Multidrug resistance among gram negative *E. coli* in chicken meat (Rafha-Saudi Arabia).

Abstract

Aims: Multidrug resistant (MDR) bacteria poses a major public health issuer globally. The genes for antibiotic resistance are transferred vertically in the form of genomic DNA and horizontally in the form of plasmids or transposons. Antibiotic are extensively used in animal farming to treat and prevent animal diseases, and at sub-therapeutic doses, they are used to promote animal growth. This extensive use of antibiotics is causing an increase in resistance among bacteria. More frequent, chicken meat available at retail shops is reported to be contaminated with a variety of drug resistant bacteria including *E. Coli*. The aim of the present study was to investigate antibiotic resistance in *Escherichia coli* strains isolated from chicken meat available in the local shops of Rafha, Saudi Arabia.
Place and Duration of Study: Department of basic health sciences, College of Pharmacy, Northern Border University, Rafha, Saudi Arabia, between February and October, 2019
Methodology: Eighty-six *E.coli* strains, isolated from chicken meat, were tested for their antibiotic resistance profile, using the disc diffusion method.
Results: All the isolated *E. coli* strains were tested against 14 antibiotics. The maximum resistance was found against penicillin G (95%) followed by amoxicillin (85%), Cephalothin (81%), Erythromycin (72%), and Tetracycline (50%). Imipenem was the most effective agent of all with only 1% resistance followed by Cefepime with almost 6% resistance.

A high percentage of the isolates (57%,) were multidrug resistant as they were non—susceptible to at least one antimicrobial in ≥3 antimicrobial classes including amoxicillin, erythromycin and tetracycline.
Conclusion: The prevalence of MDR *E. coli* in retail chicken meat is very high and could pose a serious threat to public health.

Keywords: Antimicrobial resistance (AMR), *Escherichia coli (E. coli)*, Chicken meat, Multidrug resistance (MDR), polymerase chain reaction (PCR)

1. Introduction

Antibiotic are used in the husbandry of livestock for many different purposes. They are used therapeutically for the treatment of various infectious diseases, for the metaphylactic treatment of a herd of animals when one of them gets disease, and for the prophylactic treatment against any anticipated disease. The antibiotics are also used at sub-therapeutic dose in animal food and water to promote animal growth and to improve efficiency of the food (1). New FDA Veterinary Feed Directive has recommended stopping any such use (2). This exercise has already been banned in Europe since 2006 (3).

The excessive misuse of antibiotics or antimicrobials kills the susceptible bacteria leaving behind only the resistant ones which increase in number overtime (4). Bacteria acquire resistance through many ways. Some bacteria have natural resistance against some antibiotics; some acquire genetic mutations which make them resistant to some antibiotic while some others receive resistant genes present on plasmid from other bacteria (5). Selective pressure of antibiotic overuse in human and animal has resulted in the propagation of antibiotic resistance bacteria which is linked with the attainment and spread of resistant genes among such bacteria. In this context, the probiotic bacterial strains are also considered to attain various antibiotic resistant genes and may transfer them to pathogenic bacterial strains (6).

Foods from animal origin, predominantly chicken and meat are found to be associated with food-borne diseases. Chicken may be contaminated with many different types of microorganisms including bacteria (7). Many studies have reported the drug resistant *E. coli* in poultry (8), egg (9), milk (10) and raw meat (11). Raw chicken is reported to possess highest percentages of drug resistant *E. coli* and *Salmonella spp.* (12). Gram-negative bacteria especially Enterobacteriaceae organisms including *E. coli* and *Salmonella* are mostly reported to produce β-lactamase enzymes. These enzymes break β-lactam ring of the β-lactam antibiotics. More than 1000 different types of β-lactamase enzymes have been reported so far (13). Drug resistant Enterobacteriaceae especially the ESBL-producer are increasing rapidly around the world (14) and their prevalence has shifted from the hospital to the community (15). They have been isolated from different sources including cattle, chickens, vegetables and raw milk (16, 17, 18). Recently, carbapenemase $bla_{\text{NDM-1}}$ producing bacteria were isolated from tap water (19).

In a recent report published in India, it was found that 100% of *Escherichia coli*, 92% of *Klebsiella pneumoniae*, and 78% of *Staphylococcus lentus* from the poultry forms were resistant to three or more antibiotics (20). This higher prevalence of antibiotic resistant bacteria is a direct result of antibiotics misuse in animals (21, 22). Poultry and cattle account for the biggest pools of antibacterial resistant bacteria, especially *Escherichia coli* and *Salmonella* (23, 24).

E. coli is the most commonly found gram-negative pathogen in human colon and is the most common cause of UTIs. It also causes diarrhea and bacteremia (25). Drug resistant *E. coli* strains can transfer antibacterial resistant genes not only to other *E. coli* strains but also to other common GIT bacteria (26). Therefore, it is important to study multidrug resistance in *E. coli* found in food including chicken meat.

The aim of current study was to study the spectrum of drug resistance among *E. coli* isolated from chicken meat in Rafha, Saudi Arabia.

2. Material & Methods

2.1 Sample collection:

The study was done at the Department of basic health sciences, College of Pharmacy, Northern Border University, Rafha city of Saudi Arabia, between February and October, 2019. Fresh and frozen chicken meat samples (sterile swabs) were collected in appropriate sterile containers from different chicken shops in Rafha and were immediately transported to the laboratory for bacteriological investigation.

2.2 Bacteriological analysis.

The samples were streaked onto MacConkey agar plates and incubated aerobically at 37 ^{o}C for 24 hours. The isolates which fermented lactose within 24 hours on MacConkey agar were further identified by PCR. For that purpose, isolated pink colonies were carefully picked and used for PCR identification of *E. coli* as well as disc diffusion method for antibiotic sensitivity testing.

2.3 Molecular Identification of E. coli

DNA isolation was done using Bacterial DNA preparation kit (Jena Biosciences, PP206S) according to the manufacturer's protocol. The DNA samples were run on 1% agarose gel for the conformation. For the *E. coli* identification, specific *E. coli* gene segments were amplified by PCR technique with specific primers using the extracted DNA. The PCR was done in a 200 µl PCR tube. The 20 µl reaction mixture contained 1X PCR buffer (10mM Tris HCL with 50mM KCL, pH 8.3), 2.5 mM $MgCl_2$, a 0.2mM of each dNTP, 0.2 µM of each of the primers, 0.5U Taq DNA polymerase and 2µl of the DNA template. The sequences of different primers used in this study are given in Table 1. The PCR cycling conditions were; 95°C for 1 min for one cycle followed by 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 30 seconds. The final extension was done at 72°C for 5 min. The PCR products (5µl) were analyzed using agarose gel (2%) electrophoresis (Fig. 1).

Table 1. Primers used for *E. coli* identification

Primer ID	Primer Sequence	Tm	Amplicon Size
lacZ4 F	5′- CTGCTGCTGCTGAACGGCAA 3′	59.5	243
lacZ4 R	5′- CACCATGCCGTGGGTTTCAA 3′	57.5	
M12 F	5′- GTGATCTCCAGCTACCGCTA 3′	57.5	200
M12 R	5′- CGTTGCAAACTGACGCTCTT 3′	55.4	

2.4 The Antibiotic Sensitivity Testing:

The in vitro antimicrobial sensitivity testing for the *E. coli* isolates was carried out using the disc diffusion method. Multidrug resistance was defined as resistance to three or more classes of antibiotics. The antibiotic discs used in this study (Bioanalyse^{R} 50 susceptibility discs) with their potency and standard zones of inhibition are given in Table 2. The results were analyzed using simple percentage prevalence values to find out the prevalence of drug resistant among isolated *E. coli*.

3. Results

A total of 86 *E. coli* positive samples were included in the study. The *E. coli* strains isolated from all 86 chicken samples were tested against all the 14 antibiotics included in the study. The antibiotics used with potency and standard zone diameters for the interpretation of the results are given in Table 2. All the isolates were resistant to at least one or more antibiotics. The number of resistant, intermediate and susceptible isolates against various antibiotics found in this study are given in Table 3.

Table 2. Zone diameter interpretative standards used.

#	Code	Antimicrobial agent	Potency	Zone of inhibition (mm)		
				Resistant	Intermediate	Susceptible
1	P	Penicillin G	10 U	14	-	15
2	AX	Amoxicillin	25 µg	13	14-16	17
3	AMC	Augmentin	20+10 µg	13	14-17	18
4	KF	Cephalothin 1st gen	30 µg	14	15-17	18
5	CAZ	Ceftazidime 3rd gen	30 µg	17	18-20	21
6	CTX	Cefotaxime 3rd gen	30 µg	17	18-22	23
7	FEP	Cefepime 4th gen	30 µg	14	15-17	18
8	CIP	Ciprofloxacin	5 µg	15	16-20	21
9	TE	Tetracycline	30 µg	11	12-14	15
10	E	Erythromycin	15 µg	13	14-22	23
11	C	Chloramphenicol	30 µg	12	13-17	18
12	CN	Gentamicin	10 µg	12	13-14	15
13	SXT	Cotrimoxazole (25 µg)	1.25/23.75 µg	10	11-15	16
14	IPM	Imipenem	10 µg	13	14-15	16

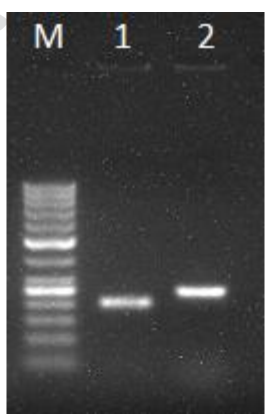

Figure 1. PCR identification of *E. coli*. Lane M: 50 bp DNA ladder, Lane 1: M12 gene fragment (200 bp), Lane 2: lacZ 4 gene fragment (243 bp)

3.1 Percentage of resistance

The maximum resistance was seen against penicillin G (95%) whereas Imipenem was the most effective agent of all with only 1% resistance (Table 3). Among other antimicrobial agents used, maximum resistance (≥ 50%) was seen against amoxicillin (85%), Cephalothin (81%), Erythromycin (72%), and Tetracycline (50%). Less than 50% isolates were resistant to Ceftazidime (44%), Cefotaxime (36%), Gentamicin (35%), Augmentin (33%), Co-trimoxazole (30%), Ciprofloxacin (29%), and Chloramphenicol (22%). Cefepime, the fourth generation cephalosporin, was the second most effective antibiotic after Imipenem seen in the current study with almost 6% resistance. The percentage of resistance, from highest to lowest are given in Fig. 2.

Table 3: Antimicrobial resistance and susceptibility pattern of *E. coli* isolates

#	Code	Name	Resistant (%)	Intermediate	Susceptible
1	P	Penicillin G	82 (95)	0	4
2	AX	Amoxicillin	73 (85)	9	4
3	AMC	Augmentin	28 (33)	47	11
4	KF	Cephalothin 1st gen	70 (81)	6	10
5	CAZ	Ceftazidime 3rd gen	38 (44)	26	20
6	CTX	Cefotaxime 3rd gen	31 (36)	28	27
7	FEP	Cefepime 4th gen	5 (6)	12	59
8	CIP	Ciprofloxacin	25 (29)	22	43
9	TE	Tetracycline	43 (50)	11	32
10	E	Erythromycin	62 (72)	22	1
11	C	Chloramphenicol	19 (22)	12	55
12	CN	Gentamicin	30 (35)	20	36
13	SXT	Cotrimoxazole (25 µg)	26 (30)	16	44
14	IPM	Imipenem	1 (1)	0	85

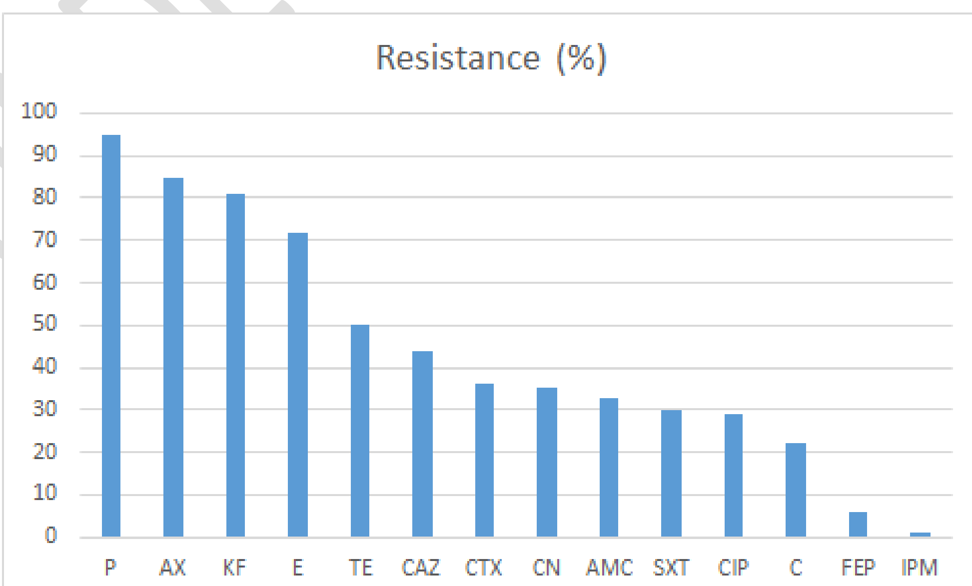

Figure 2. Antimicrobial resistance percentage, from highest to lowest. Each isolate was tested against: penicillin G (P), amoxicillin (AX), Cephalothin (KF), Erythromycin (E), Tetracyclin (TE), Ceftazidime (CAZ), Cefotaxime (CTX), Gentamicin (CN), Augmentin (AMC), Cotrimoxazole (SXT), Ciprofloxacin (CIP), Chloramphenicol (C), Cefepime (FEP), and Imipenem (IPM).

3.2 Frequency of resistance

All isolates were resistant to more than one antibiotics. The number of isolates resistant to six different antibiotics was the maximum. Overall, equal or more than 8 isolates were resistant against 3 to 8 antibiotics. Number of isolates resistant to more than eight or less than three antibiotics was less than four (Fig. 3).

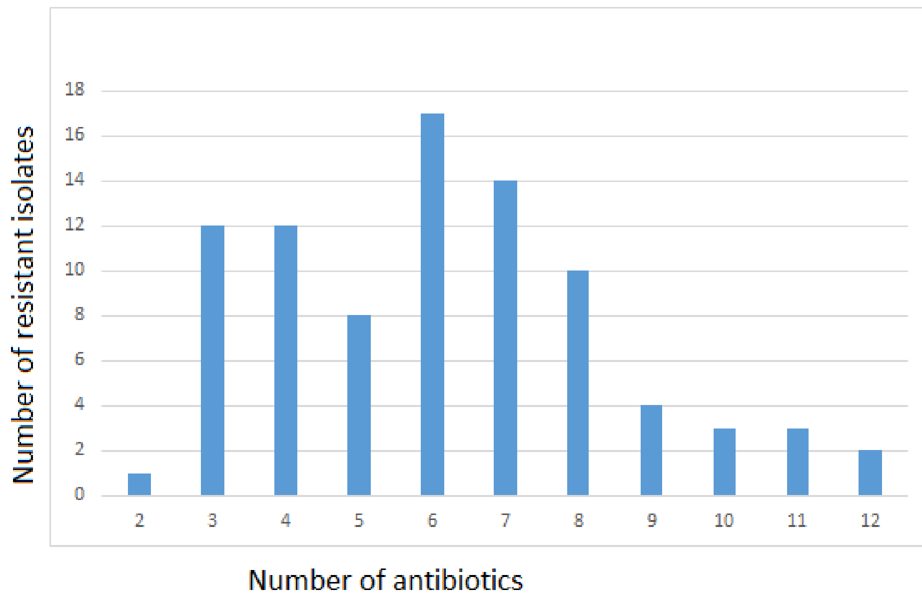

Figure 3. Frequency of resistance against two or more antibiotics.

3.3 Multi drug resistance

An isolate is defined as multidrug resistant if it is non-susceptible to at least one antimicrobial in ≥3 antimicrobial classes. A larger percentage of the isolates (57%,) were multidrug resistant as they were non—susceptible to amoxicillin, erythromycin and tetracycline. Among those resistant to these three classes, higher percentage was also resistant to Cotrimoxazole (68%), chloramphenicol (62%), ciprofloxacin (60%), and Gentamicin (56%) separately. A considerable number of the isolates were resistant to 5 or more different classes of antimicrobial agents (Table 4)

Table 4. Frequency of multidrug resistance.

Antimicrobials	Frequency	%
Ax, E, TE	50	57
Ax, E, TE, SXT	34	39
Ax, E, TE, C	31	35
Ax, E, TE, CIP	30	34
Ax, E, TE, CN	28	32
Ax, E, TE, SXT, C	18	20
Ax, E, TE, SXT, C, CIP	14	16
Ax, E, TE, SXT, C, CIP, CN	14	16

3.4 Tetracycline susceptible isolates

Since tetracycline is one of the most commonly used antibiotics used in chicken growth promotion, the data was further analyzed to see multidrug resistance against tetracycline susceptible isolates. Number of *E. coli* isolates susceptible to Tetracycline was 32 (37%). These isolates were also susceptible to Chloramphenicol (100%), Imipenem (100%), Cefepime (84%), Cotrimoxazole (81%), Ciprofloxacin (72%) and others but with lower percentages. The Tetracycline susceptible isolates were resistant to Penicillin G (100%), Amoxicillin (88%), Cephalothin (63%), erythromycin (59%) and many others but with lower percentages (Table 5).

Table 5. Tetracycline susceptible isolates (n=32) showing Resistance/susceptibility against other antibiotics used.

#	Code	Name	Resistant	Intermediate	Susceptible
1	P	Penicillin G	32 (100%)	0	0
2	AX	Amoxicillin	28 (88%)	3	1
3	AMC	Augmentin	8	21	3
4	KF	Cephalothin 1st gen	20 (63%)	5	7
5	CAZ	Ceftazidime 3rd gen	11	11	9
6	CTX	Cefotaxime 3rd gen	7	13	12
7	FEP	Cefepime 4th gen	0	5	27 (84%)
8	CIP	Ciprofloxacin	2	7	23 (72%)
9	E	Erythromycin	19 (59%)	12	1
10	C	Chloramphenicol	0	0	32 (100%)
11	CN	Gentamicin	10	8	14
12	SXT	Cotrimoxazole (25 µg)	2	4	26 (81%)
13	IPM	Imipenem	0	0	32 (100%)

4. Discussion

Antibiotics are given to chicken and other animals at sub-therapeutic doses for growth promotion, which according to Food and Drug Administration (FDA), result in antibiotic resistance and pose a threat to human health (27). Because of the extensive use of antibiotics in poultry, chicken meat has become a potential source of multi drug resistant bacteria including *E. coli* worldwide (28).

The overall prevalence of *E. coli* in chicken meat in the current study was 100%. Saikia and Joshi, 2010, reported 98% *E. coli* prevalence in chicken meat in India (29). In a study conducted in Holand, 94% of the chicken retail meat samples contained at least one ESBL producing *E. coli* (30). Other studies found 82% prevalence of *E. coli* in Bangladesh (31), 78% in India (32), 76% in Korea (33), 58% in Kenya (34), and 47% in Nigeria (35). Interestingly, a study from Saudi Arabia found only 31% *E. coli* in chicken (36).

Beta-lactam antibiotics, such as penicillins and cephalosporins are among the most commonly used antibiotics in chicken and most commonly prescribed antibiotics against bacterial infections in human (37, 38). As compared to Gram-positive bacteria, Gram-negatives usually have stronger intrinsic resistance against some antibiotics. This is because of the composition and complexity of their outer membrane and inner cell wall. The outer membrane of gram negative bacteria is made of lipopolysaccharides (LPS) and phospholipids. The inner cell wall is made of a single layer of peptidoglycans. Penicillin G is not able to penetrate the outer layer in gram negative bacteria (39). In the current study, maximum resistance was observed against penicillin drugs especially Penicillin G (95%) which is quite understandable. The results are in accordance with other studies (40, 41, 42). Resistance to Amoxicillin, Cephalothin (first generation cephalosporin) and erythromycin were also high, above 70% (85%, 81% and 72% respectively) and may be because of selective pressure due to over use of these antibiotics in poultry. These results aligned with the finding from other groups (43, 44). Interestingly, while studying AMR among Enterococcus species from food animal origin, Liu et al, 2013, (45) found similar resistance (72.8%) against erythromycin. Higher levels of resistance against amoxicillin and erythromycin among *E. coli* isolates were also reported in Bangladesh (46).

Exactly half of the isolates were resistant to tetracyclin. Other studies have reported higher levels of resistance in *E. coli* against tetracycline like 76% (47), 77% (48), 80% (49), 81% (50), and 90% (51) isolated from different types of samples from chicken.

Imipenem and Cefepime were the most effective antimicrobials against the *E. coli* isolates found in this study. Similar results were reported in another study where zero resistance to Imipenem was observed (52). On the contrary, amazingly higher level of resistance was reported against carbapenems including Imipenem (47.7%) and meropenem (41.9%) in Bangladesh. However, they could not give any clear explanation for these results as these antibiotics are not used in poultry farms in Bangladesh (53). The same study also reported an unexpectedly high resistance against Cefepime (72.1%). Similarly, astonishingly higher resistance was reported against Cefepime (95.8%) in another study (54). Close to the results reported by us, a study done in turkey reported zero resistance against Imipenem and Cefepime (55).

Two mechanisms may be responsible for Multidrug resistance in bacteria. First, bacteria may accumulate plasmids having multiple drug resistant genes. Second, they may have genes on their chromosome which code for the multidrug efflux pumps, removing the drug out of the cell. Whichever the mechanism be involved, the extensive unnecessary use of antimicrobials in poultry is the main cause of the development of multidrug resistance in bacteria especially *E. coli*. For multidrug resistant analysis, three antimicrobial classes amoxicillin, erythromycin and tetracycline were selected. Fifty-seven percent of the isolates were resistant to these three classes of antibiotics. Some of these were resistant to other classes of antibiotics as well. Some other studies have reported even much higher incidence of MDR in *E. coli* in chicken (94%) in India (56) and in Nepal (80.0%) (57). In a combined study from China and Sudan, MDR was 80% and 54.4% respectively (58). However, they defined multidrug-resistance as isolates showing resistance to two or more antimicrobial classes.

5. Conclusion:

While all isolates in our study were resistant to two or more than two drugs, seventy-three isolates were resistant to 3-8 antibiotics, with some resistant to even more (9-12) antibiotics. These results indicate that chicken meat in the city is heavily contaminated with multidrug resistant *E. coli* which is due to the extensive use antibiotics in poultry farming. Authorities in the ministries of health and food must take notice of this and take necessary measures to reduce the extensive use of antibiotics in poultry.

5. References

1. FDA U. Guidance for industry# 209. The judicious use of medically important antimicrobial drugs in food-producing animals. US Food and Drug Administration, US Department of Health and Human Services. Center for Veterinary Medicine, Rockville, MD. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf. 2012.
2. American Veterinary Medical Association. Veterinary Feed Directive (VFD) Basics. to be found under https://www. avma. org/KB/Resources/Pages/VFD123. aspx. 2018. https://www.avma.org/KB/Resources/Pages/VFD123.aspx
3. Anadón A. WS14 The EU ban of antibiotics as feed additives (2006): alternatives and consumer safety. Journal of Veterinary Pharmacology and Therapeutics. 2006;29:41-44. https://doi.org/10.1111/j.1365-2885.2006.00775_2.x
4. Lutgring JD, Machado MJ, Benahmed FH, Conville P, Shawar RM, Patel J, Brown AC. FDA-CDC Antimicrobial Resistance Isolate Bank: a publicly available resource to support research, development, and regulatory requirements. Journal of clinical microbiology. 2018;56(2). https://doi.org/10.1128/JCM.01415-17
5. Alliance for the Prudent Use of Antibiotics. General background: about antibiotic resistance. Tufts University School of Medicine. 2016;136. http://www.tufts.edu/med/apua/about_issue/about_antibioticres.shtml
6. Rabia A, Shah NP. Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. Int Food Res J. 2011;18: 59–75.
7. Rehman MU, Rashid M, Sheikh JA, Bhat MA. Molecular epidemiology and antibiotic resistance pattern of enteropathogenic *Escherichia coli* isolated from bovines and their handlers in Jammu, India. Journal of Advanced Veterinary and Animal Research. 2014; 1: 177-181. https://doi.org/ http://dx.doi.org/10.5455/javar.2014.a30
8. Bush K, and Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob. Agents Chemother. 2010; 54: 969-976. https://doi.org/10.1128/AAC.01009-09

9. Arathy DS, Vanpee G, Belot G, Mathew V, DeAllie C, Sharma R. Antimicrobial drug resistance in *Escherichia coli* isolated from commercial chicken eggs in Grenada, West Indies. West Indian Med J. 2011; 60:53–6.
10. Cizek A, Dolejska M, Novotna R, Haas D, Vyskocil M. Survey of Shiga toxigenic *Escherichia coli* O157 and drug-resistant coliform bacteria from in-line milk filters on dairy farms in the Czech Republic. J Appl Microbiol. 2008;104:852–60. https://doi.org/10.1111/j.1365-2672.2007.03602.x
11. Srinivasa Rao T, Gill JPS, Ravi Kumar GVVPS, Ghatak S. Multi drug resistance patterns of Shiga toxin - producing *Escherichia coli* (STEC) and non - STEC isolates from meats, RTE meat foods, drinking water and human diarrhoea samples of Punjab, India. Arch Clin Microbiol. 2011;2:1–12.
12. Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K. Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Revista do Instituto de Medicina Tropical de São Paulo. 2014;56(4):341-346. https://doi.org/10.1590/S0036-46652014000400012
13. Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, et al. Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. Emerg Infect Dis. 2007;13:838–46. https://dx.doi.org/10.3201%2Feid1306.061576
14. Tham J, Walder M, Melander E, Odenholt I. Prevalence of extended-spectrum beta-lactamase-producing bacteria in food. Infection and Drug Resistance. 2012;5:143-147. https://dx.doi.org/10.2147%2FIDR.S34941
15. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect Dis. 2005;5:481–93. https://doi.org/10.1016/S1473-3099(05)70189-4
16. Jakobsen L, Kurbasic A, Skjot-Rasmussen L, Ejrnaes K, Porsbo LJ, Pedersen K, et al. *Escherichia coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. Foodborne Pathog Dis. 2010;7:537–47. https://doi.org/10.1089/fpd.2009.0409

17. Ramchandani M, Manges AR, DebRoy C, Smith SP, Johnson JR, Riley LW. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli*. Clin Infect Dis. 2005;40:251–7. https://doi.org/10.1086/426819
18. Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, et al. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis. 2010;16:88–95. https://dx.doi.org/10.3201%2Feid1601.091118
19. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis. 2011;11:355–62. https://doi.org/10.1016/S1473-3099(11)70059-7
20. Bhushan C, Khurana A, Sinha R, and Nagaraju M. Antibiotic Resistance in Poultry Environment: Spread of Resistance from Poultry Farm to Agricultural Field, Centre for Science and Environment, New Delhi. 2017 (report)
21. Norstrom M, Hofshagen M, Stavnes T. Antimicrobial resistance in Campylobacter jejuni from humans and broilers in Norway. Epidemiol Infect. 2006; 134, 127–130. https://doi.org/10.1017/S0950268805004814
22. Angulo FJ, Johnson KR, Tauxe RV. Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microb Drug Resist. 2000; 6, 77–83. https://doi.org/10.1089/mdr.2000.6.77
23. Boyle F, Morris D, O'Connor J, DeLappe N, Ward J, Cormican M. First report of extended-spectrum-β-lactamase-producing Salmonella enterica serovar Kentucky isolated from poultry in Ireland. Antimicrobial agents and chemotherapy. 2010;54(1):551-3. https://doi.org/10.1128/AAC.00916-09
24. Minami A, Chaicumpa W, Chongsa-Nguan M. Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control. 2010;21(3):221–226. https://doi.org/10.1016/j.foodcont.2009.05.011
25. Salvadori M, Coleman BL, Louie M, McEwen S, McGeer A. Consumption of antimicrobial-resistant *Escherichia coli*-contaminated well water: human health impact. PSI Clin Res. 2004; 1:6-25.
26. Österblad M, Hadanen A, Manninen R, Leistevuo T, Peltonen R, Meurman O, et al. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. J

Antimicrob Chemother. 2000; 44:1479–84. https://doi.org/10.1128/AAC.44.6.1479-1484.2000

27. Price LB, Newland J, Bole A, Bortolaia V, Larsen J, Loneragan GH et al. Combating antibiotic resistance - A Policy Roadmap to Reduce Use of Medically Important Antibiotics in Livestock. Washington, D.C: George Washington University, 2017. 72 p.
28. Trkov M, Rupel T, Žgur-Bertok D, Trontelj S, Avguštin G, Ambrožič Avguštin J. Molecular characterization of *Escherichia coli* strains isolated from different food sources. Food Technology and Biotechnology. 2014;52(2):255-62.
29. Saikia P, Joshi SR: Retail market poultry meats of North-East India – a microbiological survey for pathogenic contaminants. Res J Microbiol. 2010, 5(1):36-43.
30. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, Van Den Munckhof MP, van Essen-Zandbergen A, et al., Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clinical Microbiology and Infection. 2011;17(6):873-80. https://doi.org/10.1111/j.1469-0691.2011.03497.x.
31. Jakaria A, Islam MA, & Khatun MM. Prevalence, characteristics and antibiogram profiles of *Escherichia coli* isolated from apparently healthy chickens in Mymensingh, Bangladesh. Microbes Health. 2012;**1**(1): 27–29. https://doi.org/10.3329/mh.v1i1.13710
32. Hussain A, Shaik S, Ranjan A, Nandanwar N, Tiwari SK, Majid M, et al. Risk of transmission of antimicrobial resistant *Escherichia coli* from commercial broiler and free-range retail chicken in India. Frontiers in microbiology. 2017;**8:**2120. https://doi.org/10.3389/fmicb.2017.02120
33. Park HJ, Yoon JW, Heo EJ, Ko EK, Kim KY, Kim YJ, et al. Antibiotic resistance and virulence potentials of Shiga toxin-producing *Escherichia coli* isolates from raw meats of slaughterhouses and retail markets in Korea. Journal of microbiology and biotechnology. 2015;**25**(9):1460-6. https://doi.org/10.4014/jmb.1502.02034
34. Ngai DG, Nyamache AK, & Ombori O. Prevalence and antimicrobial resistance profiles of *Salmonella* species and *Escherichia coli* isolates from poultry feeds in Ruiru Sub-County, Kenya. BMC Res Notes. 2021;**14**(1):1-6 https://doi.org/10.1186/s13104-021-05456-4

35. Adeyanju G T, & Ishola O. Salmonella and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. SpringerPlus 2014;**3**(1):139. https://doi.org/10.1186/2193-1801-3-139
36. Altalhi AD, Gherbawy YA, and Hassan SA. Antibiotic resistance in *Escherichia coli* isolated from retail raw chicken meat in Taif, Saudi Arabia. Foodborne pathogens and disease. 2010;**7**(3):281-285. https://doi.org/10.1089/fpd.2009.0365
37. Wilke MS, Lovering AL, Strynadka NC. β-Lactam antibiotic resistance: a current structural perspective. Curr Opin Microbiol. 2005;8(5):525–533. https://doi.org/10.1016/j.mib.2005.08.016
38. Pandey N, Cascella M. Beta Lactam Antibiotics; StatPearls: St. Petersburg, FL, USA. 2020. https://www.ncbi.nlm.nih.gov/books/NBK545311/
39. Doyle MP, Busta F, Cords BR, Davidson PM, Hawke J, Hurd HS, Isaacson RE, Matthews K, Maurer J, Meng J, Montville TJ. Antimicrobial resistance: implications for the food system: an expert report, funded by the IFT foundation. Comprehensive Reviews in Food Science and Food Safety. 2006 Jul;5(3):71-137. https://doi.org/10.1111/j.1541-4337.2006.00004.x
40. Agyare C, Boamah VE, Zumbi CN, Osei FB. Antibiotic use in poultry production and its effects on bacterial resistance. Antimicrobial resistance-a global threat. 2018; 5:1-20. DOI: 10.5772/intechopen.79371. Available from: https://www.intechopen.com/books/antimicrobial-resistance-a-global-threat/antibiotic-use-in-poultry-production-and-its-effects-on-bacterial-resistance.
41. Moawad AA, Hotzel H, Neubauer H, Ehricht R, Monecke S, Tomaso H, et al. Antimicrobial resistance in Enterobacteriaceae from healthy broilers in Egypt: emergence of colistin-resistant and extended-spectrum β-lactamase-producing *Escherichia coli*. Gut pathogens. 2018;10(1):1-2. https://doi.org/10.1186/s13099-018-0266-5
42. Mohamed MA, Shehata MA, Rafeek E. Virulence genes content and antimicrobial resistance in *Escherichia coli* from broiler chickens. Vet Med Int.2014: ID 195189. https://doi.org/10.1155/2014/195189
43. Dahshan H, Abd-Elall AM, Megahed AM, Abd-El-Kader MA, Nabawy EE. Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained

from poultry farms, Egypt. Environmental monitoring and assessment. 2015;187(2):1-0. https://doi.org/10.1007/s10661-014-4218-3

44. Ngogang MP, Ernest T, Kariuki J, Mouliom Mouiche MM, Ngogang J, Wade A, van der Sande MA. Microbial Contamination of Chicken Litter Manure and Antimicrobial Resistance Threat in an Urban Area Setting in Cameroon. Antibiotics. 2021;10(1):20. https://doi.org/10.3390/antibiotics10010020
45. Liu Y, Liu K, Lai J, Wu C, Shen J, Wang Y. Prevalence and antimicrobial resistance of Enterococcus species of food animal origin from Beijing and Shandong Province, China. J Appl Microbiol. 2013;114(2):555-63. https://doi.org/10.1111/jam.12054
46. Zinnah MA, Haque MH, Islam MT, Hossain MT, Bari MR, Babu SA, et al. Drug sensitivity pattern of *Escherichia coli* isolated from samples of different biological and environmental sources. Bangladesh Journal of Veterinary Medicine. 2008;6(1):13-8. https://doi.org/10.3329/bjvm.v6i1.1332
47. Rahman MA, Rahman AK, Islam MA, Alam MM. Antimicrobial resistance of *Escherichia coli* isolated from milk, beef and chicken meat in Bangladesh. Bangladesh Journal of Veterinary Medicine. 2017;15(2):141-6. https://doi.org/10.3329/bjvm.v15i2.35525
48. Shecho M, Thomas N, Kemal J, Muktar Y. Cloacael carriage and multidrug *resistance Escherichia coli* O157: H7 from poultry farms, eastern Ethiopia. Journal of veterinary medicine. 2017;2017. https://doi.org/10.1155/2017/8264583
49. Abd El Tawab AA, Ammar AM, Nasef SA, Reda RM. Prevalence of *E. coli* in diseased chickens with its antibiogram pattern. Benha Veterinary Medical Journal. 2015;28(2):224-30.
50. Adelowo OO, Fagade OE, Agersø Y. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. The Journal of Infection in Developing Countries. 2014;8(09):1103-12. https://doi.org/10.3855/jidc.4222
51. Zeryehun T, and Bedada B. Antimicrobial resistant pattern of fecal *E. coli* in selected broiler farms of eastern Harare zone, Ethiopia. International Journal of applied biology and pharmaceutical technology. 2013;4(4):298-304.

52. Vinueza-Burgos C, Ortega-Paredes D, Narváez C, De Zutter L, Zurita J. Characterization of cefotaxime resistant *Escherichia coli* isolated from broiler farms in Ecuador. PLoS ONE. 2019;14(4): e0207567. https://doi.org/10.1371/journal.pone.0207567
53. Parvin MS, Talukder S, Ali MY, Chowdhury EH, Rahman MT, Islam MT. Antimicrobial Resistance Pattern of *Escherichia coli* Isolated from Frozen Chicken Meat in Bangladesh. Pathogens. 2020;9(6):420. https://doi.org/10.3390/pathogens9060420
54. Younis G, Awad A, Mohamed N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. Vet World. 2017;10(10):1167-1172. https://doi.org/10.14202/vetworld.2017.1167-1172
55. Pehlivanlar Önen S, Aslantaş Ö, Şebnem Yılmaz E, Kürekci C. Prevalence of β-Lactamase Producing *Escherichia coli* from Retail Meat in Turkey. J Food Sci. 2015;80(9):M2023-9. https://doi.org/10.1111/1750-3841.12984.
56. Brower CH, Mandal S, Hayer S, Sran M, Zehra A, Patel SJ, et al. The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. Environmental health perspectives. 2017;125(7):077015. https://doi.org/10.1289/EHP292
57. Shrestha A, Bajracharya AM, Subedi H, Turha RS, Kafle S, Sharma S, et al. Multi-drug resistance and extended spectrum beta lactamase producing Gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal. BMC research notes. 2017;10(1):1-5. https://doi.org/10.1186/s13104-017-2917-x
58. Abdelgader SA, Shi D, Chen M, Zhang L, Hejair H, Muhammad U, Yao H, Zhang W. Antibiotics resistance genes screening and comparative genomics analysis of commensal *Escherichia coli* isolated from poultry farms between China and Sudan. BioMed research international. 2018 Aug 26;2018. https://doi.org/10.1155/2018/5327450