VOLUME-7, ISSUE-5, MAY-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper | Veterinary Science

ANTIMICROBIAL RESISTANCE PATTERN OF *E. COLI* FROM POULTRY: A REVIEW

Dr. Sujata S. Bhave* Senior Research Fellow, Department of Veterinary Public Health, Bombay Veterinary College, Parel, Mumbai - 12. *Corresponding Author

Dr. Nitish N. Joat M.V.Sc., Pathology.

ABSTRACT Antimicrobial resistance (AMR) is a worldwide threat to public health, and usage of antimicrobials and AMR in animal production is one of its contributing sources. Poultry is one of the most preferred types of meat consumed world over. Large amounts of antimicrobials are used to raise poultry flocks under intensive conditions to prevent and to treat disease, as well as for growth promotion. Antimicrobial resistant poultry pathogens may cause consequences such as treatment failure, leading to economic losses, but also act as a source of resistant bacteria/ genes (including zoonotic bacteria) that may pose a risk to human health. Here we reviewed data on AMR in *E. coli* isolated from poultry. This review highlights the AMR pattern in *E. coli* isolated from poultry and suggests the need to monitor the AMR pattern in bacteria in the poultry production systems.

KEYWORDS : *E. coli*, Poultry, Antimicrobial Resistance, Antibiotics.

Antimicrobial resistance (AMR) is a global health concern (WHO, 2014). Over recent years, the contribution of antimicrobial usage (AMU) and AMR from animals to the overall burden of AMR has emerged with substantial evidence (Marshall and Levy, 2011). The excessive use of antimicrobials in food animal production is the contributing factor in increasing the AMR burden. Due to intensification of farming practices in much of the developing world, the magnitude of usage is expected to increase considerably over coming years (Van Boeckel *et al.*, 2015). Much of the research and assumptions on the prevalence and evolution of AMR in animal production systems relate to organisms that more often than not are commensal in poultry such as *Escherichia coli* (Tadesse *et al.*, 2012; Simoneit *et al.*, 2015; Luna-Galaz *et al.*, 2016), *Enterococcus* spp., and *Staphylococcus aureus* (Bortolaia *et al.*, 2016) as well as foodborne zoonotic pathogens, such as non-typhoidal *Salmonella*(NTS) (Blackall, 1988; Van *et al.*, 2012Luna-Galaz *et al.*, 2016;) and *Campylobacter* spp. (Custer *et al.*, 2015). However, the problems of AMR producing *E. coli* in relation to food animals including poultry production have not been specifically addressed.
One of the factors accelerating the development of AMR in pathogens, as well as in commensal organisms is the indiscriminate use of antimicrobials in animal farming. In addition to the concerns due to the emergence of AMR in bacteria from poultry production, there are also human health concerns about the presence of anti-microbial residues in meat (Reig *et al.*, 2008) and eggs (Goetting *et al.*, 2011). AMR in *E. coli* isolated from poultry is also likely to lead to economic losses, resulting from the expenses on ineffective antimicrobials, as well as the burden of untreated poultry disease. Here, we review and summarize data on phenotypic resistance against antimicrobials among *E. coli*, in order to identify overall trends and high-light knowledge gaps.

Among *Enterobacteriace* family, *Escherichia coli* (*E. coli*) are ubiquitous bacteria representing diverse strains of high relevance to human having potential to cause wide spectrum of diseases (Wang *et al.,* 2009; Bauchart *et al.,* 2010). More than 90% *E. coli* strains are known as harmless commensal of GIT in warm-blooded animals; while 8-10% strains are highly diverse and adapted pathogens. Pathogenic *E. coli* strains fall in two categories: intestinal/diarrheagenic *E.coli* (DEC) and those that cause extraintestinal illness i.e. extra-intestinal pathogenic *E. coli* (ExPEC). In addition, strains which cause systemic infection in poultry i.e.collibacillosis are called as avian pathogenic *E. coli* (APEC).Since *E. coli* is ubiquitous in the gastrointestinal tract of warm-blooded animals, it has been extensively used to monitor AMR in food animals (including poultry) (EFSA, 2014, Nhung *et al.*, 2016).

World-wide,poultry is one of the most widespread food producing industries, and chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year (FAO, 2017). The relatively low production costs and the absence of cultural and religious restrictions for its consumptionare the main reasons for extensive growth of poultry production.In most countries,a large diversity of antimicrobials are used to raise poultry (Agunos *et al.*, 2012, Landoni and Albarellos, 2015), mostly through the oral route, with the aim to prevent and to treat disease, but also to enhance growth and productivity (Page and Gautier, 2012). A large number of such antimicrobials are considered to be of critical and high importance for human medicine (WHO, 2017).

Also, one likely contributing factor of antimicrobial resistance in poultry is large increasing human consumption of retail chicken over the past 30 years which has led to the changes in the scale and method of poultry production including use of antimicrobial agents for growth promotion, infection, prevention and treatment which has amplified multidrug resistant*E. coli* in food animal reservoirs. Among different meat types, poultry generally exhibits highest overall level of *E. coli* contamination and poultry associated *E. coli* tend to be more extensively antimicrobial resistant than those from other meats (Manges and Johnson, 2015). In addition, some *E. coli* strains hosted by poultry are potential source of AMR genes that may transmit to humans (Overdevest *et al*., 2011, Kheiri and Akhtari, 2016).

Here, we review and summarize data on phenotypic resistance pattern against antimicrobials among *E. coli* from poultry, in order to identify overall trends. This review is intended to highlight the antimicrobial resistance pattern of *E. coli* from poultry from different areas of world as well as India and thus justifies the need of the strict surveillance of the antimicrobial resistance pattern from poultry.

Worldwide many researchers have studied the antimicrobial resistance pattern in *E. coli* in poultry. Johnson *et al.* (2005) examined antimicrobial sensitivity pattern of *E. coli* for 12 antimicrobials in 1648 diverse food items including pork, beef, chicken, turkey and miscellaneous food such as cheese, shrimp, crab fish etc. Antibiotics used were ampicilin, amoxicillin-clavulanate, cefazolin, ceftazidime, gentamicin, tetracycline, nitrofurantoin, nalidixic acid, ciprofloxacin, sulfisoxazole, trimethoprim, and trimethoprim-sulfamethoxazole. Results showed that resistance to ciprofloxacin and nalidixic acid was highest in poultry (3.4% and 30%, respectively) than any other food items. Miles *et al.* (2006) analyzed 82 *E. coli* strains isolated from fecal samples of broiler chickens (n-34) to investigate the prevalence of antibioticresistance in fecal *E. coli* isolates from healthy broiler chickens in Jamaica. All isolates were tested by agar disc diffusion to determine their susceptibility patterns to 11 antimicrobial agents. All the avian isolates were highly resistant to kanamycin (91.2%), nalidixic acid (85.3%) and tetracycline (82.5%). Resistance to ofloxacin, ciprofloxacin, chloramphenicol and amoxicilin/clavulanate was low with 11.8%, 8.8%, 2.9% and 2.9%, respectively. None of the avian isolate showed resistance to gentamicin. Tetracycline is a commonly used first line antibiotic for many domestic animals and is commonly used before the antibiotic resistance profile of a pathogen has been determined. Resistance to tetracycline is plasmid mediated coupled with a wide variety of genetic determinants. This makes it more possible for a susceptible bacteriumto acquire resistance factors by conjugation, or by transformation(Miles *et al.*, 2006).

Johnson *et al.* (2009) tested 287*E. coli* isolates from turkey and chicken products for antibiotic resistance to cotrimoxazole, nalidixic acid and ceftiofur. Overall, 61 isolates were resistant to cotrimoxazole, 48 were resistant to nalidixic acid and 36 were resistant to ceftiofur. Chicken isolates showed equal resistance and susceptibility percentage (40%) to nalidixic acid and higher percentage i.e. 56% resistance to ceftiofur. The isolates showed equal resistance to cotrimoxazole (33%).Kazemnia *et al.* (2014) evaluated patterns of antibiotic resistance of *E. coli* strains isolated from avian collibacillosis (n-25). From 50 tested *E. coli* isolates, all of them (100%) were resistant toPenicillin and erythromycin, followed by 49 (98%) to nalidixic acid, 47 (94%) to cephalexin, 43 (86%) to amoxicilin, 42 (84%) to ampicilin, 37 (74%) to ciprofloxacin, 32 (64%) to tetracycline, 27 (54%) to cefixime and 18 (36%) to gentamicin. The high rate of frequency of resistance towards β lactam antibiotics and cephalosporin groups could be explained by the fact that these drugs are easilyavailable without physicians' prescriptions frompharmacy in developing countries.

Gai *et al.* (2015) evaluated susceptibility of the*E. coli* isolates to antimicrobial agents (n-13) for 87 isolates enumerated from intensive poultry farms in China. All *E. coli* isolates were highly resistant to trimethoprim-sulfamethoxazole, tetracycline (85.06%), sulfisoxazole (83.91%), ampicilin (66.67%), fluoroquinolones [enrofloxacin, 63.22%, ofloxacin, 50.57%] and gentamicin (57.47%). All isolates were multi-drug resistant as they were resistant to at least 2 groups of antimicrobials. Koga *et al.* (2015a) subjected total of 84 *E. coli* strains isolated from chicken carcasses in 2007, and 121 *E. coli* strains isolated in 2013 for antimicrobial susceptibility testing against 15 antimicrobials to analyze the frequency of antimicrobial resistance among *E. coli* strains isolated from commercial chicken carcasses in Parana´, Brazil. Results revealed that, in 2007, high frequency of resistance to tetracycline (70.24%), nalidixic acid (61.9%), and trimethoprim– sulfamethoxazole (58.33%) was seen. In addition, 62 (73.8%) of the strains were resistant to 3 or more antimicrobials. In 2013, an increase in the frequency of resistance to the majority of antimicrobials tested, with the exception of gentamicin, ciprofloxacin, enrofloxacin, and trimethoprim–sulfamethoxazole was seen. Resistance to tetracycline was most frequently observed in 2013 as well, with 90.91% of strains, followed by nalidixic acid (78.51%) and ampicilin (66.94%). Study observed that 79.3% of strains were resistant to 3 or more antimicrobials, and all strains were resistant to at least 1 antimicrobial tested.Rasmussen *et al.* (2015) sampled and analyzed 188 samples from imported and locally produced chicken meat to investigate AMR pattern of *E. coli*. 109 *E. coli* isolates were recovered from meat whereas the remaining 44 were isolated from the cloaca of locally reared live chickens. Antimicrobial susceptibility testing showed that no *E. coli* isolate was resistant to mecillinam, piperacilin/tazobactam, ertapenem and meropenem, and only few isolates showed resistance to gentamicin and nitrofurantoin. Antimicrobial resistance profiles of*E. coli* isolates from locally reared chickens were compared to imported chickens. Antimicrobialresistance to four antibiotic markers with highest resistance was detected more frequentlyin isolates from local chickens compared to imported chickens (tetracycline 88.9% vs.57.5%, sulphonamide 75.0% vs. 46.6%, ampicillin 69.4%vs. 61.6%and trimethoprim66.7% vs. 38.4%). This study demonstrated that the international trade of food may lead to development of multidrug resistant *E. coli* in locally produced meat.

Some researchers worked to compare the antimicrobial resistance pattern of free range chicken with that of conventionally raised ones. Koga *et al.* (2015b) tested 156 *E. coli* strains from conventionally raised and free range poultry for antimicrobial susceptibility test against 15 antimicrobials. According to the test, strains from conventionally raised poultry showed a higher frequency of antimicrobial resistance than strains from free range poultry for all antimicrobials tested. The frequency of antimicrobial resistance to strains from free-range poultry was low, except to tetracycline (60% of resistance), whereas the strains from conventional poultry showed a high frequency of resistance mainly to tetracycline, nalidixic acid, and ampicilin. Braykov *et al.* (2016) tested antimicrobial resistance in *E. coli* isolated from 262 "production birds" (commercially raised broiler chickens and laying hens) and 455 "household birds" (raised for domestic use) in order to evaluate the effects of animal agriculture on the spread of antibiotic resistance. Resistance to tetracycline was detected in 78% of production birds and 34% of household birds. More than half of the production bird isolates were resistant to sulfisoxazole and trimethoprim/sulfamethoxazole (69% and 63%, respectively) compared to 20% and 17%, respectively, of the isolates from household birds. The lowest resistance was to gentamicin (16% of production and 1% of household bird isolates) and amoxicillin/clavulanate (18% and 2%, respectively).The high frequency of antimicrobial resistance in strains from conventional poultry carcasses, primarily to tetracycline, nalidixic acid, and ampicillin, can be related with the selective pressure due to the high use of antimicrobials and/or the contamination of environment in aviculture industries. However,low frequency of antimicrobial resistance in strains from free range poultry, except to tetracycline can be attributed to the restriction or absence of use of antimicrobials in family agriculture (Koga *et al.*, 2015b).

In Indian context, Joshi *et al.* (2012) evaluated antibiotic resistance profile of *E. coli* isolates from colibacillosis in layers in and around Pantnagar. For the study, a total of 20 isolates of *E. coli* were recovered from 35 cases of colibacillosis in layers during necropsy. Antibiogram was studied via disc diffusion method against 12 antibiotics. A high degree of resistance was seen against cephalexin (73.68%) followed by neomycin (31.58%), enrofloxacin(31.58%), pefloxacin (26.31%). Minimum resistance was against amikacin (5.2%). In contrast, Chloramphenicol was 100% susceptible against all isolates.From this study, it is clear that multiple antibiotic resistant zoonotic *E. coli* are high in poultry birds in and around Pantnagar, India. Jana and Mondal (2013) studied a total of 83 raw poultry meat samples from West Bengal, India with the objective to determine the antibiogram of *E. coli* isolates associated with samples. Thirteen 'O' serogroups of *E. coli* isolates were tested with 12 different antimicrobial agents. The results showed that *E. coli* isolates were 100% sensitive to chloramphenicol, followed by amikacin (92.31%), gentamicin (84.62%), ciprofloxacin (69.23%), erythromycin (15.38%), oxytetracycline (15.38%), cefixime (7.69%), sulphafurazole (7.69%) and vancomycin (7.69%). Intermediate sensitivity showed to kanamycin (61.54%) followed by methecillin (38.46%), erythromycin (23.08%), ciprofloxacin (15.38%) and amikacin (7.69%). For novobiocin, 100% of the isolates showed resistant followed by cefixime (92.31%), sulphafurazole (92.31%), vancomycin (92.31%), oxytetracycline (84.62%), erythromycin (61.54%), methicillin (61.54%), ciprofloxacin (15.38%), gentamicin (15.38%) and kanamycin (7.69%). The possible explanation for this resistance of *E. coli* against the antibiotics as found in the study could be due to the indiscriminate use of these antibiotics in poultry. This high resistance might be attributed to transmissible drug resistance and resistance might also develop due to mutational changes (Jana and Mondal, 2013).

Such widespread occurrence of antimicrobial resistance in *E. coli* of poultry origin suggests that poultry may play considerable role as a reservoir for antimicrobial resistant *E. coli*. It is further suggested that more extensive surveillance on the phenotypic and genotypic resistance pattern of *E. coli* from poultry origin should be undertaken because transmission of resistant plasmids to humans from food animals (poultry) can occur. These typesof studies will help in tracing out the origin of antimicrobial resistant bacteria. The obtained data of antimicrobial resistance from several published reports indicated that most *E. coli* recovered from poultry are multi - drug resistant. Long term prospective studies on examining *E. coli* isolates from demarcated geographic locations are required to more accurately identify temporal and spatial differences in antimicrobial resistance pattern in strains of poultry origin.

References:

1. Agunos A, Leger D, Carson C (2012). "Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada". Can. Vet. J., Vol. 53, pp. 1289–300.
2. Bauchart P, Germon P, Bree A, Oswald E, Hacker J, Dobrindt U (2010). "Pathogenomic comparison of human extra-intestinal and avian pathogenic Escherichia coli - search for factors involved in host specificity or zoonotic potential". Microb.Pathog., Vol. 49, pp. 105-115.
3. Blackall P J (1988). "Antimicrobial drug resistance and the occurrence of plasmids in Haemophilus paragallinarum". Avian Dis., Vol. 32, pp. 742–7.
4. Bortolaia V, Espinosa-Gongora C, Guardabassi L (2016). "Human health risks associated with antimicrobial-resistant enterococci and Staphylococcus aureus on poultry meat". Clin. Microbiol. Infect.,Vol. 22, pp. 130–40.
5. Braykov N P, Eisenberg J N S, Grossman M, Zhang L, Vasco K, Cevallos W, Muñoz D, Acevedo A, Moser K A, Marrs C F, Foxman B, Trostle J, Trueba G, Levy K (2016)."Antibiotic Resistance in Animal and Environmental Samples Associated with Small-Scale Poultry Farming in Northwestern Ecuador". Appl. Environ. Sci. Vol. 1, pp. e00021-15.
6. Custer B, Steele JA, Wilcox BA, Xu J (2015). "Intensified food production and correlated risks to human health in the Greater Mekong Subregion: a systematic review". Environ. Health, Vol.14, pp. 43.
7. EFSA (2014). "The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012". EFSA J., Vol. 12, pp. 3590.
8. Food and Agriculture Organization of the United Nations (2017). FAOSTAT: Live Animals Data. Available from: http://www.fao.org/faostat/en 12.
9. Gai W, Wang J, Cui Z, Qu Z, Cui J, Du X, Huang X, Zhao J (2015). Molecular classification and drug resistance analysis of Escherichia coli isolated from poultry in China. Int. J. Clin. Exp. Med., Vol. 8, pp. 836-844.
10. Goetting V, Lee KA, Tell LA (2011). Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. J. Vet. Pharmacol. Ther., Vol. 34, pp. 521–56.
11. Jana A., Mondal A (2013). Serotyping, pathogenicity and antibiogram of Escherichia coli isolated from raw poultry meat in West Bengal, India. Veterinaria Italiana, Vol. 49,pp. 361-365.
12. Johnson J R, Kuskowski M A, Smith K, O'Bryan T T, Tatini S (2005). "Antimicrobial-resistant and extraintestinal pathogenic Escherichia coli in retail foods". J. Infect. Dis., Vol. 191, pp. 1040–9.
13. Johnson J R, McCabe J S, WhiteD G, Johnston B, Kuskowskiand M A, McDermott P (2009)."Molecular analysis of Escherichia coli from retail meats (2002–2004) from the United States National Antimicrobial Resistance Monitoring System". Clin. Infect. Dis., Vol. 49, pp. 195–201.
14. Joshi S, Singh R, Singh S P (2012)."Antibiotic resistance profile of Escherichia coli isolates from Colibacillosis in and around Pantnagar, India". Vet. World, Vol. 5, pp. 405-408.
15. Kazemnia A, Ahmadiand M, Dilmaghani M (2014)."Antibiotic Resistance Pattern of Different Escherichia coli Phylogenetic Groups Isolated from Human Urinary Tract Infection and Avian Colibacillosis". Iran. Biomed. J., Vol.18, pp. 219-224.
16. Kheiri R, Akhtari L (2016). "Antimicrobial resistance and integron gene cassette arrays in commensal Escherichia coli from human and animal sources in IRI". Gut. Pathog.,Vol. 8, pp. 40.
17. Koga V L, Rodrigues G R, Scandorieiro S, Vespero E C, Oba A, de Brito B G, de Brito K C T, Nakazato G, Kobayashi R K T (2015a)."Evaluation of the antibiotic resistance and virulence of Escherichia coli strains isolated from chicken Carcasses in 2007 and 2013 from Parana, Brazil". Foodborne Pathog. Dis. Vol. 12, pp. 479-485
18. Koga V L, Scandorieiro S, Vespero E C, Oba A, de Brito B G, K. de Brito C T, NakazatoG, Kobayashi R K T (2015b)."Comparison of Antibiotic Resistance and Virulence Factors among Escherichia coli Isolated from Conventional and Free Range Poultry". Bio. Med. Res. Int., Vol. 2015, pp. 1-8
19. Landoni M F, Albarellos G (2015). "The use of antimicrobial agents in broiler chickens".Vet. J.,Vol. 205, pp. 21–7.
20. Luna-Galaz G A, Morales-Erasto V, Peñuelas-Rivas CG, Blackall P J, Soriano-Vargas E (2016). "Antimicrobial sensitivity of Avibacterium paragallinarum isolates from four Latin American countries", Avian Dis., Vol. 60, pp. 673–6.
21. Manges A R and Johnson J R (2015). "Reservoirs of Extraintestinal Pathogenic Escherichia coli". Microbiol. Spectrum, Vol. 3, pp. UTI-0006-2012.
22. Marshall B M and Levy SB (2011). "Food animals and antimicrobials: impacts on human health", Clin. Microbiol. Rev., Vol. 24, pp. 718–33.
23. Miles T D, McLaughlin W., Brown P D (2006). Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. BMC Vet. Res., Vol. 2, pp. 7.
24. Nhung NT, Cuong N V, Thwaites G, Carrique-Mas J (2016)."Antimicrobial usage and antimicrobial resistance in animal production in southeast Asia: a review". Antibiotics (Basel), Vol. 5, pp. E37.
25. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C., van der Zwaluw K., Huijsdens X., Kluytmans J. (2011) Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. Emerg. Infect. Dis.,Vol. 17, pp. 1216–22.
26. Page S W, Gautier P (2012). "Use of antimicrobial agents in livestock". Rev. Sci. Tech.,Vol. 31, pp. 145–88.
27. Rasmussen M M, Opintan J A, Frimodt- Møller N, Styrishave B (2015)."Beta-lactamase producing Escherichia coli isolates in imported and locally produced chicken meat from Ghana".PLoS ONE,Vol. 10, pp. e0139706.
28. Reig M and Toldra F (2008). "Veterinary drug residues in meat: concerns and rapid methods for detection". Meat Sci., Vol. 78, pp. 60–7.
29. Simoneit C, Burow E, Tenhagen B A, Käsbohrer A (2015). "Oral administration of anti-microbials increase antimicrobial resistance in E. coli from chicken-a systematic review",Prev. Vet. Med.,Vol. 118, pp. 1–7.
30. Tadesse D A, Zhao S, Tong E, Ayers S, Singh A, Bartholomew M J, McDermott P F (2012)."Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002", Emerg. Infect. Dis., Vol. 18, pp. 741–9.
31. Van Boeckel T P, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP,Teillant A, Laxminarayan R(2015)."Global trends in antimicrobial use in food animals",ProcNatlAcadSciUSA,Vol. 112, pp. 5649–54.
32. Van T T, Nguyen H N, Smooker P M, Coloe P J (2012). "The antibiotic resistance characteristics of non-typhoidal Salmonella enteric isolated from food-producing animals, retail meat and humans in South East Asia". Int. J. Food Microbiol., Vol. 154, pp. 98–106.
33. Wang M C, Tseng S S, WU A B, Huang J J, Sheu B S, Wu J J (2009). "Different roles of host and bacterial factors in Escherichia coli extra-intestinal infections".Clin.Microbiol.Infec.,Vol 15, pp. 372-379.
34. World Health Organization. Antimicrobial Resistance: Global Report on Surveillance. (2014).Available from: http://www.who.int/drugresistance/ documents/ surveillancereport/en/
35. World Health Organization (2017).Critically Important Antimicrobials for Human Medicine, 5th Revision. Available from: http://www.who.int/ foodsafety/ publications/ antimicrobials-fifth/en/