3%. The evisceration process at the abattoir is the main way that *Salmonella* contaminates carcasses. Additionally, there are unhygienic conditions with reference to the equipment, personal hygiene, and storage temperature and seldom were the cutting tables cleaned or sanitized before being used.

In accordance with **EOS 1651, (2005)** there should be no *Salmonella* present in chicken meat or chicken meat products. The findings showed that 3 (3%) of the chicken meat and products that were analyzed did not meet Egyptian standards.

The outcomes of the serological identification of the tested *E. coli* isolates were found in agreement with **Edris et al. (2015)** who could isolate *E. coli* from chicken meat with serological identification revealed the presence of O55: H7, O78, O125:H18, O128:H2 , O127:H6, O26 ,O111:H4 and O124 serotypes. **Hassanin et al. (2020)** who isolated 11 strain of *E.coli* from different parts from chicken carcasses and isolates that were serotyped showed the presence of O111:H2, O55:H7, O146:H21 and O125:H21. Whereas, our results for serological identification of isolated *Salmonella* spp. were in line with the findings of **Xiao et al. (2023),** who stated that *S. enterica* serovar Havana was identified among 31 different *Salmonella* serotypes isolated from poultry meat in Shanghai during 2021. **Santos et al. (2014)** isolated *Salmonella enterica* serovar Havana from raw, unprocessed chickens. Also in accordance with **Green *et al.*, (2018)** who recorded that *Salmonella* serotypes *S.* Enteritidis, *S.* Heidelberg, *S.* Kentucky and *S.* Montevideo were commonly found to infect poultry meats. **Almeida et al. (2015)** reported that the first food poisoning outbreak in Brazil brought on by *Salmonella enterica* serovar Alachua, which was isolated from a food sample.

In this study, 100% of examined *E.coli* isolates were resistant to amoxicillin clavulinic acid (AMC) and tetracycline (TE) which agree with **Abo-Almagd et al. (2023)** and **Ramadan et al. (2020**) whose declared high resistance of *E.coli* to amoxicillin clavulinic acid (AMC) and tetracycline (TE). Nine isolates out of 12 *E. coli* isolates (75%) exhibit resistant to 3 different or more antimicrobials classes, classifying them as multidrug-resistant strains (MDR) which aligns with **Alam et al. (2023)** who detected the multidrug-resistant *E. coli* isolates with 70%. The increased frequency of resistance patterns against three or more classes of antimicrobials may be associated with the varying antibiotic treatment protocols used for the different livestock species **Bogaard et al., (2001).** In addition, **Parvin et al. (2020**) who

reported 100% rates of (MDR) *E .coli* isolates in chicken meat .

In this study, all *Salmonella enterica* isolates (100%) were resistant to amoxicillin clavulinic acid (AMC) and trimethoprim–sulfamethoxazole (SXT), but all *Salmonella* isolates were intermediate resistant and susceptible to cefotaxim (CTX) and cefoperazone (CEP) antibiotics which was similar to **Sabeq et al. (2022)** whose reported that all *Salmonella* isolates were susceptible to third and fourth-generation cephalosporins antibiotics.

Salmonella enterica serovar Alachua was showed phenotypic multidrug resistant (MDR) with percentage of (33.3%) to at least three different antibiotics classes amoxicillin clavulinic acid (AMC), tetracycline (TE), gentamycin (CN), enrofloxacin (ENR), trimethoprim-sulfamethoxazole (SXT), fosfomycin (FO) which agree with **Nkuchia et al. (2010)**, but *Salmonella enterica* serovar Havana was resistant to amoxicillin clavulinic acid (AMC) and trimethoprim–sulfamethoxazole (SXT) which differ with **Firoozeh et al. (2011**) whose reported that *Salmonella enterica* serovar Havana showed phenotypic multidrug resistant to at least five different antibiotics classes and with **Almeida et al., (2015)** whose reported that all the *Salmonella enterica* serovar Alachua isolates from clinical and food samples were susceptible to cefotaxime (CTX), amoxicillin–clavulanic acid (AMC), streptomycin (S), gentamicin (CN), trimethoprim–sulfamethoxazole (SXT) and tetracycline (TE) .

Antimicrobial resistance in *E. coli* and *Salmonella enterica* serovars is an important issue for public health. This study shows the phenotypic antimicrobial resistance and detect genotypic *bla* TEM, *tet*A, *sul*1 resistance genes in six *E. coli* randomly selected from 12 (6/12) *E.coli* isolates which showed multidrug resistance to different antimicrobial classes and three *Salmonella* isolates isolated from meat and giblets of chicken. In the present study, *E. coli* resistance coding genes (*bla*TEM, *tet*A) detect with percentage of 100% (6/6) but (*sul*1) gene detected with percentage of 66.7% (4/6). These results similar to **Ramadan et al. (2020**) whose reported higher frequencies of tetA, *bla*TEM and *sul*1 resistance genes from the examined *E. coli* isolates.

Tetracycline resistance was a specific focus due to the prevalence of the using of this drug in the poultry field **(Imam et al. 2020).** In previous study of **Alam et al. (2023)** whose reported that (84.4%) of E.*coli* isolates encoded *tet*A gene which similar to the present study but **Adelowo et al. (2014**) found that *tet*A was encoded in 21% of *E. coli*, which was lower than this study. Also these results differs from **Abo-Almagd et al. (2023)** whose reported that *E. coli* isolates from chicken carcasses had harbored *bla* TEM gene with (64%). Moreover *Salmonella enterica* resistance coding genes (*bla* TEM, *tet*A, *sul*1) detected with 100% (3/3) for each gene which agree with **Shabana et al. (2019)** whose detected *bla* TEM gene in 100% of *Salmonella enterica* isolates and differ with Abd **El-Twab et al. (2016**) whose mentioned that *Salmonella enterica* serovar Havana was not harbored *tet*A gene. In previous study of **Zhu et al. (2017**) reported that (50.5%) of *Salmonella* isolates were harbored *sul*1 gene of sulfonamide-resistance which differ with the present study.

The critical need to proper antimicrobial drugs usage to reduce spreading of multidrug resistant (MDR) bacteria species. Therefore, it was necessary to replace the antimicrobial drugs by many compounds recognize as safe for the environment, such as organic acids, which have an antimicrobial activity and are generally used as preservatives in food **(Borges et al. 2013).** Organic acids such as tartaric, citric, propionic, lactic, malic and acetic acids had antibacterial activity on different pathogenic bacteria species **(Lingham et al. 2012).** The mechanism of action of these organic acids is likely due to the ability of these acids to enter bacterial cell membrane and acidify the cytoplasm of those cells, preventing bacterial growth **(Salsali et al. 2008)**.

Referring to results of agar well diffusion method which revealed variation of the inhibition zone of two organic acids (acetic and lactic acids) in concentrations 0.5%, 1% and 2% for each acid against eight *E. coli* serovars and two *Salmonella* serovars which showed antimi-

crobial resistance to different antimicrobial classes. The two tested organisms show sensitivity to acetic acid more than lactic acid which differ withother authors whose recommended that lactic acid demonstrated greater efficacy as an antibacterial agent than citric, propionic and acetic acid (**Pundir and Jain, 2011, Daskalov, 2012**).

Acetic acid exhibits an inhibitory zone against *E .coli* isolates at concentrations (0.5%) was in a range (10 to 17 mm), at concentrations (1%) showing a range (12 to 20 mm), and at concentrations (2%) showing a range (16 to 22 mm), that parallel to **Wali and Abed, (2019)** whose reported that acetic acid (0.5%) was capabled to inhibit bacterial growth at concentrations range (13 to 18 mm) and **Abdullah and Al-shwaikh, (2009)** who found that acetic acid minimum inhibition zone at concentrations (1%) range between (10 to 15mm) and (14 to 20 mm) at concentration (2%), respectively.Moreover, in order to extend the shelf life of poultry, beef, and pork meat and eliminate bacteria like *Salmonella* and *Escherichia coli*, acetic acid has been suggested as an antimicrobial agent **(Sakhare et al. 1999).**

On the other hand, the inhibitory zone of lactic acid against *E .coli* isolates at concentrations 0.5% displaying a range 9 to 18 mm, at concentrations 1% displaying a range 10 to 19 mm and at concentrations 2% displaying a range 13 to 21 mm , that agree with **Stanojevic´-Nikolic´ et al. (2016)** whose found that as the concentration of lactic acid increased, there was a corresponding increase in the inhibition zone, while differ with **Yesillik et al. (2011)** whose indicated that lactic acid was ineffective against *E. coli* .

Similarly, the inhibitory zone of acetic acid against *Salmonella* serovars was at range (11 to 15 mm) at concentrations of 0.5%, (13 to 15 mm) at concentrations of 1%, and (17 to 22 mm) at concentrations of 2%. Also, lactic acid exhibits an inhibitory zone spanning to the range (9 to 12 mm) at concentrations of 0.5%, (12 to 15 mm) at concentrations of 1%, and (15 to 19 mm) at concentrations of (2%.‹(that parallel to **Yesillik et al. (2011)** whose indicated that *Salmonella* typhimurium was inhibited from growing at a concentration of 9 mg/mL of lactic acid, resulting in a 22.6 mm inhibition zone. The present results agree with previous researches demonstrated the antimicrobial activity of lactic acid against Salmonella enteritidis, L. monocytogenes, and *E. coli* **(Eswaranandam et al. 2004** and **Anang et al. 2007).**

These present results differed with **Jankuloski et al. (2014)** whose reported that inhibitory zone of lactic acid and acetic acid was at range (2 to 3 mm) and (3 to 4 mm), respectively at concentrations of (2% to 10%**)** against *Salmonella enteritidis***.**

CONCLUSION

This study showed the importance of chicken meat and giblets as an essential source to food-borne bacteria, *E. coli* and *Salmonella*, which carrying antimicrobial resistance genes of different antimicrobial drugs leading to spread of antimicrobial resistant (AMR) bacteria species and a possibility of transferring resistance genes between humans, animals, and the environment and that has a significant public health problem. In order to reduce the prevalence of *E. coli, Salmonella*, and other food-borne contaminants, it is crucial to use hand sanitizers and modern disinfection methods. Additionally, washing hands thoroughly before selling chicken meat and wearing hand gloves, head coverings and nose masks, chicken carcasses are properly chilled to prevent growing of bacteria, also cooking to a high temperature of 100°C, which helps destroy pathogens before they are consumed and finally antibiotics should not be used carelessly since they will eventually lose all of their ability to combat microorganisms. The organic acids as acetic acid and lactic acid had antimicrobial activity against the pathogenic food-borne bacteria, so this study referred to the significance of using these two organic acids against *E. coli* and *Salmonella* in vitro as way to reduce using of antimicrobial drugs and restrict the transmitting the resistance genes between humans, animals, and the environment offering a promising strategy to mitigate the transmission risk

of these pathogens in chicken processing plants.

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