



Research Article

Prevalence of Multiple Drug Resistance among Avian Pathogenic *E. coli* Isolates from Commercial Poultry

Abdul Latif¹, Abdul Wadood*¹, Javid Iqbal², Zainab Syed², Durdana¹, Saba Sabir Malik¹, Mehraj Gull², Zubair Luqman³, Misbah Aslam⁴, Hafiz Muhammad Ali³ and Rashid Ahmed Magsi⁵

¹Department of Microbiology, University of Balochistan, Quetta, Pakistan.

²Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

³University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

⁴Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

⁵Livestock and Dairy Development Department, Balochistan, Quetta, Pakistan.

* Corresponding Author : wadoodcasvab@gmail.com

Abstract

Infections associated with Avian Pathogenic *E. coli* are responsible for huge economic losses for poultry industry worldwide. Particularly, its association with colibacillosis, a complex syndrome which is characterized by lesions of multiple organs i.e. peritonitis, pericarditis, air Sacculitis, osteomyelitis, salpingitis and yolk sac infections is responsible for high mortality and morbidity. Moreover, it causes respiratory tract infections among poultry birds, followed by septicaemia. Liver samples were collected from commercial poultry birds from the various retail shops located in Peshawar City. Bacteria were identified by biochemical and molecular methods. Out of all the tested isolates n=85, 98% were identified as Avian Pathogenic *E. coli* (APEC). Identified APEC samples were further tested against 23 different antibiotics including amoxicillin (89.40%), levofloxacin (62.40%), ciprofloxacin (71.80%), tobramycin (14.10%), gentamycin (34.10%), neomycin (53.00%), streptomycin (81.00%), tigecyclines (0.00%), oxytetracyclines (96.50%), doxycycline (61.20%), nitrofurantoin (1.00%), chloramphenicol (63.50%), cefixime (7%), cefepime (4.70%), ceftazidime (8.30%), cefotaxime (8.00%), cephalothin (43.50%), trimethoprim, sulfamethoxazole (77.60%), lincomycin (100%), augmentin (4.70%), carbapenem (4%) and polymyxin B (15%). Out of all n=85 isolates 99.9% were multi-drug resistant. Furthermore, ESBL encoding TEM, OXA, SHV were detected in following percentages 53.60%, 19.50%, 9.70% respectively genes

Keywords: Avian Pathogenic *E. coli*, Drug Resistance, Commercial Poultry

ARTICLE INFORMATION

Received: 25.08.2019

Revised: 17.12.2019

Accepted: 29.12.2019

DOI: 10.31580/pjmls.v2i4.1168

© Readers Insight Publication

INTRODUCTION

Escherichia coli (*E. coli*) is rod shaped, gram negative, coliform bacterium of genus *Escherichia* which is commonly found in lower intestine of warm blooded organisms. Cells are about 2.0 µm long and 0.25–1.0 µm in diameter, with a cell volume of 0.6–0.7 µm³. Most of the environmental *E. coli* strains are harmless, but some can cause severe food poisoning in their hosts, and are responsible for product recalls due to food contamination. The harmless strains are the part of the normal flora of the gut, and can benefit their hosts by generating vitamin K₂, and help in avoiding colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. *E. coli* is excluded into the environment within fecal matter. The bacterium grows immensely in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly later.

E. coli can be divided into two group's i.e commensals and pathogenic. The pathogenic group is further divided into two other subgroups: diarrheagenic *E. coli* (DEC) and extra intestinal pathogenic *E. coli* (ExPEC). The DEC further characterized into six pathotypes, among them the most prevalent enteroaggregative *E. coli* (EAEC) is an important pathogen associated with diarrhea (1). The

ExPEC pathotype is further classified in six main sub pathotypes: uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC), mammary pathogenic *E. coli* (MPEC), sepsis/new-born meningitis associated *E. coli* (NMEC), sepsis-associated pathogenic *E. coli* (SePEC), and endometrial pathogenic *E. coli* (EnPEC). The DEC pathotypes is classified into eight other sub pathotypes: enterotoxigenic *E. coli* (ETEC), entero pathogenic *E. coli* (EPEC), entero haemorrhagic *E. coli* (EHEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). Poultry have an extensive history and while tracing the origin, many variations are seen (2). For many years, poultry and man have coexisted. Humans mostly use them as a source of food i.e. eggs and meat.

Human body require macronutrients i.e carbohydrates, fats and proteins, but these nutrients must be consumed in balanced form as it is necessary for healthy lifestyle. Meat is the best source of protein in the diet because it has all the vital amino acids which are essential for enzyme and protein synthesis in the body (3).

E. coli is native colonist of gastrointestinal tracts of human and animals (warm blooded). Extra intestinal *E. coli* reports for bulk of 85% of infections of urinary tract (UTIs) in humans and is the major etiology of community-acquired UTIs (4). Meat,



which is sold on grocery stores especially poultry meat was found to be carrying *E. coli* (5). Variety of foods is probable source of Shiga Toxin producing *E. coli* (STEC). Poultry meat largely get contaminated with *E. coli* during massaging, due to poor handling in processing of meat, cross contamination and contamination may occur from human to food through handlers. *E. coli* has been developed worldwide from chicken meat. It might be caused by increased use of antibiotics (6). *E. coli* is incubated in 72-120 hours and the clinical signs are comprised of diarrhea which after few days' converts into bloody diarrhea, abdominal cramps, fever may absent. The clinical marks of *E. coli* septicemia are mainly referred to bacteremia, toxemia and the consequences due to localization of bacteria at multiple tissue spaces throughout the host body (7).

Antibiotics also known as antimicrobial drug can be natural, synthetic, semi-synthetic and these drugs are used against bacterial infections and diseases, through different routes i.e. parental, oral or topical (8). These are often used in poultry settings as a growth promoter because of prevention through diseases and increase feed efficiency. When antibiotics use as growth promoter, it increases the feed efficacy and increases the standards of poultry industry as well and these things allow the person to get the standard chicken meat and eggs at reasonable rate. And its use has also enhanced the physical condition by reducing the disease incidence. In spite of all these positive factors, unfortunately poultry meat contains high concentration of drug residues which cause antibiotic resistance (9). Antibiotics are mainly used in poultry sector as anaphylactic agents and therapeutic agents. The exact mechanism of antibiotics through which they enhance the growth is still not known but many mechanisms are proposed, such as: (i) other competitive organism is eradicated so enough nutrients are there, (ii) nutrients are absorbed more capably by intestinal epithelium, (iii) number of strong pathogenic microorganisms are less which are responsible for subclinical diseases, (iv) production of toxins by microflora of intestine is lessened (10). Major classes of antimicrobial agents (antibiotics) which are used in poultry include penicillin (11), aminoglycosides, cephalosporins, macrolides (e.g. tylosin, erythromycin), sulfonamides, quinolones/fluoroquinolones and tetracycline (12).

The use of antibiotics has a vital role in the development of bacterial resistance against number of antibiotics. Antibiotics which are used in agriculture field are a big reason of antibiotic resistance. The fact regarding the high antibiotic resistance due to misuse of antibiotics in the animal farm and poultry is ignored (13). Increased contact to antimicrobial drugs which is used for disease prophylaxis and treatment, and growth promotion of intestinal microbes is one of the reason for the appearance of resistant strains (14). Rather than treating with diseases, 90% of the drugs used as growth promoters. Resistant organisms emerge from agricultural settings and enter into human settings, thus forming trans-border resistance. Antibiotic resistance is in fact a defensive tool which is used by those bacteria which generate antibiotics to defend themselves from their own harmful and dangerous products and for protection by other microorganisms. Resistance can be transfer from one organism to other and also can be develop by mutation and then these strains having resistance spread in population and become dominant. The rate of antibiotics resistance is high in the pathogenic and commensal bacteria, if more antibiotics are used. I-e direct relation of antibiotics and antibiotics resistance (8).

The enteric bacteria which are resistant, isolated from poultry seeking attention because of the direct and indirect effect on human health e-g by increasing both mortality and morbidity, and indirect effect include the treatment costs of the

diseases caused by resistant bacteria (15). Humans keep high risk due to contact to these resistant pathogens from animals through direct contact by using contaminated food and water and also the contact to other infected humans. The resistant determinant which is present in animals also contaminate the meat which is taken from them (8). AB resistant epidemiology is generated by the concern of transmitting drug resistant food borne bacteria to human settings by eating food that is contaminated with strong pathogen or by direct or indirect contact with animals, which contributing towards human illnesses with higher rates of treatment failures (16).

Antibiotic resistance can be transferred by many mechanisms from bacteria to daughter bacteria and to members of same or different species. Two sites are present in bacteria where encoding of genotypic traits of antibiotic resistance is done. These sites are Chromosomes, extra chromosomal i.e. plasmids. Only some bacteria are sensitive to antibiotics while others are not. Generally, there are three requisites of sensitivity i.e. Mechanism of entrance into bacteria, absence of drug modifying and inactivating enzymes. Any type of alteration in above mentioned site could make sensitive bacteria to resistant. Plasmids are Extrachromosomal genetic elements can cause antibiotic resistance in bacteria specially R-plasmids or R-factors. Among many species of bacteria specially pathogens of livestock and humans, these plasmids are widely distributed. Few of them are also responsible for transferring antibiotic resistance among species belonging to different genera. Several mechanisms are present for conferring antibiotic resistance in R-plasmids. These mechanisms include, Modification of the metabolic pathways resulting in circumvention of antibiotic effects, Inactivation of the drugs by encoding such enzymes that can change its chemical composition, Decrease in binding capacity of drug by alteration in its target site, Reduction in the accumulation of drug inside the cell by altering the permeability of cell or by efflux of the drug actively. These mechanisms of antibiotic resistance are usually determined by same plasmid that can be transmissible or in transmissible. Hence, the chances of survival of bacteria in the presence of different microbial agents can be increased by resistance genes present in R-plasmids resulting in development of evolutionary pressure chiefly due to utilization of antibiotics. This consequently causes in distribution and acquisition of resistance genes by R-plasmids to several antibiotics.

Virulence factors of APEC are adhesion, Type-1 fimbriae, P fimbriae, Curli fimbriae, S-fimbriae, Capsule, Colicin, Temperature sensitive hemagglutinin (TSH), Iron acquisition system (Iron is needed by bacteria by bacteria for several biological reactions e-g electron transport, production of nucleotides, peroxides reduction. And for survival of bacteria) and Serum resistance (It is a significant factor contributing to APEC virulence factor and is chiefly contributed by K1 capsular polysaccharide. Bacterial capsules, lipopolysaccharides, colicins and outer membrane proteins are structures of bacteria mediating them in resistance to complement) (17). Toxins (Enterotoxins, temperature labile toxins, verotoxins, temperature stable toxins and vacuolating toxins are some of the examples that are synthesized by APEC).

METHODOLOGY OF THE STUDY

Sampling

Total 110 chicken livers were collected from various shops of Peshawar, Pakistan. The shops were located in different areas of Peshawar city. Firstly, areas were selected according to the population then one or two poultry shops were randomly selected for sampling from each selected area. These liver



samples were collected in sterile and tightly covered falcon tubes, and were taken to the laboratory in a cool bank for further processing.

Isolation of *E. coli* from Chicken's Liver

Frozen samples were then allowed to thaw at room temperature for some hours and then transferred to 50ml Brain Heart Infusion (BHI) broth present in another set of purified falcon tubes. BHI broth having liver samples was incubated for 24hours at 37 °C. After incubation 500 µl of inoculum was moved to MacConkey agar plates, and then these plates were incubated at 37 °C for 18-24hour. Then colonies which seemed to be *E. coli* were transferred to MacConkey agar for more purification. Then these purified cultures were stored for small duration of time on MacConkey agar plates and slants having nutrient agar. While long term storage was achieved by glycerol preservation method (GPM) at 200° C.

Identification of *E. coli*

Initial identification of *E. coli* was done by morphological identification (on MacConkey agar), Gram Staining and by lactose fermentation on MacConkey agar. It has also been identified by Eosin Methylene Blue agar (EMB) to distinguish it (*E. coli*) from *shigella* and *salmonella* as well as from other Gram negative and lactose fermenting bacterial species. Further *E. coli* was identified by biochemical testing such as indole test, urease test, citrate test and triple iron sugar test.

Antibiotic susceptibility testing (AST)

Once *E. coli* has been confirmed morphologically and biochemically so it is subjected for antibiotic susceptibility testing against 23 antibiotics (AB) commonly used in humans and poultry. Drugs belonging to different classes were: Fluoroquinolones include levofloxacin (LEV 30µg) and ciprofloxacin (CIP 30µg). Penicillin include amoxicillin (AML 30µg) and ampicillin (AMP 30µg), Aminoglycosides include gentamycin (CN 30µg) and tobramycin (TOB 30µg), neomycin (N 30µg) and streptomycin (S 30µg), Tetracycline's include doxycycline (DO30µg), tigicycline (TGC 30µg), oxytetracycline(OT 30µg), phenicols include chloramphenicol (C30µg), cephalosporins cefexime (CFM 30µg), cefotaxime (CTX 30µg) and cephalothin (KF 30µg), cefepime (FEP 30µg), ceftazidime (CAZ 10µg), and nitrofurans include nitrofurantoin (30µg) Folate pathway inhibitors contain trimethoprim Sulfomethaxazole (SXT 30µg) ,Lincosamides include lincomycin (MY 30µg),β-lactamase inhibitors contain augmentin (AUG 30µg), polymixin include polymixin b (PB30µg). Carbapenems(10µg) include imipenem and meropenem. Antibiotic susceptibility testing was done according to CLSI guidelines 2015 by disc diffusion assay.

Antibiotic Discs

Antibiotic discs of oxoid (UK) were used, having different classes: fluoroquinolones include levofloxacin (LEV 30µg) and ciprofloxacin (CIP 30µg) , penicillins include amoxicillin (AML 30µg) and ampicillin (AMP 30µg), aminoglycosides include gentamycin (CN 30µg) and tobramycin (TOB 30µg), neomycin (N 30µg) and streptomycin (S 30µg), tetracyclines include doxycycline (DO30µg), tigicycline (TGC 30µg), oxytetracycline(OT 30µg), phenicols include chloramphenicol (C30µg), cephalosporins cefexime (CFM 30µg), cefotaxime (CTX 30µg) and cephalothin (KF 30µg), cefepime (FEP 30µg), ceftazidime (CAZ 10µg) and nitrofurans include nitrofurantoin (30µg), folate pathway inhibitors contain trimethoprim sulfomethaxazole (SXT 30µg), lincosamides include lincomycin

(MY 30µg), β-lactamase inhibitors contain augmentin (AUG 30µg), polymixin include polymixin b (PB30µg), carbapenems(10µg) include imipenem and meropenem. And according to recommendations the working discs were kept at 2-8° C while discs without working to be stored at -20° C, and discs were placed at room temperature one hour prior to use.

MDRs Identification

Total 11 classes of antibiotics were used comprising different antibiotics. *E. coli* isolates were reported to MDRs (multi drug resistant) when they showed resistance to at least 3 or more than 2 classes of antibiotics.

Molecular Identification

Morpho-cultural and biochemical identification was followed by highly authentic and precise molecular identification. For molecular identification, a series of steps and techniques were done, i-e: starting from DNA extraction to PCR and then finally gel electrophoresis.

Then the isolation of *E. coli* from media was done by pellet formation. Genomic extraction of DNA was done by phenol chloroform DNA extraction which was followed by DNA estimation and quantitation techniques. Molecular detection of *E. coli* was performed by PCR optimization techniques. Detection of TEM gene, OXA gene, SHV gene and PSE gene had done under PCR specified conditions and optimization followed by agarose gel electrophoresis.

Data Analysis

SPPS and SEM (Smart PLS) was used to analyze the data.

RESULTS

After collection and isolation of *E. coli* on MacConkey agar media. Round and pink colonies of *E. coli* were obtained. Morphological identification of *E. coli* has confirmed the identification by giving gram negative rods (gram staining), green metallic sheen on EMB media and round pink colonies on lactose fermentation MacConkey agar. Biochemical testing (citrate test, indole test, urease test, and TSI test) confirmed the presence of *E. coli* by giving results favoring *E. coli* identification. Antibiotic susceptibility testing revealed resistant and sensitive strains of *E. coli* against carbapenem. MDR pattern of APEC shows resistant patterns of *E. coli* against penicillin, fluoroquinolones, aminoglycosides, tetracycline, nitrofurans, phenicols and cephalosporins (Fig. 1). *E. coli* had also showed resistance against folate pathway inhibitors, Beta lactamase inhibitors, polymixins and carbapenem.

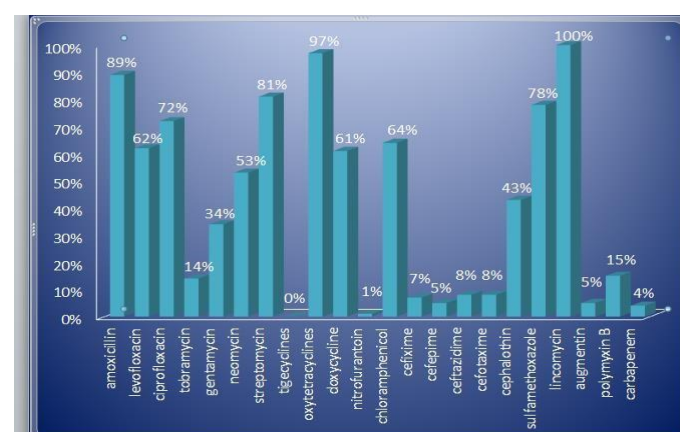


Fig. 1. APEC resistant patterns against different antibiotics

Multidrug Resistant (MDR) *E. coli*

Among 85 isolates, 84 (98.9%) are reported to be MDR, because they display resistance to 3 or more than 3 classes of antibiotics (Fig. 2).

Screening of ESBLs genes have been done by plasmid DNA extraction (Fig. 3 and 4). Bands of plasmid DNA were observed on gel. TEM gene were detected and its ladder and bands were observed on gel. Other genes i.e. OXA gene, SHV and PSE genes were also detected on gel in the form of band and ladder. All gene have been detected on 2% agarose gel.

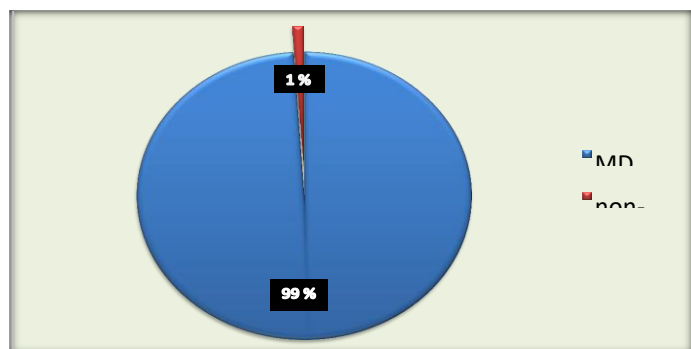


Fig. 2. Pie chart shows % of MDRs and non MDR.

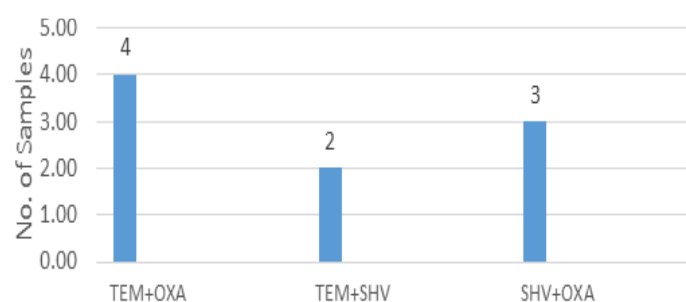


Fig. 3. Graph showing coexistence of two ESBLs genes.

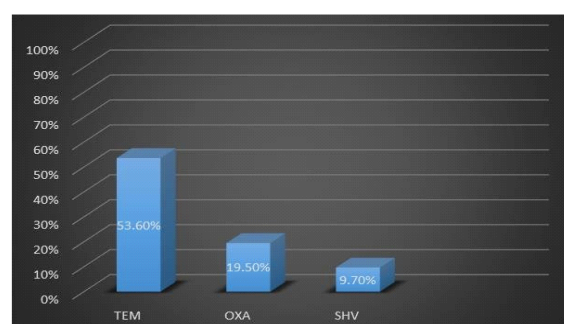


Fig. 4. Graph showing prevalence of ESBL genes.

DISCUSSION

Avian pathogenic *E. coli* (APEC) is an important source of transmission of diseases and antibiotic resistance and hence a big cause of economic loss in poultry industry. Out of 110 samples of chicken liver 85 (77%) were positive for *E. coli* which is a big percentage. The presence of *E. coli* in meat is not only an infectious dose for handler and consumer but also a potential source of cross contamination. Our results are supported by different studies e-g a research held in Bangkok in 2014 showed that 38 out of 152 (25%) samples were confirmed with *E. coli* (18). Another study from Vietnam conducted in 2014 showed the confirmed presence of *E. coli* i.e. (19).

One research from Iran showed that 57 out 360 (15%) chicken samples were found to be contaminated from *E. coli* (20). The difference of percentages might be due to different geographical conditions. Another study from Nigeria showed the 43.4% prevalence of *E. coli* (21). Similarly, a study at Helsinki in 2012 showed that *E. coli* was isolated from 207 out of 219 (94.5%) poultry meat products (22). A study conducted by Alexandria University Egypt showed that 68% of the samples of chicken meat were found to be having *E. coli* (23). A study conducted by University of veterinary and animal sciences (UVAS) Lahore Pakistan in 2016 showed that out of 200 samples of cloacal swabs 126 (63%) were found to be confirmed with *E. coli* (18). A research from India showed the occurrence of 74 different bacteria in 110 raw meat samples and the prevalence of *E. coli* is 98%. It is supposed to be considered that poor handling of animal carcass and meat and use of contaminated water and instruments as well as cross contamination from environment is the major reasons of contamination of poultry meat with *E. coli*. So, poor handling can be a reason of different prevalence rate of *E. coli* in different studies. In our study from 85 samples of chicken liver 83 were found to be multi drug resistance (MDRs). And it is obviously because of the frequent use of antibiotics as growth promoters and anaphylactic agents in poultry industry. A study report in Vietnam support our result which validate a very high (81.3%) prevalence of MDR *E. coli*, isolated from small-scale chicken farms and households (19). Another study from Thailand show 100% prevalence rate that all of the samples were found to be MDR *E. coli*. In our study, resistance to different antibiotics are different i.e. ciprofloxacin is 71.80%, ampicillin is 96.5%, nitrofurantoin is 11%, ceftazidime is 8.30%, cefotaxime is 8%, sulphamethoxazole-trimethoprim is 77.60%, resistance to chloramphenicol is 63.50%, oxytetracycline is 96.50%, streptomycin is 81%, carbapenem 4%. While a study in Iran showed the resistance to oxytetracycline 43.40%, chloramphenicol 20.75%, sulfadimethoxine-trimethoprim 39.62%. Similarly, a study conducted at COMSATS university Islamabad, Pakistan, confirmed the resistance to ciprofloxacin 44%, nitrofurantoin 28%, ceftazidime 16%, cefotaxime 16%, sulphamethoxazole-trimethoprim 84%, ampicillin is 44% (24). The difference in resistance pattern percentages might be due to difference of antibiotic usage. A study report from Pakistan shows antibiotic resistance against Amoxicillin and Tetracycline with % resistance of 71.4% and 57.1% respectively in commercial broiler, whereas 80.3% and 82.1% in backyard poultry respectively and the percentage resistance against streptomycin and trimethoprim was detected to be high in backyard poultry i.e. 64.2% and 53.5% as compared to the commercial broiler with 28.5% and 35.7% respectively. Likewise, other study shows that 92% isolates showed resistance to tetracycline and ampicillin. (35/38). The resistance against kanamycin were 15.8% (6/38), whereas against streptomycin 23.7%. Another study from India shows the antibiotic resistance of *E. coli* (90% to tetracycline and ampicillin, 20% to gentamicin, norfloxacin, and ciprofloxacin, 40% chloramphenicol and 10% resistance to cotrimoxazole). Another study from India showed that 90.77% of *E. coli* was resistant to nitrofurazone, 83.08% to tetracyclines, 76.92% to cotrimoxazole, 83.08% to ciprofloxacin and 81.54% to chloramphenicol (25). A study conducted in 2015 at Turkey showed 68.4% of the poultry products were proved to be positive for ESBLs (extended spectrum beta lactamases) producing *E. coli* (26). Similarly, another study conducted in Gabon in 2014 showed that 23% of the samples of chicken meat were having ESBLs producing *E. coli*. Another study from

Germany shows that 43.9% of the isolates were found to be ESBLs generating *E. coli*.

Another study from Turkey held in 2016 showed 80% of ESBLs producing *E. coli*. A research performed in Slovakia showed 60% resistance to ampicillin, 18% to tetracycline, 40% to streptomycin, 14% to florfenicol and 17 % to cotrimoxazole (27). The prevalence of ESBLs genes in our study is TEM 53.60%, OXA 19.05%, SHV 9.70% and PSE 0%. The isolates show highest prevalence for TEM and no sample was found to be carrying PSE gene. A study from Iran showed the prevalence of TEM 37.7%, CTXM 60.3% and VEB 13.2%. Another study from Germany showed the prevalence of SHV 43.9%, CTX-M 41.2% and TEM 8.6%.

While in our study TEM has highest prevalence followed by OXA, this might be due to difference of geographical location and difference in resistance pattern. Another study from Turkey showed the prevalence for TEM, CTX-M and SHV 96.4%, 53.7% and 34% respectively. A research conducted in India showed the prevalence of TEM 1.53% in chicken meat while no isolate was found positive for SHV. Another study from Netherlands showed that in chicken meat most prevalent genotype was CTX-M 58.1% and the prevalence of TEM and OXA was 14%. Another study showed the prevalence of CTX-M 62% and TEM 13% in poultry (18). Another study showed the prevalence of TEM<, SHV, CTX-M among asymptomatic carrier poultry birds and diseased bird, 28% 56%, 73% 0%, 2% 32% respectively. It shows that TEM and SHV are prevalent among asymptomatic carriers while CTX-M shows high prevalence in diseased birds. An analysis of Dutch meat showed the prevalence of CTX-M1 49%, TEM 26%, SHV12, SHV2 16% and 4% respectively.

Conclusion

The overall study shows the high resistance pattern of APEC against a number of antibiotics commonly used and high percentage of ESBLs genes. It proves the inappropriate use of antibiotics in poultry industry, easily available drugs and flaws in monitoring by regulatory authorities. The high prevalence of virulence genes among APEC show that they are not only a threat to poultry but to human as well. A large scale study is needed to view the resistance pattern across the country and a close attention should be given to the use of antimicrobials.

Acknowledgments

I am thankful to Dr Zahoor, Dr Fazal ur Rehman, dr Ali Akber, Dr Zara, Dr Abdul Wadood, Javaid Iqbal, Zainab Syed, Durdana, Mehraj Gull and Saba Sabir Malik for their help and continuous support in my entire research work.

References

1. Zil-e-Huma, Tareen AM, Kaleem U, Tauseef M Asmat, Asim I, Mustafa MZ, Irshad A, Saddeq UR. Incidence of Diarrheagenic *Escherichia coli* Pathotypes in Children Suffering from Diarrhea in Tertiary Care Hospitals, Quetta, Pakistan. *Pakistan Journal of Zoology*. 2019;51:2015-21.
2. Elson H, Gleadthorpe A, Vale M, Mansfield U. Housing and husbandry of laying hens: Past, present and future. *Lohmann Information*. 2011;46:16-24.
3. McAfee AJ, McSorley EM, Cuskelly GJ, Moss BW, Wallace JM, Bonham MP, et al. Red meat consumption: An overview of the risks and benefits. *Meat science*. 2010;84:1-13.

4. Zhang L, Foxman B. Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. *Front Biosci*. 2003;8:e235-e44.
5. Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, et al. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. *Emerging infectious diseases*. 2012;18:415.
6. Machado E, Ferreira J, Novais Â, Peixe L, Cantón R, Baquero F, et al. Preservation of integron types among *Enterobacteriaceae* producing extended-spectrum β -lactamases in a Spanish hospital over a 15-year period (1988 to 2003). *Antimicrobial agents and chemotherapy*. 2007;51:2201-4.
7. Grassl GA, Finlay BB. Pathogenesis of enteric *Salmonella* infections. *Current opinion in gastroenterology*. 2008;24:22-6.
8. Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, et al. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*. 2004;53:28-52.
9. Donoghue DJ. Antibiotic residues in poultry tissues and eggs: human health concerns? *Poultry Science*. 2003;82:618-21.
10. Feighner SD, Dashkevich MP. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholytaurine hydrolase activity. *Appl Environ Microbiol*. 1987;53:331-6.
11. Caetano R, Kaplan MS, Huguet N, Conner K, McFarland BH, Giesbrecht N, et al. Precipitating circumstances of suicide and alcohol intoxication among US ethnic groups. *Alcoholism: clinical and experimental research*. 2015;39:1510-7.
12. Mathew AG, Cissell R, Liamthong S. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne pathogens and disease*. 2007;4:115-33.
13. Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: perspective, policy, and potential. *Public health reports*. 2012;127:4-22.
14. Gyles CL. Antimicrobial resistance in selected bacteria from poultry. *Animal health research reviews*. 2008;9:149-58.
15. Warren R, Ensor V, O'Neill P, Butler V, Taylor J, Nye K, et al. Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β -lactamases in the UK. *Journal of Antimicrobial Chemotherapy*. 2008;61:504-8.
16. Ye Y, Yang Q, Fang F, Li Y. The camelliagenin from defatted seeds of *Camellia oleifera* as antibiotic substitute to treat chicken against infection of *Escherichia coli* and *Staphylococcus aureus*. *BMC veterinary research*. 2015;11:214.
17. Nakazato G, Campos TAd, Stehling EG, Brocchi M, Silveira Wd. Virulence factors of avian pathogenic *Escherichia coli* (APEC). *Pesquisa Veterinária Brasileira*. 2009;29:479-86.
18. Akbar A, Sitara U, Ali I, Khan MI, Phadungchob T, Anal AK. Presence of *Escherichia coli* in poultry meat: a potential food



safety threat. International Food Research Journal. 2014;21:941.

19. Nguyen VT, Carrique-Mas JJ, Ngo TH, Ho HM, Ha TT, Campbell JI, et al. Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong Delta of Vietnam. *Journal of Antimicrobial Chemotherapy*. 2015;70:2144-52.

20. Momtaz H, Rahimi E, Moshkelani S. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinari medicina*. 2012;57:193-7.

21. Adeyanju GT, Ishola O. *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus*. 2014;3:139.

22. Lyhs U, Ikonen I, Pohjanvirta T, Raninen K, Perko-Mäkelä P, Pelkonen S. Extraintestinal pathogenic *Escherichia coli* in poultry meat products on the Finnish retail market. *Acta Veterinaria Scandinavica*. 2012;54:64.

23. Samaha I, Ibrahim H, Hamada M. Isolation of some enteropathogens from retailed poultry meat in Alexandria Province. *Alex J Vet Sci*. 2012;37:17-22.

24. Chakravarty MS, Ganesh P, Amaranth D, Shanthi Sudha B, Subhashini M. *Escherichia coli*-occurrence in the meat of shrimp, fish, chicken and mutton and its antibiotic resistance. *European Journal of Experimental Biology*. 2015;5:41-8.

25. Bauermeister LJ, Bowers JW, Townsend JC, McKEE SR. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. *Journal of food protection*. 2008;71:1119-22.

26. Beninati C, Reich F, Muscolino D, Giarratana F, Panebianco A, Klein G, et al. ESBL-producing bacteria and MRSA isolated from poultry and turkey products imported from Italy. *Czech Journal of Food Sciences*. 2015;33:97-102.

27. Moura A, Araújo S, Alves MS, Henriques I, Pereira A, Correia A. The contribution of *Escherichia coli* from human and animal sources to the integron gene pool in coastal waters. *Frontiers in microbiology*. 2014;5:419.