A Study On The Association Between Enterotoxigenic Potentiality And Antimicrobial Resistance In Bacteria Isolated From Avian Origins

Samah Eid *, Heba Badr* and Ahmed Samy**
*Bacteriology Dept., **Biotechnology Dept.,
Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Nadi El-Seid St., P.O. Box 246, Dokki, Giza 12618, Egypt.
Email: samaheid@ymail.com

ABSTRACT

Antibiotic susceptibility pattern was studied in food poisoning implicated bacteria isolated from a variety of avian origin. Ten salmonella isolates, sixty E.*coli* isolates ,and sixty coagulase positive staphylococci isolates were included in the study. The phenotypic antibiotic susceptibility patterns of studied isolates revealed that 100% of tested isolates fulfilled the criteria of multidrug resistant bacteria . Conventional PCR was applied to investigate the presence of antibiotic resistance genes, and enterotoxin genes. Testing the ten Salmonella isolates by PCR detected *Stn* genes, *tet*A, and bla^{TEM} genes with rates of 100%, 60%,and 40% , respectively; Neither *Aac* gene , nor *qn*Rs genes was detected. Fifteen E.*coli* isolates were tested by PCR , 20%, 100%, 100% ,46.67% , 0% , and 13.33% were positive for *Aac*, *tet*A, bla^{Tem} , *qn*Rs , *Stx*1, and *Stx*2, respectively. PCR results of fifteen staphylococcus isolates revealed that zero %, 100%, 100% of the tested isolates were positive for *Aac, tet*K, and *bla*Z, respectively. PCR revealed that non of the tested isolates were positive for *sea* nor *see* genes. Meanwhile, 60%, 86.7%, and 20% of staphylococcus isolates were positive for *seb, sec,* and *sed* genes, respectively. It could be concluded that poultry, poultry products , and poultry environments could impose public health hazard through disseminating multidrug resistant bacteria with enterotoxigenic potentialities.

Key Words: multidrug resistance MDR bacteria, enterotoxin genes, PCR, antibiotic resistance genes .

INTRODUCTION

Antimicrobial resistance is a serious concern in both Human and Veterinary Medicine. Resistant bacterial infections are associated with increased morbidity, mortality, and treatment expenses compared to their susceptible counterparts,*(1)*.The wide spread non-human use of antimicrobial agents that are regarded as critically or highly important for use in humans creates a reservoir of resistant bacteria and resistance genes. The spread of use of antibiotics adds to the burden of antimicrobial resistance in human medicine and may shorten the time that these valuable antimicrobial agents will be available for effective treatment of infections in humans, *(2)*.

Antibiotics have been used successfully in poultry for different purposes such as growth promotion, prophylaxis, or therapeutics. However, their use in animal production and human therapy has resulted in increased bacterial resistance to many antibiotics, *(3)* .

Many epidemiological studies and research have implicated foods of animal origin as major vehicles associated with illnesses caused by Escherichia *coli*, Campylobacter, Salmonella and Yersinia spp, *(4)*.

Antimicrobial resistance was more frequent in pathogenic than in other E. *coli* strains. Also the resistance genes found in ETEC isolates were different from those of other E.

coli isolates and that clear associations exist between specific resistance and virulence genes, *(5)*.

Coagulase Positive Staphylococci"CPS" cause staphylococcal food poisoning. Recently, these bacteria have received increasing attention due to their potential role in the dissemination of antibiotic resistance markers, *(6)*.

Most cases of salmonellosis in human are the consequence of consuming contaminated poultry, pork, beef and eggs,*(7)*.Multi drug resistant strains of Salmonella are ubiquitous in poultry and poultry environments *(8)*. All strains of Salmonella enterica investigated were found to carry the Salmonella enterotoxin gene (*stn*) as determined by PCR *(9)*.

The aim of the presented study was to investigate the association between the antibiotic resistant genes and enterotoxin genes in food poisoning implicated bacteria isolated from avian origin.

MATERIAL AND METHODS

Bacterial isolates

Bacterial isolates obtained from Senior author's previous studies were used . Sampling and isolation were conducted during the period from January 2012 till June 2014, at Sharkia Governorate. Ten salmonella isolates were obtained from feral pigeon, feral pigeon houses, chilled chickens, and duck flocks. Sixty E.*coli* isolates were obtained from diseased broiler flocks, turkey flocks, edible chicken parts, and from backyard balady eggs. Sixty coagulase positive Staphylococcus isolates were obtained from chilled chicken, edible chicken parts, and from backyard balady eggs. All isolates used for the study were preserved at −70°C in brain heart infusion broth (Becton Dickinson, Sparks, MD) containing 20% glycerol until further investigations, *(5)*.

Viability and Purity

Viability of isolates was tested by sub-culturing a loopful of each preserved isolate into Buffered Peptone Water "Oxoid", and allow preliminary non selective enrichment by incubation at 37 C for 24 hours. Broth cultures of Salmonella isolates , E.*coli* isolates , and coagulase positive Staphylococcus isolates were tested for their purity by subculturing into selective media to obtain pure, separate characteristic colony of each microorganism according to *(10-12)* respectively.

Serotyping

E. *coli* isolates and Salmonella isolates were serotyped in Reference Laboratory for Veterinary Quality Control on Poultry Production using commercially available kits (Test Sera Enteroclon, Anti –Coli, SIFIN Berlin, Germany), and (TestSera Enteroclon, Anti –Salmonella, SIFIN Berlin, Germany).

Antibiogram

Antibiotic susceptibility of Salmonella, E.*coli*, and coagulase positive Staphylococcus isolates were tested against 12, 13,and 12 antibiotics of the most commonly prescribed in Veterinary field. Disc diffusion technique of Kirby –Bauer was applied according to *(13)*. Interpretation of the results based on the diameter of the inhibition zones produced was done according to *(14)*.

Table 1. Cycling conditions of different primers during cPCR.

Tested gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No.of cycles	Final extension
tetA(A)	94°C	94°C	50°C	72°C	35	72°C
	5 min.	45 sec.	45 sec.	45 sec.		10 min.
bla_{TEM}	94°C	94°C	54°C	72°C	35	72°C
	5 min.	45 sec	45 sec	45 sec		10 min.
aac(6')-Ib-cr	94°C	94°C	52°C	72°C	35	72°C
	5 min.	30 sec.	30 sec	30 sec		10 min.
qnrS	94°C	94°C	53°C	72°C	35	72°C
	5 min.	45 sec.	45 sec.	45 sec.		10 min.
*Stx*1 , *Stx*2	94°C	94°C	58°C	72°C	35	72°C
	10 min.	1 min.	1 min.	1 min.		10 min.
blaZ , *tetK*	94°C	94°C	54°C	72°C	35	72°C
	5 min.	30 sec.	30 sec.	30 sec.		10 min.
Sea, Seb, Sec ,	94°C	94°C	50°C	72°C	35	72°C
See	5 min.	30 sec.	30 sec.	30 sec.		10 min.
Sed	94°C	94°C	48°C	72°C	35	72°C
	5 min.	30 sec.	30 sec.	30 sec.		10 min.
stn	94°C	94°C	59°C	72°C	35	72°C
	10 min.	45 sec.	45 sec.	45 sec.		10 min.

Table 2. Sequence of primers used for enterotoxin and antibiotic resistance genes .

Primer	Sequence	Amplified product	Reference	Tested Pathogen
tetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576 bp	*(15)*	Salmonella Spp, E.*coli*
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	*(16)*	Salmonella Spp, E.*coli*
aac(6')-Ib-cr	CCCGCTTTCTCGTAGCA TTAGGCATCACTGCGTCTTC	113 bp	*(17)*	Salmonella Spp, Staphylococci, E.*coli*
qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417 bp	*(18)*	Salmonella Spp, E.*coli*
*stx*1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	*(19)*	
*Stx*2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp		E.*coli*
blaZ	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173 bp	*(20)*	
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp		Staphylococci
Sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102 bp	*(21)*	
Seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164 bp		
Sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451 bp		Staphylococci
Sed	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278 bp		
See	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209 bp		
stn	TTG TGT CGC TAT CAC TGG CAACC ATT CGT AAC CCG CTC TCG TCC	617 bp	*(22)*	Salmonella

RESULTS AND DISCUSSION

Live poultry are potential sources for infection, animals are more likely to shed pathogens because of stress induced by prolonged transportation, confinement, crowding, and increased handling, ***(23)***.

Table 3. Serotypes Of Salmonella Isolates

SN	Serotype	Antigenic formula	Source of isolation	No of isolates
1	Salmonella Abortusequi	4,12: ---; enx	Feral Pigeon	3
2	Salmonella Entritidis	1,9,12: gm ; ---	Chilled chicken , ducklings	2
3	Salmonella Typhimurium	1,4,(5)12: I ; 1,2	Chilled chicken , ducklings	4
4	Salmonella Anatum	3,10 {15}{15,23}: e,h; 1,6(Z64)	ducklings	1
	Total number of isolates	----------	----------	10

Isolation of different Salmonella Spp from avian origin including chicken, duck, turkey, eggs, pigeon ,and chicken meat with predomination of Salmonella Entritidis and Salmonella Typhimurium were recorded by many researchers as ***(24,25)***. Isolation of Salmonella Abortusequi from pigeon was justified by ***(26)*** who reported that Free-ranging birds can be sub-clinical carriers for about 2000 Salmonella species, thus represented as a reservoir of Salmonellae , they also recorded that Salmonella species vary with geographic location and the types of food consumed .Our results also agreed with ***(27)*** who isolated Salmonella Anatum, Salmonella Typhimurium, and Salmonella Entritidis from ducklings in Sharkia Governorate during 2013 which is the same period of isolation in the present study (Table 3).

In the present study, serotyping of E.*coli* isolates confirmed the identification of 22 serotypes. Among isolated E.*coli* O 111 was identified , this result was in agreement with that recorded by ***(28)*** who concluded that variations in distribution of serotypes according to geographic region occur, but in most studies the commonly identified serotypes have been O1, O2, O18, O35, O36, O78, and O111. ***(28)*** also recorded that some APEC are untypeable. ***(29)*** recorded that high prevalence of resistance seems to be a characteristic of O111 and O26 strains. They elucidated the prevalence and molecular basis of antimicrobial resistance in E. *coli* O111. (Table 4).

Table 4. Serotypes of E.*coli* Isolates

SN	Serogroup	Serotype	Source of isolation	No of isolates	% of serotype /no of isolates
1	Poly 1	O 26	Diseased broilers ,Diseased turkey	3	5.0 %
		O 44	Diseased broilers	1	1.7 %
		O 114	Diseased broilers	5	8.3 %
		O 125	Diseased broilers	4	6.7 %
2	Poly 2	O 55	Diseased broilers , Diseased turkey	4	6.7 %
		O 91	Diseased broilers, Chilled eggs	3	5.0 %
		O 111	Diseased broilers	3	5.0 %
		O125	Chilled eggs	3	5.0 %
		O126	Diseased broilers	1	1.7 %
		O 166	Chilled eggs	1	1.7 %
3	Poly 3	O 25	Diseased broilers	1	1.7 %
		O118	Diseased broilers	1	1.7 %
		O 145	Diseased broilers, Chilled eggs	2	3.3 %
4	Poly 4	O6	Chilled eggs	3	5.0 %
		O27	Chilled eggs	8	13.3 %
		O 159	Chilled eggs	3	5.0 %
5	Poly 5	O25	Chilled eggs	1	1.7 %
		O153	Chilled eggs	1	1.7 %
6	Poly 6	O115	Chilled eggs	1	1.7 %
		O169	Chilled eggs ,Chicken parts	4	6.7 %
7	Poly8	O29	Chilled eggs ,Diseased turkey	6	10.0 %
		O152	Chilled eggs	1	1.7 %
	Total			60	100 %

The introduction of antimicrobial agents in commercial feed for cattle, pigs, and poultry started in the early 1950s *(30)* . In Europe, concern for the spread of antimicrobial resistant bacteria from the large reservoirs in food animals led the countries of the European Union to abandon the use of antimicrobial agents for growth promotion in food animals by 1 January 2006, *(31)*. Antimicrobial treatment of animals cannot reliably eliminate infection, prevent shedding, or protect against reinfection. In addition, treatment of animals can prolong shedding and contribute to antimicrobial resistance,*(23)*. They reported also that among the aminoglycosides, only gentamicin and apramycin are relevant for human therapy. Gentamicin and apramycin were introduced in Veterinary therapy in the early 1980s in several European countries. Several cephalosporins are widely used in Veterinary clinical therapy *(30)*. Also, they observed that the most of antimicrobial agents used for growth promotion in animals were mainly active against gram-positive bacteria, whereas many of the antimicrobial agents used in veterinary clinical therapy are broad spectrum and are mainly active against gram-negative bacteria, such as Salmonella, and E. *coli* . Quinolones are broad-spectrum antimicrobial agents that are highly effective in the treatment of a variety of infections in both humans and animals. The US Food and Drug Administration withdrew the quinolones such as enrofloxacin, which were used to treat E. *coli* infections in poultry, *(2)*.

The results of testing antibiotic susceptibility pattern of Salmonella ,E.*coli* , and CPS isolates performed in this study revealed that, 100% of tested isolates from the three tested pathogens fulfilled the criteria of multidrug resistance "MDR"pathogens as described by *(14);* As all isolates were resistant to more than 3 drugs that belong to 3 different antibiotic groups.

The results for isolation rates of MDR Salmonella detected in the present study was in accordance with those of *(32,33).* They detected MDR salmonella with rates of 100% ,and 72.7%. Lower isolation rate was detected by *(34)* who recorded an isolation rate of 31%.

Recovery rate of MDR E.*coli* isolates in the study was similar to those recorded by *(35,32)* who detected MDR E.*coli* with a rate of 100% .On the other hand, lower isolation rate was detected by *(36)* who recorded MDR E.*coli* with an isolation rate of 10.9%.

The recovery rate of MDR CPS in the study was in agreement with those of *(37, 32)* who recorded isolation rates of 100% , 77.2%, respectively. While lower recovery rate was reported by *(38),* who recorded an isolation rate of 44.12%.

Table 5. Antibiotic Susceptibility Pattern Of Salmonella Isolates Tested By Disc Diffusion Technique.

Antibiotic group	SN	Chemotherapeutic agent	Susceptible S	Intermediate I	Resistant R
penicillins	1	Penecillin "P" (10ug)	30.0%	0%	70.0%
	2	Amoxicillin "AMX"(25ug)	50.0%	0%	50.0%
	3	Ampicillin "AMP" (10ug)	30.0%	10.0%	60.0%
Quinolones	4	Norfloxacin "NOR" (10ug)	80.0%	0%	20.0%
	5	Ciprofloxacin "O" (5ug)	80.0%	0%	20.0%
Aminoglycosides	6	Kanamycin "K" (20ug)	40.0%	0%	60.0%
	7	Neomycin "N" (30ug)	0%	20.0%	80.0%
	8	Gentamycin "GM" (10 ug)	70.0%	0%	30.0%
	9	Streptomycin "S" (10 ug)	30.0%	20.0%	50.0%
Tetracyclins	10	Doxycyclin "DO" (30 ug)	80.0%	0%	20.0%
	11	Tetracycline "TE" (30 ug)	90.0%	0%	10.0%
	12	Oxytetracycline"OT"(30ug)	90.0%	0%	10.0%

Salmonella isolates antibiogram as shown in (Table 5) revealed the prevalence of high resistance rates against Penicillin, amoxicillin ,and ampicillin with resistance rates of 70%, 50%,and 60%, respectively. These results agreed with those recorded by *(32,39)* who recorded resistance rates of (100% ,and 100%) and (100% ,and 88%) against penicillin and ampicillin, respectively. Higher resistance rate against amoxicillin (100%) was recorded by *(40).* Antibiotic susceptibility pattern of Salmonella isolates against aminoglycosides revealed high resistance rates 60%,80%,and

50% against kanamycin , neomycin ,and streptomycin, respectively. Lower resistance rate (30%) was recorded against gentamycin. Almost similar resistance rate (50%) against kanamycin was recorded by *(39)*. Results of gentamycin agreed with that of *(39)* as they observed a resistance rate of 24%, in addition they recorded a lower resistance rate (30%) for streptomycin . In contrast, *(40)* recorded the highest susceptibility rates (100%) for both gentamycin and neomycin .

Low resistance rates (20%)of salmonella isolates were detected against both ciprofloxacin, and norfloxacin . This result partially disagreed with that of *(25)* who recorded a resistance rate (73%) against ciprofloxacin. Amazingly identical similar resistance rates were recorded by *(39,40)* they recorded 20%, 13.5% resistance rates of salmonella against both norfloxacin, and ciprofloxacin, respectively. The results disagreed with that registered by *(32)* who recorded 83% resistance rates against ciprofloxacin.

Low resistance rates 20%, 20%, and 10% of Salmonella isolates were detected against doxycyclin, tetracycline ,and oxytetracyclin , respectively. These results were in contrast with that recorded by *(32)*, who recorded resistance rates (83%) of Salmonella isolates against both doxycyclin and tetracycline.Meanwhile *(39)* recorded resistance rates (100%) against tetracyclines. These results also, disagreed with those of *(40)* who recorded resistance rates (50%), and (100%) against doxycycline ,and tetracycline ,respectively.

The result of antibiotic susceptibility of E.*coli* isolates were shown in (Table 6). This study revealed that E.*coli* isolates exerted 95 %, 83.4%, and 83.3% resistance rates against penicillin, amoxicillin and ampicillin, respectively.

Table 6. Antibiotic Susceptibility Pattern Of E.*coli* Isolates Tested By Disc Diffusion Technique

Antibiotic group	SN	Chemotherapeutic agent	Susceptible S	Intermediate I	Resistant R
penicillins	1	Penecillin "P" (10ug)	5.0%	0%	95.0%
	2	Amoxicillin "AMX" (25ug)	8.3%	8.3%	83.4%
	3	Ampicillin "AMP" (10 ug)	16.7%	0%	83.3%
Quinolones	4	Norfloxacin "NOR" (10ug)	75.0%	0%	25.0%
	5	Ciprofloxacin "O" (5ug)	70.0%	5.0%	25.0%
	6	Enrofloxacin "ENR"(10ug)	11.7%	5.0%	83.3 %
Aminoglycosides	7	Kanamycin "K" (20ug)	50.0%	0%	50.0%
	8	Neomycin "N" (30ug)	25.0%	0%	75.0%
	9	Gentamycin "GM" (10 ug)	50.0%	0%	50.0%
	10	Streptomycin "S" (10 ug)	8.3%	8.3%	83.3%
Tetracyclins	11	Doxycyclin "DO" (30 ug)	3.3%	0%	96.7 %
	12	Tetracyclin "TE" (30 ug)	5.0%	0%	95.0%
	13	Oxytetracyclin "OT" (30ug)	3.3%	0%	96.7%

Lower resistance rate (65%) against penecillin was detected by *(32)* . Almost similar resistance (70.4%, and 84.62%) against amoxicillin were detected by *(40,41)*.Similar resistance rates (89%, 81.5%) against ampicillin were recorded by *(41,42)*. Lower resistance rates (53% , and 15%) , and (47% , and 60%) against amoxicillin and ampicillin , respectively were recorded by *(43, 44)*.

Antibiotic susceptibility of E.*coli* isolates revealed resistance rates (50%,75%, 50%, and 83.3%) against kanamycin, neomycin, gentamycin, and streptomycin ,respectively. These findings were similar to resistance rates that recorded by *(40,43)* they recorded

resistance rates (77% ,and 69.24%) against kanamycin. Lower resistance rate (23.08%) against neomycin was recored by **(40).** Also, lower resistance rates (35%, and 14%) against gentamycin were recorded by *(40,45).* Similar results of resistance against streptomycin (81%, and 100%) were recorded by *(41,43),* respectively. While lower resistance rate (43%) was detected by *(42).*

Regarding resistance rates of doxycyclin, tetracycline, and oxytetracyclin resistance rates were(96.7%, 95%, and 96.7%), respectively. This result agreed with the result recorded by*(43)* against doxycyclin(88%), meanwhile disagreed with the result obtained by *(40)* against doxycyclin (53.75%) .The resistance profiles against tetracycline agreed with that recorded by *(32,40)* they recorded (94%, and100%), respectively against tetracycline. Lower resistance rates against tetracycline (45%, and 33%) were reported by *(42,44),* respectively. Resistance rates against oxytetracyclin was in agreement with that recorded by *(43)* who recorded resistance rate (95%) against oxytetracyclin.

S. *aureus* is well established as a clinical and epidemiological pathogen and the potentially pathogenic role of S. *aureus* as a food-borne pathogen should not be neglected. Antibiotic-resistant isolates might be transmitted to humans by the consumption of food products containing such resistant and multi-resistant bacteria *(46).*

Concerning antibiotic sensitivity of CPS isolates results were shown in (Table7). Resistance rates toward penicillin ,amoxicillin, ampicillin, and oxacillin were 56% , 30%, 12% , and 85% , respectively. Higher resistance rate to penicillin (100%) was detected by *(47)* .In addition, they recorded antibiotic resistance rate to amoxicillin 23%. Higher resistance rates to ampicillin (100%) were detected by *(33 , 37)* . Lower resistance rate against ampicillin (53%) was detected by *(32).* Lower resistance rates to oxacillin (19%) was recorded by *(46).*

Table 7. Antibiotic Susceptibility Pattern Of Coagulase Positive Staphylococci Isolates Tested By Disc Diffusion Technique

Antibiotic group	SN	Chemotherapeutic agent	Susceptible S	Intermediate I	Resistant R
Penicillins	1	Penecillin "P" (10ug)	40.0%	4.0%	56.0%
	2	Amoxicillin "AMX" (25ug)	68.0%	2.0%	30.0%
	3	Ampicillin "AMP" (10 ug)	88.0%	0%	12.0%
	4	Oxacillin "OX" (1ug)	10.0%	5.0%	85.0%
Cephalosporins	5	Cefotaxim "CTX" (30ug)	30%	4.0%	66.0%
Quinolones	6	Norfloxacin "NOR" (10ug)	54.0%	10.0%	36.0%
	7	Ciprofloxacin "O" (5ug)	75.0%	0%	25.0%
Aminoglycosides	8	Neomycin "N" (30ug)	80.0%	10.0%	10.0%
	9	Gentamycin "GM" (.10 ug)	32.0%	0%	68.0%
	10	Streptomycin "S" (10 ug)	20.0%	5.0%	75.0%
Tetracyclins	11	Tetracyclin "TE" (30 ug)	10.0%	5.0%	85.0%
	12	Oxytetracyclin "OT" (30ug)	5.0%	0%	95.0%

Also, antibiotic resistance to cefotaxim was 66%. Higher resistance rate to cefotaxim (100%) was recorded by *(37).* Antibiotic susceptibility profile to norfloxacin, and ciprofloxacin revealed 36%, and 25%, respectively. Nearly similar resistance rate (41%) was detected to ciprofloxacin by *(32)* . Lower resistance rate to ciprofloxacin (15.4%) was detected by *(47).*While no resistance (0%) was detected by *(37).*

Studying resistance profile of neomycin, gentamycin, and streptomycin revealed resistance rates (10%, 68%, and 75%, respectively). The results of antibiotic susceptibility against the studied aminoglycosides were in disagreements with

those detected by *(33)* who recorded resistance rates of (0%) against both streptomycin ,and gentamycin.

This study revealed high antibiotic resistance to tetracycline, and oxytetracyclin with resistance rates (85%, and 95%, respectively). In addition similar antibiotic resistance rates of CPS isolates (100% , and 85%) to tetracycline were recorded by *(32, 47)* ,respectively. On the other hand, lower resistance to oxytetracyclin (28%) was detected by *(32)*.

Table 8. Molecular profile for enterotoxin and antibiotic resistance genes of Salmonella isolates by PCR .

Serial NO.	Strain code	*Stn* gene (617 bp)	Antibiotic resistance genes			
			Aac (113bp)	*tet* (A) (576bp)	βla*tem* (516bp)	*qnR*s (417 bp)
1	1	+ve	-ve	+VE	+VE	-VE
2	2	+ve	-ve	+VE	+VE	-VE
3	55(4)	+ve	-ve	-VE	-VE	-VE
4	55(5)	+ve	-ve	+VE	-VE	-VE
5	5	+ve	-ve	-VE	-VE	-VE
6	2	+ve	-ve	+VE	-VE	-VE
7	7	+ve	-ve	-VE	-VE	-VE
8	6	+ve	-ve	+VE	+VE	-VE
9	55(3)	+ve	-ve	-VE	-VE	-VE
10	4	+ve	-ve	+VE	+VE	-VE
Detection rate		100%	0%	60%	40%	0%

Table 9. Molecular profile for enterotoxin and antibiotic resistance genes of E.*coli* isolates by PCR .

Serial NO.	Strain Code	*STX*1 (614bp)	*STX*2 (779bp)	Antibiotic resistance genes			
				Aac (113bp)	*tet* (A) (576bp)	βla*tem* (516bp)	*qnR*s (417)
1	Z18	-ve	-ve	-ve	+ve	+ve	+ve
2	H61	-ve	-ve	-ve	+ve	-ve	+ve
3	20 LV	-ve	-ve	+ve	+ve	+ve	+ve
4	H3	-ve	+ve	+ve	+ve	+ve	-ve
5	T2	-ve	+ve	-ve	+ve	+ve	-ve
6	2L	-ve	-ve	-ve	+ve	+ve	-ve
7	E22	-ve	-ve	+ve	+ve	+ve	+ve
8	E31	-ve	-ve	-ve	+ve	+ve	-ve
9	E49	-ve	-ve	-ve	+ve	+ve	-ve
10	Z9	-ve	-ve	-ve	+ve	+ve	-ve
11	2LV	-ve	-ve	-ve	+ve	+ve	+ve
12	1L	-ve	-ve	-ve	+ve	+ve	-ve
13	1LV	-ve	-ve	-ve	+ve	+ve	+ve
14	H1	-ve	-ve	-ve	+ve	+ve	+ve
15	21 LV	-ve	-ve	-ve	+ve	+ve	-ve
Detection rate		0%	13.33%	20%	100%	93.3%	46.67%

Table 10. Molecular profile for enterotoxin and antibiotic resistance genes of Coagulase Positive Staphylococci isolates by PCR.

Serial No.	Strain code	Staphylococcal enterotoxin genes					Antibiotic resistance genes		
		sea (102 bp)	*seb* (164bp)	*sec* (452bp)	*Sed* (274bp)	*see* (209bp)	*Aac* (113bp)	*Tet*(k) (360bp)	*BlaZ* (173bp)
1	Z10	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
2	E 100	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
3	Z9	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
4	6	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
5	33	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
6	H5	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
7	Z14	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
8	40	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
9	E96	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
10	23	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
11	31	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
12	Z13	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve
13	24	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
14	Z15	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
15	103	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
Detection rate		0%	60%	86.67%	20%	0%	0%	100%	100%

The isolates involved in this study were tested by conventional PCR technique to investigate the presence of enterotoxins as well as antibiotic resistance genes.

It has been proposed that Salmonella enterotoxin gene (*stn*) is a putative virulence factor and the causative agent of diarrhea ***(48)***. In the present study *stn* gene was tested in the ten salmonella isolates .The results revealed that 100% salmonella isolates were positive for *stn* gene .These findings confirmed that *stn* gene is widely distributed among Salmonella irrespective to the serovars and source of isolation, the same finding was concluded by ***(22)***.Other reports highlighted the possibility of using this gene in routine screening of pathogenic Salmonellae. As the ***stn*** gene contained a unique sequence for Salmonella spp. the role of ***stn*** in Salmonella virulence is still debated. Some research groups reported that, there was no detected differences of virulence phenotypes between wild-type and an ***stn*** gene-deleted (*Δstn*) mutant ***(49)***. However a recent published data indicated that ***stn*** regulates the localization and levels of *Omp*A gene in Salmonella that is important for maintenance of membrane integrity, and in turn for bacterial homeostasis ***(50)***. Salmonella isolates were tested for the presence of antibiotic resistance genes using PCR. Phenotypic and genotypic study for antibiotic profile were in agreement for penicillins, and tetracyclins ; As results revealed that zero%, 60% , 40% and zero% of the tested isolates were positive for *Aac*, *tet*A, bla^{TEM} , and *qnR*s, respectively. While, phenotypically 30%, 20%, 70% and 20% of the tested isolates were resistant to gentamycine, doxycycline, penicillin and ciprofloxacin, respectively. The accordance of phenotypic and genotypic results for penecillins and tetracyclins was also recorded by ***(51)*** as they found that 90.5% of the penicillin resistant isolates carried the bla^{TEM} gene, and that 100% of the tetracycline resistant isolates carried the *tet*A gene.While the results of this study failed to pointed out the association between antibiotic resistance and enterotoxin genes. ***(52)*** stated that 100% of Salmonella *enterica* investigated were found to carry *stn* gene as examined by PCR, and that

81.2% possessed the bla^{TEM}, *tet*A and *aac* (6')-Ib-cr resistance genes.

Estimating the statistical association using chi-square and Cramer'sV analysis revealed significant statistical association between the demonstrated phenotypic and genotypic antibiotic resistance and also between the studied antibiotic resistance genes and salmonella enterotoxin gene *stn* where $P < 0.05$, (Figure 1).

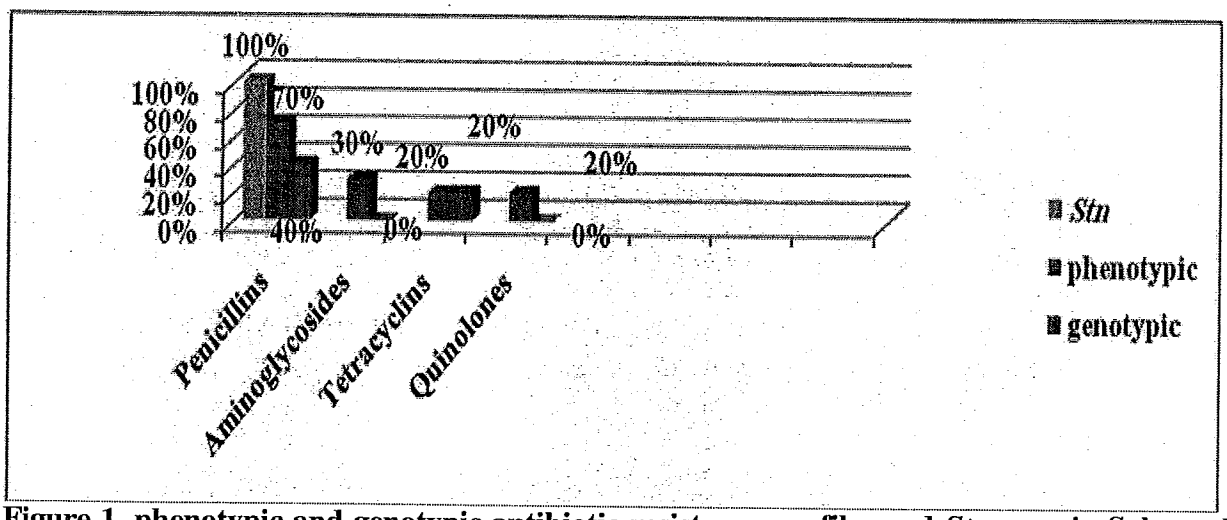

Figure 1. phenotypic and genotypic antibiotic resistance profiles and *Stn* gene in Salmonella isolates.

Shiga toxin producing Escherichia *coli* (STEC) have emerged as important enteric foodborne zoonotic pathogens of considerable public health significance, worldwide. STEC comprise a diverse group that elaborate one or both Shiga toxins (*stx*1 and *stx*2) which can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in human beings *(53)*. In the present study ***stx***1, and ***stx*** 2 genes were tested in fifteen E.*coli* isolates which were selected on the basis of having the highest phenotypic resistance antibiotic profile. The results showed that none of these isolates carry ***stx***1 gene. These findings agreed with the results were reported by *(54)*.In contrast, these results were in disagreement with those reported by *(55)* who reported the presence of ***stx***1 gene in 97.56% of the tested E.*coli* isolates .Out of tested fifteen tested E.*coli* isolates, PCR detected *stx*2 gene in two isolates only .This low incidence rate agreed with that recorded by *(55)* who found 5 isolates out of 82 were positive for presence of *stx*2. The low detection rate of *stx* genes among the tested E.*coli* isolates of the present study was reasonable due to the failure of the present study to isolate " O 157 " which is considered as the most implicated Shiga toxin producing serotype in poultry meat as recorded by *(55)* . In the present study PCR results and phenotypic antibiotic susceptibility profiles were identical for tetracyclins, penicillins, and quinolones in the studied E.*coli* isolates. PCR results of antibiotic resistance genes for E.*coli* isolates revealed that 20%, 100%, 100% and 46.67% of the tested isolates were positive for *Aac*, *tet*A, bla^{TEM} , and *qnRs* genes ,respectively. While, phenotypically 50%, 75%, 95% and83% of the tested E.*coli* isolates were resistant to neomycin, tetracyclin, ampicillin and ciprofloxacin, respectively. **(5)**,and**(1)** reported that the consistently higher prevalence of antimicrobial resistance observed in ETEC was confirmed by the presence of associations between antimicrobial resistance and virulence genes. They applied a more detailed analysis which showed that the strongest associations were between *tet*A and *est*A genes, and between *tet*A and *paa* genes. They also concluded that the use of antimicrobial agents may select for bacteria carrying virulence genes, and stated that associations generate

hypotheses about the physical relationships between genes that dictate coselection.

Estimating the statistical association using chi-square and Cramer'sV analysis revealed that significant statistical association between phenotypic and genotypic antibiotic resistance was estimated between each of ampicillin , ciprofloxacin and neomycin, and bla^{TEM} gene ,*qnRs* gene, and *Aac* gene, respectively where $P < 0.05$. No statisitical significant association was estimated between tetracycline and *tet*A gene. Statistical association was also estimated between all tested antibiotic resistance genes and *Stx* 1 and *Stx* 2 genes with $P < 0.05$, no significant statistical association was estimated between *Aac* gene and *Stx*1, where $P > 0.05$.

The result of insignificant statistical association estimated between phenotypic resistance against tetracycline and genotypic detection of *tet*A gene was justified by the scientific fact recorded by ***(56)*** who reported that a threat may be posed by isolates with silent, but intact, antibiotic resistance genes, such isolates possess wild type genes that are not expressed, but may convert to resistance by activating expression of the silent genes ,of those silent acquired resistance genes; *aadA*, *bla* (OXA-2), *strAB*, *sul1*, and *tet(A)* genes.In addition, ***(57)*** concluded that resistance of gram negative enteric bacteria as E.*coli* and salmonella were mainly mediated by *tet*AC with a few isolates displaying *tet*A, B, D, G, E, H, and J. They observed that *tet*A is located frequently on transposons such as Tn1721, and the gene has been widespread among Gram-negative bacteria, the mechanism of resistance by chromosomal genes is activated by induction or mutation caused by the stress of exposure to antibiotics in natural and clinical environments ; thus confirm the switching from silent to expressing state of *tet*A gene in response to exposure to antibiotics.

It is well documented that strains of Staphylococcus *aureus* produce a variety of extracellular toxins, including enterotoxins. Six groups of staphylococcal enterotoxins (SEs) have been recognized: SEA, SEB, SEC, SED, SEE, and SEG. These enterotoxins are small peptides (26 to 29 kDa) and have a great deal of similarity at the amino acid level . They are the main source of food poisoning and cause intensive intestinal peristalsis, ***(21).***

In the present study 15 Staphylococcal isolates were tested for presence of *sea* , *seb,sec* ,*sed,* and *see* enterotoxins genes. Isolates were selected on the basis of having the highest phenotypic antibiotic resistance profiles. The results revealed that non of the tested isolates were positive for neither *sea* nor *see* genes. While 9, 13, and 3 staphylococci isolates were positive for *seb, sec,* and *sed* genes, respectively. ***(58)*** concluded that there is no prevailing type of SEs, apart from the strains isolated from foods involved in staphylococcal gastroenteritis. However, ***(59)*** determined that, 100% of 146 S. *aureus* isolates harbored SE genes, they also determined that 36 (11.7%) isolates out of the investigated 146 isolates had *sea* genes. ***(60)*** determined that 37 (22.3%) out of 166 S. a*ureus* isolates harbored *sea, seb*, and *sec* genes ; They also recorded that *sea* was the most frequent detected SE gene as it was detected among 32 (86.5%) out of the tested 37 staphylococci isolates; Those results were in disagreement with the findings of the present study. It is worth to observe that genes encoding SEs have different genetic supports, most of which are mobile genetic elements such as; phages (*sea, see*), transposons (*seb*), plasmids (*seb* and *sed*), pathogenicity islands (*seb,* and *sec*) or chromosomal genes (*seb*). Thus may share in the explanation of the variety and differences of its incidence recorded among researches and studies. PCR results of antibiotic resistance genes of the tested staphylococci isolates revealed that zero %, 100%, and 100% of the tested isolates were positive for *Aac, tet*K, and *bla*Z, respectively. While, phenotypically 68%, 85%, and 56% of the tested isolates were resistant to gentamycin, tetracyclins, and penicillins, respectively .The prevalence of multidrug resistant coagulase positive staphylococci isolates which carried enterotoxin genes was in accordance with that recorded by ***(6)*** who used PCR to identify *sea, seb, sec, sed,* and *see* genes and revealed that 70% of the isolates harbored at least one enterotoxin gene, with *sea* and *sed* being the most frequently encountered ones.They also

reported that high frequency of isolates resistant to penicillin, teicoplanin, oxacillin, and clindamycin was observed, and 80% of CPS were found to be resistant to at least one of the 11 tested antimicrobials. The results of investigating Coagulase Positive Staphylococci also agreed with that reported by *(61)* who recorded that staphylococcal isolates tested in their study revealed resistance phenotypes to antibiotics widely administered in humans, such as tetracycline and that forty strains were positive for at least 1 enterotoxin-encoding gene, the genes most frequently detected were *sea* (28.6%) and *seb* (27.5%). The results also agreed with *(37)* indicated that enterotoxigenic are more resistant to antibiotic than non enterotoxigenic staphylococci isolates.

Estimating the statistical association using Chi -square and Cramer'sV analysis revealed significant statistical association between the detected phenotypic and genotypic antibiotic resistance patterns and also between the studied antibiotic resistance genes and enterotoxin genes SEs where $P < 0.05$,(Figure2).

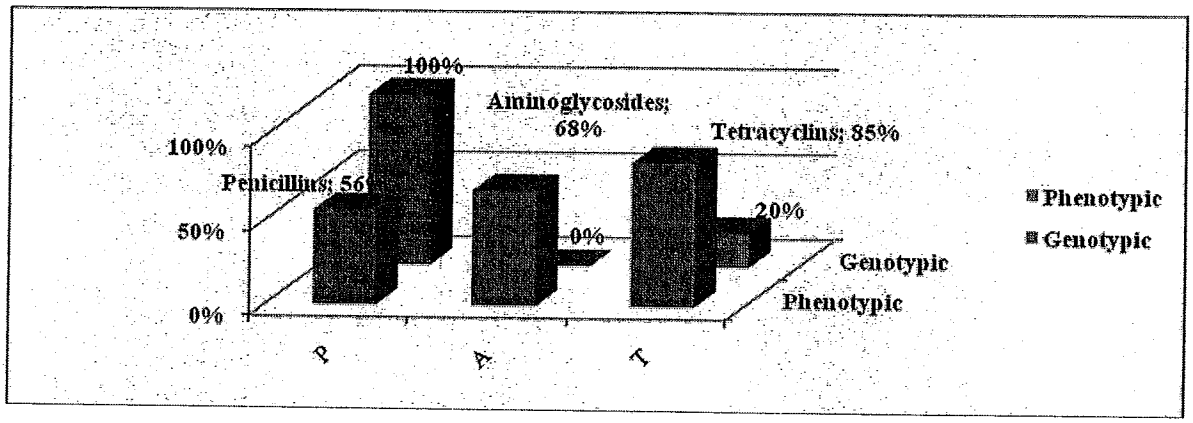

Figure 2. Genotypic vs phenotypic resistance profile of Staphylococci isolates.

Finally, it could be concluded that multidrug resistant E.*coli* , Salmonella ,and Staphylococci are distributed throughout poultry, and poultry products . Those multidrug resistant strains are proven to be carrier of enterotoxin genes; Imposing a serious potential biological risk to public health. It's recommended to conduct further studies to support the generation of an acceptable solid conclusion about the associations between acquisition of enterotoxins genes , and antibiotic resistance genes in food poisoning implicated bacteria. It's also recommended to verify and reactivate monitor and control biosecurity programs ,and food safety programs in poultry farms ,and poultry products, respectively.

REFERENCES

1. ***Rosengren, L. B.; Waldner, C. L. and Smith, R. J. R. (2009):*** Associations between Antimicrobial Resistance Phenotypes, Antimicrobial Resistance Genes, and Virulence Genes of Fecal *Escherichia coli* Isolates from Healthy Grow-Finish Pigs. Appl Environ Microbiol. 2009 March; 75(5): 1373–1380.Published online.
2. ***Anette, M. H. and Ole, E. H. (2008):*** Human Health Hazards from Antimicrobial-Resistant *Escherichia coli* of Animal Origin, Oxford Journals, Medicine, Clinical Infectious Diseases.2008; Volume 48, Issue 7, Pp. 916-921.
3. ***Castanon, J. I.(2007):***History of the use of antibiotic as growth promoters in European

poultry feeds. Poultry Science. 2007; 86, 2466–2471.

4. ***Cretikos, M.; Telfer, B. and McAnulty, J. (2008):*** Enteric disease outbreak reporting, New South Wales, Australia, 2000 to 2005. NSWPublic Health Bullettin. 2008; 19(1–2), 3–7.

5. ***Patrick, B.; Rebeccah, T.; Carlton, L. G.; Richard, R.; Nicol, J. H. L.; Vivian, N.; Scott, A. M.; Robert, F. and Archambault, M. (2005):*** Antimicrobial resistance and virulence genes of Escherichia *coli* isolates from swine in Ontario. Appl Environ Microbiol. 2005 November; 71(11): 6753–6761.

6. ***Martins, P.D.; Almeida, T.T., Basso, A.P.; Moura, T.M.; Frazzon, J.; Tondo, E.C. and Frazzon, A.P. (2013):*** Coagulase-positive staphylococci isolated from chicken meat: pathogenic potential and vancomycin resistance.Foodborne Pathog Dis. 2013; Sep;10(9):771-6.

7. ***Akond, M. A.; Momena S.; Saidul ,A.; Hassan, S. M. R.; Rahman, M.d. M. and Mozammel ,H. (2006):*** Frequency of drug resistant Salmonella spp. isolated from poultry samples in Bangladesh.Stamford Journal of Microbiology, December 2012. Vol. 2, Issue 1,ISSN: 2074-5346.

8. ***Alambed,J.I.;Fofana,R.B.A.;Seydi,M.,and Akakpo,A.J. (2006):*** Antimicrobial Resistance of Salmonella isolated from poultry carcasses inDakar (Senegal). Braz J Microbiol .2006;37 (4): 57-61.

9. ***Dinjus,U.; Hänel,I.; Müller,W.; Bauerfein d,R. and Helmuth,R.(1997):*** Detection of the induction of Salmonella enterotoxin gene expression by contact with epithelial cells with RT-PCR. FEMS Microbiol Lett. 1997 ;Jan 15;146(2):175-9.

10. ***ISO 6579: (2002), COR: (2004).*** Microbiology of food and animal feeding stuffs- Horizontal method for the detection of Salmonellaspp. 4th Edition. www.iso.org/iso/

11. ***Kreig, N.R.; Holt, J.G.; Williams and Wilkins.(1984):*** Bergeys Manual of Systematic Bacteriol 1984.,1:428, East Preston street, Baltimor, M.D.21202, USA.

12. ***ISO 6888-1:(1999) , AMD :(2003).*** Microbiology of food and animal feeding stuffs —Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)

13. ***Quinn, P.J.; Carter, M.E.; Markey, B. and Carter, G.R. (1999):***Clinical Veterinary Microbiology. Mosby Publishng Col, London, UK,PP:327-344.

14. ***CLSI,(Clinical and Laboratory Standards Institute (2011):*** Performance Standards for Antimicrobial Susceptibility Testing; Twenty First Informational Supplement, M100S21 ,standard by Clinical and Laboratory Standards Institute, 01/01/2011 .

15. ***Randall, L.P.; Cooles, S.W.; Osborn, M.K.; Piddock, L.J.V. and Woodward, M.J. (2004):***Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica*isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy.*53, 208–216.

16. ***Colom, K.; Pèrez,J.; Alonso, R.; Fernández,A.;Lariño,E. and Cisterna, R. (2003):*** Simple and reliable multiplex PCR assay for detection of bla_{TEM},bla_{SHV} and bla_{OXA-1} genes in Enterobacteriaceae. FEMS Microbiology Letters 223 (2003) 147-151.

17. ***Lunn, A.D.; Fàbrega, A.; Sánchez, J. and Vila, J. (2010):*** Prevalence of mechanisms decreasing quinolone-susceptibility among Salmonella spp. clinical isolates. International Microbiology. 13:15-20.

18. ***Robicsek, A.; Strahilevitz ,J.;Sahm, D.F.; Jacoby, G.A. and Hooper, D.C. (2006):*** qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates fromthe United States. Antimicrob Agents Chemother 50:2872-2874.

19. ***Dipineto, L.; Santaniello, A.; Fontanella, M.; Lagos, K.; Fioretti, A. and Menna, L.F. (2006):***Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Letters in Applied Microbiology 43 (2006) 293–295.

20. ***Duran, N.; Ozer, B.; Duran, G.G.; Onlen, Y. and Demir, C. (2012):*** Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J Med Res 135, pp 389-396.

21. ***Mehrotra, M.; Wang, G. and Johnson, W.M. (2000):*** Multiplex PCR for Detection of Genes for Staphylococcus *aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin1,and Methicillin Resistance. journal of clinical microbiology.Vol.38, No. 3,1032-1035.

22. ***Murugkar, H.V.; Rahman, H. and Dutta, P.K. (2003):*** Distribution of virulence genes in *Salmonella* serovars isolated from man & animals. Indian J Med Res.,117:66-70

23. ***CDCP ;Centers for Disease Control and Prevention, MMWR Editorial. Compendium of Measures to Prevent Disease Associated with Animals in Public Settings,(2011):*** Recommendations and Reports / Vol. 60 / No. 4 May 6, 2011. National Association of State Public Health Veterinarians, Inc.U.tteeveCeteootoreet.

24. ***Kusumaningrum, H. D.; Suliantari and Dewanti, H. R. (2012):*** Multidrug resistance among different serotypes of Salmonella isolates from fresh products in Indonesia. International Food Research Journal 19(1): 57-63 (2012).

25. ***Getenet, B.; Satheesh, N.; Daniel, A.; Yohannes, M.; Howard, E. and John, W.(2011):*** Multidrug resistant Salmonella Concord is a major cause of salmonellosis in children in Ethiopia . J Infect Dev Ctries 2011; 5(1):023-033.

26. ***Lillehaug, A.; Monceyron, J. C.; Bergsjø, B.; Hofshagen, M.; Tharaldsen, J.; Nesse, L.L. and Handeland, K. (2005):*** Screening of feral pigeon (Colombalivia), mallard (Anasplaty-rhynchos) and graylag goose (Anseranser) populations for Campylobacter spp., Salmonella spp., avian influenza virus and avian paramyxo virus. Acta Vet Scand., 46(4): 193-202.

27. ***Ismail, Rehab, I.H.(2013):*** Some Studies On Bacterial Causes Of Respiratory Troubles In Ducklings .M.V.Sc.Thesis (Avian and Rabbit Medicine Dept), Fac.Vet. Med.;Zagazig University.

28. ***Swayne, D. E. (2013):*** Colibacillosis in Diseases of Poultry, Thirteenth Edition, ch18; 751-806

29. ***Beatriz, G.; Ernst, J.; Andreas, S.; Reiner, H.; Beatriz, E. C. G. and Lothar, B.(2006):*** Phenotypic and genotypic characterization of antimicrobial resistance in Escherichia coli O111 isolates. Journal of Antimicrobial Chemotherapy (2006) 57, 1210–1214 doi:10.1093/jac/dkl127 Advance Access publication 7 April 2006.

30. ***Anette, M. H. and Ole, E. H.(2014):*** Human Health Hazards from Antimicrobial-Resistant Escherichia coli of Animal Origin, Frederick J. Angulo, Section Editor. Downloaded from http://cid. oxfordjournals.org/ by guest on October 25, 2014.

31. ***E U. Regulation (2003):***European Union. Regulation no. 1831/2003 of the European parliament and of the council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Union 2003.

32. ***Ahmed, G.; Zunita, Z.; Saleha, A. A.; Siti, K. B.;Jalila A. and Sharina O. (2012):*** High Prevalence Of Multidrug Resistant Bacteria In Selected Poultry Farms in Selangor, Malaysia. Asian Journal Of Animal and Veterinary Advances 7(9):891-897.

33. ***Okonko, O.; Nkang, A.O. ; Eyarefe, O.D. ; Abubakar, M.J. ; Ojezele ,M.O. and Amusan ,T.A. (2010):***Incidence of Multi-Drug Resistant (MDR) Organisms in Some Poultry Feeds.Sold in Calabar Metropolis, Nigeria. British Journal of Pharmacology and Toxicology 1(1): 15-28.

34. ***M'ikanatha, N.M.; Sandt, C.H.; Localio, A.R.; Tewari, D.; Rankin, S.C.; Whichard, J.M.; Altekruse, S.F.; Lautenbach, E.; Folster, J.P.; Russo, A.; Chiller, T.M.; Reynolds, S.M. and McDermott, P.F. (2010):*** Multidrug-resistant Salmonella isolates from retail chicken meat compared with human clinical isolates. Foodborne Pathog Dis. 2010 Aug;7(8):929-34.

35. ***Muhammad ; Akond , A.; Saidul, A.; Hassan , S.M.R. and Momena, S. (2009):*** Antibiotic Resistance of *Escherichia Coli* Isolated From Poultry and Poultry Environment of Bangladesh. Internet Journal of Food Safety, Vol.11, 2009, p. 19-23.

36. ***Arathy, D.S.; Vanpee, G.; Belot, G.; Mathew, V. C.; DeAllie, R. and Sharma (2011):*** Antimicrobial Drug Resistance in Escherichia *coli* Isolated from Commercial.Chicken Eggs in Grenada, West Indies. West Indian Med J 2011; 60 (1): 54.

37. ***Suleiman,A.;Zaria,L.T.;Grema,H.A. and Ahmadu, P.(2013):***Antimicrobial resistant coagulase positive Staphylococcus aureus from chickens in Maiduguri,Nigeria Sokoto Journal ofVeterinary Sciences, Volume 11 (Number 1). June, 2013 .

38. ***Anacarso, I.; Condò, C.; Sabia, C.; Messi, P.; Niederhausern, S. ;Bondi, M. and Iseppi, R. (2013):***Antimicrobial Resistance and Other Related Virulence Factors in Staphylococcus Spp isolated from Food, Environmental and Humans in Italy .Folia Microbiologica 09/2013, Universal Journal of Microbiology Research. 07/2013; 1(1):1-9.

39. **Akond, M. A.; Momena, S.; Alam, S. ; Hassan, S. M. R. ; Mahbubur, M.d.; Rahman and Hoq, M. (2012):** Frequency of drug resistant Salmonella spp. isolated from poultry samples in Bangladesh. Stamford Journal of Microbiology, December 2012. Vol. 2, Issue 1.ISSN: 2074-5346.

40. ***Hassan, M.M. ;Khaled, B. A.; Ahaduzzaman, M.d. ; Alam, M.; Faruk, S. A. M.d.and Inkeyas, U.(2014):*** Antimicrobial Resistance Pattern against E.*coli* and Salmonella in layer poultry. Research Journal for Veterinary Practitioners 2 (2): 30 -35.

41. ***José, V. L.;Liliane, V. M.;Danielle, C. O. N.; Roberta, F. V.; Batistaa, J. E. C. ; Silvaa, A. F. B. ; Ralpha, M. T.; Vaz, R. V. ; Rabellob, C.B.; Silvac, I. M. M. and Evêncio, J. (2013):***Zoonotic potential of multidrug-resistant extraintestinalpathogenic Escherichia *coli* obtained from healthy poultrycarcasses in Salvador, Brazil. Brazj infect dis . 2 0 1 3; 1 7(1):54–61.

42. ***Gregova, G. ; Kmetova, M. ; Kmet, V.; Venglovsky, J.; Feher, A. (2012):*** Antibiotic resistance of Escherichia coli isolated from a poultry slaughterhouse. Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 1, 75-77.

43. ***Zahraei, T.; Salehi and Bonab ,F. S. (2006):***Antibiotics Susceptibility Pattern of Escherichia coli Strains Isolated from Chickens with Colisepticemia in Tabriz Province, Iran. International Journal of Poultry Science 5 (7): 677-684, 2006 ,ISSN 1682-8356,© Asian Network for Scientific Information, 2006 .677.

44. ***Ružauskas, M.; Šiugždinienė, R. ; Krikštolaitis, R.; Virgailis,M. and Zienius M. D. (2010):***Prevalence and antimicrobial resistance of e. *coli* isolated from chicken liver sold in retail markets. ISSN 1392-2130. veterinarija ir zootechnika (Vet Med Zoot). T. 52 (74). 2010.

45. ***El-Rami, F. E.; Rahal, E. A. ; Sleiman ,F. T. and Abdelnoor, A. M. (2012):*** Identification of Virulence Genes amongAntibacterial-Resistant Escherichia coli . Isolated from Poultry, Advanced Studies in Biology, Vol. 4, 2012, no. 8, 385 – 396.

46. ***Ortega, E. ; Abriouel, H.; Lucas, R. and Gálvez, A. (2010):*** Multiple Roles

of Staphylococcus aureus Enterotoxins: Pathogenicity, Superantigenic Activity, and Correlation to Antibiotic Resistance. Toxins (Basel). Aug 2010; 2(8): 2117–2131.Published online Aug 10, 2010.

47. ***Otalu, O. J.R.; Junaidu, K.; Chukwudi, O. E. and Jarlath ,U.V. (2011):*** Multi-drug Resistant Coagulase Positive Staphylococcus aureus from Live and Slaughtered Chickens in Zaria, Nigeria. International Journal of Poultry Science 10 (11): 871-875, 2011.

48. ***Chopra, A. K.;Peterson, J. W.; Chart, P. and Prasad, R.(1994):*** Molecular characterization of an enterotoxin from Salmonella typhimurium.Microb.Pathog. 16, 85–98.

49. ***Wallis, T. S. M.; Wood, M.; Watson, P.; Paulin, S. and Jones, M. (1999):***Sips, Sops, and SPIs but not stn influence Salmonella enteropathogenesis. Adv. Exp. Med. Biol. 473, 275–280

50. ***Nakano, M. Yamasaki, E.Ichinose, A.; Shimohata, T. Takahashi ,A.; Akada, J.K.; Nakamura, K.; Moss, J.; Hirayama, T. and Kurazono, H. (2012):*** Salmonella enterotoxin (Stn) regulates membrane composition and integrity. Dis Model Mech. 5(4):515-21.

51. ***Hur , J.I.; Kim, J.H.; Park, J.H.; Lee ,Y.J. and Lee, J.H. (2011):***Molecular and virulence characteristics of multi-drug resistant Salmonella Enteritidis strains isolated from poultry. Vet J. 2011 Sep;189(3):306-11.

52. ***Rahman, H. (1999):***Prevalence of enterotoxin gene (stn) among different serovars of Salmonella. Indian J Med Res. 1999 Aug;110:43-6.

53. ***Gyles, C.L.(2007):*** Shiga toxin-producing Escherichia coli: an overview. J AnimSci 85:E45–E62.

54. ***Magwedere, K.; Dang, H.A.; Mills ,E.W.; Cutter ,C.N.; Roberts, E.L. and DeBroy, C. (2013):*** Incidence of Shiga toxin-producing Escherichia coli strains in beef, pork, chicken, deer, boar, bison, and rabbit retail meat. J Vet Diagn Invest. 2013 Mar;25(2):254-8.

55. ***Momtaz ,H. and Jamshidi ,A. (2013):*** Shiga toxin-producing Escherichia coli isolated from chicken meat in Iran: Serogroups, virulence factors, and antimicrobial resistance properties. Poult. Sci. 92 :1305–1313.

56. ***Enne ,V.I.; and Bennett, P.M. (2010):*** Methodsto determine antibiotic resistance gene silencing.Method Mol. Biol.; 642: 29-44.

57. ***Onyango, D. M.; Kakai, R. and Waindi, E. N. (2011):*** Tetracycline efflux pump indifferent *Salmonella enterica* isolated from diarrhea patients in two rural health centers in Western Kenya. Iranian Journal of Clinical Infectious Diseases .2011;6(1):24-30.

58. ***Geniogeorgis, C.A. (1989):*** Present state of knowledge on staphylococcal intoxication. IntJ Food Microbiol, 9: 327-360.

59. ***Omoe, K.; Ishikawa, M.; Shimoda ,Y.; Hu , D.; Ueda, S. and Shinagawa, K.(2002):*** Detection of *seg*, *seh*, and *sei*genes in *Staphylococcusaureus*isolates and determination of the enterotoxin productivities of *S. aureus*isolates harboring *seg*, *seh*, or *sei*genes. *JClinMicrobiol*, 40: 857-862.

60. ***Lim,S.; Joo,Y.; Moon, J.;Lee ,A.; Nam, H.; Wee, S.and Koh, H. (2004):*** Molecular typing of enterotoxigenic Staphylococcus *aureus* isolated from bovine mastitis in Korea. J VetMed Sci, 66: 581-584.

61. ***Moura ,T. M.; Campos ,F. S.; d'Azevedo, P. A.; Sand ,S.T. V. D.; Franco, A.C.; Frazzon ,J. and Frazzon, A. P. G. (2012):*** Prevalence of enterotoxin-encoding genes and antimicrobial resistance in coagulase-negative and coagulase-positive Staphylococcus isolates from black pudding. Rev. Soc. Bras. Med. Trop. vol.45 (5) :579-85.

الملخص العربي

دراسة عن الأرتباط بين مسببات التسمم المعوي ومقاومة المضادات الحيوية في البكتريا المعزولة من الطيور

سماح عيد عبد السلام * ،هبة بدر محمود* ، أحمد سامي أحمد**
باحث بكتريولوجيا*، باحث البيوتكنولوجيا**
المعمل المرجعي للرقابة البيطرية على الأنتاج الداجني،معهد بحوث صحة الحيوان.

أجريت دراسة نمط المقاومة للمضادات الحيوية على عدد ١٠ ،٦٠،٦٠ معزولة منتمية لميكروب السالمونيلا، الميكروب القولوني النموذجي والمكور العنقودي الذهبي على التوالي . تم عزل الميكروبات محل الدراسة من عينات متنوعة ذات أصل داجني . تبين أن جميع المعزولات ذات نمط متعدد المقاومة للمضادات الحيوية حيث أظهرت المعزولات مقاومة لأكثر من ثلاث عقاقير ينتمي كل منها الى مجموعة مختلفة من المضادات الحيوية . تم تطبيق اختبار انزيم البلمرة المتسلسل (PCR)على المعزولات التي أظهرت أعلى نسب مقاومة للمضادات الحيوية وذلك للأستدلال على تواجد جينات المقاومة للمضادات الحيوية وكذلك جينات التسمم المعوي.تبين أن جميع معزولات السالمونيلا تحمل جين التسمم المعوي ***stn*** بنسبة ١٠٠% .كما تحمل جينات *tet*A , bla^{TEM} بنسب ٦٠%و ٤٠% على التوالي . بينما لم يستدل على امتلاك أي من معزولات السالمونيلا لجيني المقاومة للمضادات الحيوية *Aac* أو *qnR*s . أكد اختبار ال PCR أن معزولات الميكروب القولوني النموذجي تحمل جينات المقاومة للمضادات الحيوية *Aac, tetA*, bla^{Tem} , *qnR*s بنسب 20%, 100%, 100% ,46.67% على التوالي .ثبت عدم امتلاك أي من معزولات القولوني النموذجي لجين الشيجا ١ بينما امتلكت ١٣,٣٣% من المعزولات لجين الشيجا ٢. فحص معزولات المكور العنقودي باستخدام ال PCR أثبت تواجد جينات *Aac,* *tet*K, and *blaZ* بنسب0% ,100% ,100% 0 على التوالي .جاءت نتائج الPCR سلبية لجيني التسمم المعوي نوعي *sea*، see بينما *تواجدت جينات sed* بنسب *seb, sec,* 20% ,86.7% ,60% من معزولات المكور العنقودي على التوالي.