Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

The prevalence of extended spectrum *β–lactamase* producing *E. coli* isolated from chickens in Sharqia Governorate

Ashraf A. Abd El Tawab [1], Ahmed S. A. Ibrahim[2], Reem I. Ali [3] *

[1]*Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Banha University, Banha, P.O. Box 13518, Egypt.*

[2] *Animal Health Research Institute, Agricultural Research Center, Dokki – Giza, Egypt.*

[3] *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Banha University, P.O. Box 13518, Egypt.*

ARTICLE INFO

Keywords

Antibiotic resistance

Chickens.

E. coli

ESBL

Received 27/02/2021
Accepted 22/03/2021
Available On-Line 01/04/2021

ABSTRACT

E. coli producing extended spectrum beta-lactamase (ESBL) in poultry has a major concern due to the possible transmission between them and human that may cause a public health threat. Our study is directed to estimate the prevalence of resistance pattern and characterization of ESBL reducibility and genes in *E. coli* isolated from chicken respiratory tract from chicken farms in Sharqia Governorate. 250 samples from 50 chicken farms were collected. Isolation and identification of *E. coli* serotypes were performed. In addition, Antibiotic Resistance Profile (ARP) against β-lactam antibiotics, ESBL phenotypic screening and PCR for ESBL genes; SHV and TEM, were done. The results showed that 140 isolates out of 250 total isolates (56%) were morphologically and biochemically positive for *E. coli*. Different sero-groups of isolated *E. coli* exhibited high resistance rates against 14 antibiotics. 35.7 % of *E coli* isolates were recorded to be phenotypically ESBL-positive producing bacteria. Genotypically, ESBL genes including *bla TEM*and *bla SHV* genes were detected in 50 *E. coli* samples in (100%) and 30 (60%), respectively. In conclusion, the high prevalence of *E. coli* producing ESBL genes in poultry farms of Sharqia Governorate would account for economic and public health threat in the society

1. INTRODUCTION

Chicken accounts for the greatest rate of farming among species, with meat productivity reaches over 90 billion tons per year(FAO 2017). Antibiotics are not only given to humans, food-producing animals and agricultural production to treat infections, they are also used in food animal production as growth promoters to improve productivity. The rapid rise of antibiotic resistant pathogens negates effective therapy.Extra-intestinal Pathogenic *E. coli* (ExPEC) is the systemic or local infection caused by avian pathogenic *E. coli* outside the gut. Colibacillosis due to Ex-PEC affects 4–6 weeks aged broiler chickens and was characterized by septicemia or sub- acute fibrinous air sacculitis, pericarditis, peritonitis, and salpingitis (Kabir 2010; Suarez et al. 2020). Colibacillosis has an a negative eminent economic impact on poultry production as it increases mortality rates, condemnation of diseased carcasses at slaughter houses, and prophylaxis and treatment cost (Kabir 2010).

Avian pathogenic *E. coli* showed different resistance patterns against antibiotics permitted for poultry including chloramphenicol, sulfonamides, tetracyclines, aminoglycosides, and fluoroquinolones (Rahman et al. 1970; Bass et al. 1999; Li et al. 2007). There is a cause for worry that ESBL genes will be developed in avian pathogenic *E. coli* strains (Zhao et al. 2001)

Commensal *E. coli* strains could develop antibiotic resistance (ABR) and disseminate the ABR genes to the pathogenic bacteria. This may contribute to the spread of resistant genes from poultry to human(Anon 1997; Aarestrup 1999; Schwarz et al. 2001; Golkar et al. 2014).

ESBLs are enzymes that are capable to destruct the beta-lactam ring of cephalosporins. They can hydrolyze 3rd generation cephalosporines such as ceftriaxone, ceftazidime, and cefotaxime. Globally, ESBLs are of major concern; where their frequencies are increasing in wide areas of the world (Ghafourian et al. 2012). A variety of drugs that is authorized to be used in veterinary medicine is responsible for the emergence of ESBL-producing Gram negative Enterobacteriaceae (Bush et al. 1995). *E. coli* producing ESBL are not only prevalent in poultry farms, but were also spotted in other farm animals and meat products (Doi et al. 2010). TEM and SHV are the most prevalent ESBL types(Cantón and Coque 2006). These ESBL genes that has been evidently noticed in food-producing animals and the food chain constitutes a possible pathway of transmission from animals to humans (Leverstein-van Hall et al. 2011).

It is very imperative to screen the resistance patterns to antibiotics not only in human pathogens but also in commensal and pathogenic bacteria in animals. The aim of present study was to determine the prevalence of ESBL-producing *E. coli* and genotypic characterization of the

* Corresponding author: gogo.hamozo@gmail.com.

ESBL-related *bla* genes, including, *bla TEM* and *bla SHV* of chicken farms in Sharqia Governorate.

2. MATERIAL AND METHODS

2.1. Sample collection, bacterial isolation and identification

A 250 samples (lung and trachea) were collected from diseased chickens showed clinical signs of salmonellosis and Colibacillosis and PM lesions, from 50 commercial intensive chicken farms (3,000 to 15,000 chicken) distributed throughout different localities in El Sharkia Governorate, Egypt, during the period of April 2018 to January 2019. These samples were collected aseptically in sterile bags and transported as soon as possible to the Reference Laboratory for Quality Control on Poultry Production (RLQP), Dokki, for bacteriological examination. Twenty-five grams of samples were homogenized in 250 mL of buffered peptone water (BPW)and incubated at 37 °C for 24 h for pre-enrichment (ISO 6579, 2002). The technique of isolation and identification of *E.coli* isolates is recommended by (Swayne 1998).

2.2. Serological identification of E. coli

According to Edwards and Ewing, (1986), *E. coli* isolates were serotyped in animal health research institute, Dokki, Giza. Polyvalent and monovalent diagnostic *E. coli* antisera were used.

2.3.Antimicrobial Susceptibility

All *E. coli* isolates were tested by agar diffusion for antimicrobial susceptibility against 14 antibiotics(Table 1) according to Clinical and Laboratory Standards Institute (CLSI) guidelines and were clinically categorized with breakpoints (CLSI,2017).Multi-resistant isolates i.e., resistant to 3 or more antimicrobial categories, were selected for further examinations(Magiorakos et al. 2012).

Table 1 Standard zone of inhibition of the antimicrobial agents used in antimicrobial susceptibility tests according to (CLSI, 2017).

Antimicrobial discs	Code	Disc Potency mg/disc	Interpretation Zone diameter (mm)		
			Resistant	Intermediate	Sensitive
Ampicillin (oxoid)	AMP	10 µg	≤13	14-16	≥17
Chloramphenicol (oxoid)	C	30 µg	≤12	13-17	≥18
Norfloxacin (oxoid)	NOR	10 µg	≤12	13-16	≥17
Ciprofloxacin (oxoid)	CIP	5 µg	≤15	16-20	≥21
Oxytetracycline (oxoid)	OT	30 µg	≤11	12-14	≥15
Sulphamethazole-trimethoprim (oxoid)	SXT	25µg	≤10	11-15	≥16
Colistinsulphate (oxoid)	CT	10 µg	≤10	----	≥11
Apramycin (oxoid)	APR	15 µg	≤12	13-14	≥15
Streptomycin (oxoid)	S	10 µg	≤11	12-14	≥15
Cephalexin (oxoid)	CL	30 µg	≤14	15-17	≥18
Cefotaxime (oxoid)	CTX	30 µg	≤14	15-22	≥23
Amoxicillin-clavulinic acid (bioanalyse)	AMC	30 µg	≤13	14-17	≥18
Ceftazidime (bioanalyse)	CAZ	30 µg	≤14	15-17	≥18
Doxycycline (oxoid)	DO	30 µg	≤10	10 -13	≥14

2.3. Phenotypic screening of ESBL

A Mueller-Hinton agar(Oxoid) plate were inoculated with confirmed multi-resistant strains in the form of bacterial suspension of 0.5 McFarland, and the cefotaxime and ceftazidime disks are applied. The inhibition zone surrounding the disk/tablet with the cephalosporin alone is compared to the zone around the cephalosporin disk/tablet combined with clavulanic acid. The test was considered to be positive if the zone around cephalosporin disk/tablet combined with clavulanic acid is ≥ 5 mm bigger than that around cephalosporin disk alone(CLSI, 2017).

2.4. Bacterial DNA extraction

The isolates were streaked on nutrient agar and incubated for 14-16 hr at 37 °C. A single colony was picked up from the media plate and inoculated to 5 ml liquid culture media, then incubated overnight at 37 °C. Genomic DNA was then extracted at the Reference Laboratory for Quality Control on Poultry Production (RLQP), using the G-spinTM Total DNA Extraction Kit (INTRON Biotechnology, Korea) according to the manufacturer's recommendation.

2.5. Detection of antibiotic-resistance genes

Oligonucleotide primers used in PCR were supplied from Metabion (Germany) and Biobasic (Canada) (Table 2)**.** 12.5 µL of Emerald AmpMax PCR MasterMix (Takara,Japan),6 µL of template DNA, 1 µL of 20 Pmol of each primer,and4.5 µL of water constitute the 25-µL master mix. Applied Biosystem 2720 thermal cycler was used to perform the PCR reaction.

The PCR conditions involved an initial denaturation for 3 min at 95 °C followed by 30 cycles of 95 °C for 30 s, specific annealing temperature for 1 min, and extension at 72 °C for 30 s) followed by a final extension at 72 °C for 5 min. Fifteen microliters of each PCR product were loaded in 1.5% agarose gel (Applichem). Electrophoresis was done in 1× TBE (Tris Boric acid EDTA) buffer using 5 V/cm gradients. A100 bp DNA ladder (Fermentas) was used to determine the fragment sizes. The PCR photos were photographed and analyzed by using a gel documentation system (Alpha-Innotech, Biometra, Germany) through its computer software.

Table 2 Oligonucleotide primers sequences sources

Gene	Primer sequence (5'-3')		Length of amplified product	Reference
blaTEM	F	ATCAGCAATAAACCAGC	516 bp	Colom *et al*., (2003)
	R	CCCCGAAGAACGTTTTC		
blaSHV	F	AGGATTGACTGCCTTTTTG	392 bp	
	R	ATTTGCTGATTTCGCTCG		

F: forward primer, R: reverse primer

3. RESULTS

3.1. The incidence of *E. coli*among the examined chicken samples:

A total of 250 chickens from 50 broiler and layer chickenfarms were examined for detection of *E. coli*. Table (3) showed that 56% of isolates were positive for *E. coli* while 110 isolates were found to be negative for *E. coli* (44%).

Table 3 Incidence of *E. coli* isolated from chickens.

Bacterial sp.	Number of farms	Number of samples	Results			
			+ve	%	-ve	%
E. coli	50	250	140	56	110	44

The percentage was calculated according to the total number of samples (250).

3.2. Results of serotyping of *E. coli* isolates:
E. coli isolates were serotyped using specific eight polyvalent, then 43 monovalent group O somatic antisera. (table 4)

3.3. Sensitivity of *E. coli* serotypes to different antimicrobial agents:
The antibiotic resistance profile of 140 strains of *E. coli*was made against 14 different antibiotic discs. As shown in tables (5) *E. coli* O groups were found to be 100% resistant to streptomycin, cephalexin, oxytetracycline and deoxycycline followed by ampicillin 94.6% and sulphamethazole-trimethoprim, cefotaxime and amoxicillin-clavulinic acid with a resistance percentage 92.9%. Twenty-four out of 140 strains of *E. coli* were resistant to apramycin, in addition to 115 strains (82.1%) exhibited resistance pattern against norfloxacin, ciprofloxacin and colistin sulphate. Ceftazidime showed the least resistance percentage (64.2%) to *E. coli* strains.

Table 4 Serotyping of *E. coli* strains isolated from chicken samples.

Monovalent E. coli serogroups	No.	%	*E. coli* O antigen serotypes
O86a	25	17.8	1
O55	10	7.1	2
O166	5	3.5	2
O111	25	17.8	1
O125	50	35.7	2
O127	10	7.1	1
O157	15	10.7	3

The percentage was calculated according to the total number of *E. coli*isolates (140).

Table 5 Results of antibiograms against *E.coli* strains isolated from chickens.

	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ampicillin, AMP, 10 µg, (Oxoid)	135	96.4	5	3.5	0	0
Chloramphenicol, C, 30µg, (Oxoid)	80	100	0	0	0	0
Norfloxacin, NOR, 10 µg, (Oxoid)	115	82.1	5	3.6	20	14.3
Ciprofloxacin, CIP, 5 µg,(Oxoid)	115	82.1	10	7.1	15	10.8
Oxytetracycline, OT, 30 µg, (Oxoid)	140	100	0	0	0	0
Sulphamethazole-trimethoprim, SXT, 25µg,(Oxoid)	130	92.9	5	3.6	5	3.6
Colistin sulphate, CT, 10 µg, (Oxoid)	115	82.1	0	0	25	17.9
Apramycin, APR, 15 µg, (Oxoid)	120	85.7	20	14.3	0	0
Streptomycin, S, 10 µg, (Oxoid)	140	100	0	0	0	0
Cephalexin, CL, 30 µg, (Oxoid)	140	100	0	0	0	0
Cefotaxime, CTX, 30 µg, (Oxoid)	130	92.9	10	7.1	0	0
Amoxicillin-clavulinic acid, AMC, 30 µg, (bioanalyse)	130	92.9	0	0	10	7.1
Ceftazidime, CAZ, 30 µg (bioanalyse)	90	64.2	20	14.2	30	21.4
Doxycycline, DO, 5 µg (Oxoid)	140	100	0	0	0	0

The percentage was calculated according to the total number of *E. coli*isolates (140).

3.4. Multidrug resistance and phenotypic ESBL production patterns for *E. coli*strains:
As presented in Table (6) the MAR Index analysis revealed that all *E. coli* isolates had a very high MAR index value (>0.2). O 86a, O 125, O55, O127 and O111 *E. coli* strains showed the highest MAR index (1.0). Phenotypically, 35.7 % of *E. coli* isolates were recorded to be ESBL producers.

3.5. Detection of *blaSHV* gene of *E. coli*:
Bla SHV is the gene responsible for resistance of the isolated *E. coli* to Beta-lactam antibiotics. 30 *E. coli* samples out of 50 isolates that are multidrug resistant and showed phenotypic ESBL productivity (60%) exhibiting positive amplification of 516 bp fragment of primer specific for (*blaSHV*) gene from the extracted DNA. Also, the positive control showed 516 bp fragments whereas no amplification were observed with the negative control (Figure 1).

3.6. Detection of *bla TEM* gene of *E. coli*:
BlaTEM gene is responsible for resistance of the isolated *E. coli* to beta-lactam antibiotics. 50 *E. coli* samples out of 50 isolates that are multidrug resistant and showed phenotypic ESBL productivity (100%) exhibiting positive for this gene. amplification of 516 bp fragment of primer specific for (*blaTEM*) gene from the extracted DNA. Also, the positive control showed 516 bp fragments, whereas no amplification was observed with the negative control (Figure 2).

Table 6 MARS index analysis of *E. coli* isolates.

	No.	No. of antibiotics to which the isolate was resistant (a)	MAR index(a/b)	Phenotypic ESBL production
O111	11	13	0.92	- ve
O157	15	12	0.85	- ve
O125	11	13	0.92	- ve
O86a	10	14	1	- ve
O125	22	14	1	- ve
O125	12	12	0.85	- ve
O111	11	12	0.85	- ve
O55	4	14	1	- ve
O127	7	8	0.57	- ve
O166	5	13	0.92	- ve
O55	6	13	0.92	+ ve
O86a	5	13	0.92	- ve
O125	5	10	0.71	- ve
O127	10	14	1	+ ve
O111	6	14	1	- ve
O86a	7	12	0.85	+ ve
O86a	3	11	0.78	+ ve

MAR: the multiple antibiotic resistance, a/b: 'a' represents the number of antibiotics to which the particular isolate was resistant and 'b' the number of antibiotics to which the isolate was exposed (14).

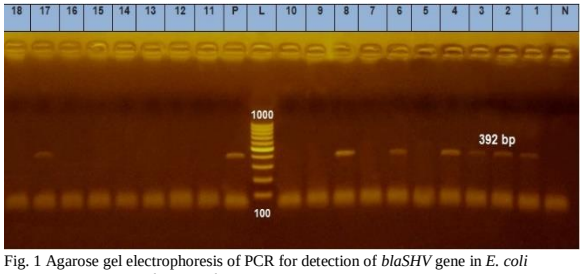

Fig. 1 Agarose gel electrophoresis of PCR for detection of *blaSHV* gene in *E. coli* isolates showing amplification of 392 bp in examined samples. L (Ladder): DNA ladder (100–1000 bp); Lanes 1-4, 6, 8, 17: positive samples. Pos: positive control; Neg: negative control.

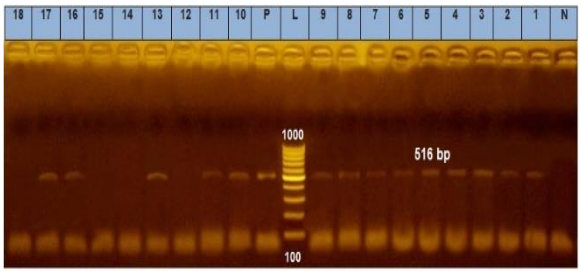

Fig. 2 Agarose gel electrophoresis of PCR for detection of *bla TEM* gene in *E. coli* isolates showing amplification of 516 bp in examined samples. L (Ladder): DNA ladder (100–1000 bp); Lanes 1-9,10,11,13,16,17: positive samples; Pos: positive control; Neg: negative control

4. DISCUSSION

The object of our study was to explore the prevalence of multi drug resistant pathogenic, or commensal *E. coli* isolated from the respiratory tract of infected chickens with CRD signs and screen the AR genes against the most importantly used antibiotic groups in human. These genes constitute a potential threat if transmitted to contact human through either the zoonotic or commensal bacteria.

Colibacillosis in poultry may be primary or secondary infections and induces a diversity of localized or systemic infections caused by *E. coli*(Rodriguez-Siek et al. 2005).

In this study, the prevalence of *E. coli* in chickens was 56%and the results may be due to high stocking rates of birds in poor aeriated houses or because of *Mycoplasma gallisepticum*infection of the flock, which lead to or exacerbated colisepticaemia. Environmental pressures and respiratory viral infections may also predispose chickens to the disease (Chamani 2013).Closely comparable result attained by Abd El Tawab et al., (2015) who reported that the incidence of *E. coli*in winter between inspected chickens was 60.9% which was more than that in summer 41%(El-Wanis. 2015).These results were agreed with that of Ruzauskas et al., (2010), where the prevalence of *E. coli* that contaminates raw chicken liver were 41.7%.Also, similar results obtained by Sarba et al., (2019), who isolated *E. coli* from 40.4% of samples from colisepticaemia chickens.

In this work, 140 out of 250 *E.coli* isolates obtained from chickens were sero-grouped in 7 O groups with the most chief serotype was *E.coli*O 125in 35.7 % (50/140) of all isolates and these results go hand to hand with the previous study where O125 was the most prevalent (61.3 %) sero-group associated with colibacillosis in poultry (Sarba et al. 2019).

Through this research, the antibiogram was carried out against different *E. coli* serotypes using 14 different antibiotic discs. The results revealed that, about 100 % of *E coli* isolates were found to be multi-resistant as they resist at least 3 antibiotics, this resistance pattern, the so called multiple antimicrobial resistance (> or =3 antimicrobials). *E coli* isolates exhibited high resistance profile against the 14 antibiotics. In previous Egyptian study performed on broiler chickens, it was detected a high phenotypic resistance rates of *E coli* to penicillin, streptomycin, trimethoprim/sulphamethoxazole, and tetracycline(El-Wanis. 2015).

E coli isolates (35.7 %) were recorded to be phenotypically ESBL-positive producing bacteria. In accordance to our results, in Sweden about 34.0% of broilers were stated to carry ESBL *E. coli* in their guts (Börjesson et al. 2013). 48.8% of *E. coli* isolates which were obtained from retail chicken meat shops were ESBL positive in Malaysia (Aliyu et al. 2016).

ESBL genes including *bla* TEM and *bla* SHV genes were prevalent in 50 *E. coli* samples (100%), 30 (60%), respectively. In agreement to this results, El-Wanis (2015) found the percentage of *bla* TEM gene from the isolated *E. coli* strains was 94.73% (18 out of 19 strains) which go hand with the results of Colom et al. (2003) who detected *bla* TEM gene in 45 out of 51 Amoxicillin-clavulanate resistant *E. coli* isolates with 88.2%. However, these results disagreed with Overdevest et al. (2011) who obtained lower percentage about 14%.Beta-lactamases encoding genes; *bla* SHV and *bla*TEM were prevalent in the APEC isolates in Jordan at a rate of 1.8 and 72.9%, respectively (Ibrahim et al. 2019). This varies from the results of Huijbers et al., (2014) in the Netherlands who found more incidence of *bla-SHV* (17%) but lower *blaTEM* (9.1%)among *E. Coli* producing ESBL in broiler and people existing or employed with broiler farms (El-Wanis. 2015).

5. CONCULOSIONS

The results of this study showed elevated prevalence of *E. coli* isolated from respiratory tract infected chicken especially O 125 in Sharqia Governorate. Phenotypically, *E. coli* isolates revealed high MARs profile against 14 antibiotic discs, in addition to ESBL producing capabilities. Two plasmid associated ESBL genes including *bla TEM* and *bla SHV* were screened and found to be eminently prevalent. The transfer of these genes to human comprises a great public health risk.

6. REFERENCES

1. Aarestrup, F. M. 1999. “Association between the Consumption of Antimicrobial Agents in Animal Husbandry and the Occurrence of Resistant Bacteria among Food Animals.” *International Journal of Antimicrobial Agents* 12(4):279–85.
2. Abd El Tawab, A., A. Abd El Aal, M. Mazied, and A. EL Morsy. 2015. “Prevalence of E. Coli in Broiler Chickens in Winter and Summer Seasons by Application of PCR with Its Antibiogram Pattern. ”*Benha Veterinary Medical Journal*29(2):119-128.
3. Aliyu, A. B., A. A. Saleha, A. Jalila, and Z. Zunita. 2016. “Risk Factors and Spatial Distribution of Extended Spectrum β-Lactamase-Producing- Escherichia Coli at Retail Poultry Meat Markets in Malaysia: A Cross-Sectional Study.” *BMC Public Health* 16(1):1–9.
4. Anon. 1997. “The Medical Impact of Antimicrobial Use in Food Animals. Report of a WHO Meeting.” 13–17.
5. Anon. n.d. *The Traditional Enteropathogenic Escherichia Coli (EPEC) Serogroup O125 Comprises Serotypes Which Are Mainly Associated with the Category of Enteroaggregative E. Coli*.
6. Bass, Lydia, Cynthia A. Liebert, Margie D. Lee, Anne O. Summers, David G. White, Stephan G. Thayer, and John J. Maurer. 1999. “Incidence and Characterization of Integrons, Genetic Elements Mediating Multiple-Drug Resistance, in Avian Escherichia Coli.” *Antimicrobial Agents and Chemotherapy* 43(12):2925–29.
7. Börjesson, Stefan, Maria Egervärn, Mats Lindblad, and Stina Englunda. 2013. “Frequent Occurrence of Extended-Spectrum Beta-Lactamase- and Transferable AMPC Beta-Lactamase-Producing Escherichia Coli on Domestic Chicken Meat in Sweden.” *Applied and Environmental Microbiology* 79(7):2463–66.
8. Bush, K., G. A. Jacoby, and A. A. Medeiros. 1995. “A Functional Classification Scheme for β-Lactamases and Its Correlation with Molecular Structure.” *Antimicrobial Agents and Chemotherapy* 39(6):1211–33.
9. Cantón, Rafael, and Teresa M. Coque. 2006. “The CTX-M β-Lactamase Pandemic.” *Current Opinion in Microbiology* 9(5):466–75.
10. Chamani, Mohammad. 2013. *Detection and Investigation of Escherichia Coli in Contents of Duodenum, Jejunum, Ileum and Cecum of Broilers at Different Ages by PCR*.
11. CLSI. 2017. *Performance Standards for Antimicrobial*.CLSI, Clinical and Laboratory Standard, and Institute. 2017. *Performance Standards for Antimicrobial Susceptibility Testing*.
12. Colom, Karmele, Javier PÃ©rez, Rodrigo Alonso, Agueda FernÃ¡ndez-Aranguiz, Eva LariÃ±o, and RamÃ³n Cisterna. 2003. “Simple and Reliable Multiplex PCR Assay for Detection of BlaTEM, BlaSHV and BlaOXA-1 Genes in Enterobacteriaceae.” *FEMS Microbiology Letters* 223(2):147–51.
13. Doi, Y., D. L. Paterson, P. Egea, A. Pascual, L. López-Cerero, M. D. Navarro, J. M. Adams-Haduch, Z. A. Qureshi, H. E. Sidjabat, and J. Rodríguez-Baño. 2010. “Extended-Spectrum and CMY-Type β-Lactamase-Producing Escherichia Coli in Clinical Samples and Retail Meat from Pittsburgh, USA and Seville, Spain.” *Clinical Microbiology and Infection*16(1):33–38.
14. Edwards, Philip, R. Ewing, William. 1986. “Edwards and Ewing's Identification of Enterobacteriaceae. ”*International Journal of Systematic Bacteriology*36(4) 581-582.
15. El-Wanis., Nehal Mahmoud Nabil; Ashraf Awad Abd El-Tawab; Ahmed Mohamed Ahmed Ammar; Soad Abd El-Aziz Abd. 2015. “MOLECULAR STUDIES ON ANTIMICROBIAL RESISTANCE GENES IN SALMONELLA ISOLATED FROM POULTRY.” *A Thesis Submitted for PhD Degree in*

Veterinary Medical Science)Bacteriology, Immunology and Mycology), Banha University.

16. FAO. 2017. "Food and Agricultural Organization Catalogue." *United Nations: Food and Agricultural Organization,*.
17. Ghafourian, Sobhan, Zamberi Sekawi, Vasanthakumari Neela, Afra Khosravi, Mohammad Rahbar, and Nourkhoda Sadeghifard. 2012. "Incidência de Klebsiella Pneumoniae Produtoras de Beta-Lactamases de Espectro Estendido Em Pacientes Com Infecção Do Trato Urinário." *Sao Paulo Medical Journal* 130(1):37–43.
18. Golkar, Zhabiz, Omar Bagasra, and Donald Gene Pace. 2014. "Bacteriophage Therapy: A Potential Solution for the Antibiotic Resistance Crisis." *Journal of Infection in Developing Countries* 8(2):129–36.
19. Huijbers, P. M. C., E. A. M. Graat, A. P. J. Haenen, M. G. van Santen, A. van Essen-Zandbergen, D. J. Mevius, E. van Duijkeren, and A. H. A. M. van Hoek. 2014. "Extended-Spectrum and AmpC β-Lactamase-Producing Escherichia Coli in Broilers and People Living and/or Working on Broiler Farms: Prevalence, Risk Factors and Molecular Characteristics." *Journal of Antimicrobial Chemotherapy* 69(10):2669–75.
20. Ibrahim, Rekaz A., Tillie L. Cryer, Shawkat Q. Lafi, Ehab Abu Basha, Liam Good, and Yaser H. Tarazi. 2019. "Identification of Escherichia Coli from Broiler Chickens in Jordan, Their Antimicrobial Resistance, Gene Characterization and the Associated Risk Factors." *BMC Veterinary Research* 15(1):1–17.
21. ISO 6579. 2002. "4th Ed: Microbiology –General Guidance on Methods for the Detection of Salmonella." *International Organize for Standardization* Geneve, Switzerland.
22. Kabir, S. M. Lutful. 2010. "Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns." *International Journal of Environmental Research and Public Health* 7(1):89–114.
23. Leverstein-van Hall, M. A., C. M. Dierikx, J. Cohen Stuart, G. M. Voets, M. P. van den Munckhof, A. van Essen-Zandbergen, T. Platteel, A. C. Fluit, N. van de Sande-Bruinsma, J. Scharinga, M. J. M. Bonten, and D. J. Mevius. 2011. "Dutch Patients, Retail Chicken Meat and Poultry Share the Same ESBL Genes, Plasmids and Strains." *Clinical Microbiology and Infection* 17(6):873–80.
24. Li, Xin Sheng, Gui Qin Wang, Xiang Dang Du, Bao An Cui, Su Mei Zhang, and Jian Zhong Shen. 2007. "Antimicrobial Susceptibility and Molecular Detection of Chloramphenicol and Florfenicol Resistance among Escherichia Coli Isolates from Diseased Chickens." *Journal of Veterinary Science* 8(3):243–47.
25. Magiorakos, A. P., A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, and D. L. Monnet. 2012. "Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance." *Clinical Microbiology and Infection* 18(3):268–81.
26. Overdevest, Ilse, Ina Willemsen, Martine Rijnsburger, Andrew Eustace, Li Xu, Peter Hawkey, Max Heck, Paul Savelkoul, Christina Vandenbroucke-Grauls, Kim van der Zwaluw, Xander Huijsdens, and Jan Kluytmans. 2011. "Extended-Spectrum β-Lactamase Genes of Escherichia Coli in Chicken Meat and Humans, the Netherlands." *Emerging Infectious Diseases* 17(7):1216–22.
27. Ozaki, H., Y. Matsuoka, E. Nakagawa, and T. Murase. 2017. "Characteristics of Escherichia Coli Isolated from Broiler Chickens with Colibacillosis in Commercial Farms from a Common Hatchery." *Poultry Science* 96(10):3717–24.
28. Rahman, MA, MA Samad, MB Rahman, and SML Kabir. 1970. "*In Vitro* Antibiotic Sensitivity and Therapeutic Efficacy of Experimental Salmonellosis, Colibacillosis and Pasteurellosis in Broiler Chickens." *Bangladesh Journal of Veterinary Medicine* 2(2):99–102.
29. Rodriguez-Siek, Kylie E., Catherine W. Giddings, Curt Doetkott, Timothy J. Johnson, Mohamed K. Fakhr, and Lisa K. Nolan. 2005. "Comparison of Escherichia Coli Isolates Implicated in Human Urinary Tract Infection and Avian Colibacillosis." *Microbiology* 151(6):2097–2110.
30. Ruzauskas, Modestas, Ričardas Krikštolaitis, and Marius Virgailis. 2010. *Prevalence and Antimicrobial Resistance of E. Coli Isolated from Chicken Liver Sold in Retail Markets*.
31. Sarba, Edilu Jorga, Kebede Abdisa Kelbesa, Morka Dandecha Bayu, Endrias Zewdu Gebremedhin, Bizunesh Mideksa Borena, and Ayichew Teshale. 2019. "Identification and Antimicrobial Susceptibility Profile of Escherichia Coli Isolated from Backyard Chicken in and around Ambo, Central Ethiopia." *BMC Veterinary Research* 15(1).
32. Schwarz, S., C. Kehrenberg, and T. R. Walsh. 2001. "Use of Antimicrobial Agents in Veterinary Medicine and Food Animal Production." *International Journal of Antimicrobial Agents* 17(6):431–37.
33. Suarez, David L., Patti J. Miller, Guus Koch, Egbert Mundt, and Silke Rautenschlein. 2020. "Newcastle Disease, Other Avian Paramyxoviruses, and Avian Metapneumovirus Infections." Pp. 109–66 in *Diseases of Poultry*. Wiley.
34. Swayne, David E. 1998. *Laboratory Manual for the Isolation and Identification of Avian Pathogens*. American Association of Avian Pathologists, University of Pennsylvania.
35. Zhao, S., D. G. White, P. F. McDermott, S. Friedman, L. English, S. Ayers, J. Meng, J. J. Maurer, R. Holland, and R. D. Walker. 2001. "Identification and Expression of Cephamycinase BlaCMY Genes in Escherichia Coli and Salmonella Isolates from Food Animals and Ground Meat." *Antimicrobial Agents and Chemotherapy* 45(12):3647–50