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PREVALENCE OF ANTIBIOTIC RESISTANCE AGAINST *ESCHERICHIA COLI* ISOLATES FROM BROILER MEAT MARKETED IN KATHMANDU VALLEY

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ABSTRACT

Antibiotic resistance is day by day becoming greater health concern. Tremendous use of antibiotic in poultry is known to be a major cause for antibiotic resistant in human. An experiment of meat samples was purchased from various meat shops and examined for bacterial contamination with resistant E. coli. (Total=384) Samples were taken for the study and among them, 189 isolates were evaluated for their antibiotic susceptibilities and the presence of antibiotic resistance. Results of antibiograms revealed that E. coli isolates were resistant to one or more of the antibiotics tested. Ciprofloxacin (42.32%) is higher resistance from the for-E. coli. Colistin Sulphate (2.11%) is less resistance for E. Coli. Whereas Chroramphenicol (42.32%), Gentamicin (41.79%), Levofloxacin (34.92%), Amikamicin (7.4%), resistance was most frequently observed. Fifteen E. coli strains showed the multidrug resistance phenotypes and harbour at least three antibiotic resistances. The results indicated that production methods influenced the frequency of antibiotic resistant E. coli on poultry products available to consumers. Future research to identify the specific practices that cause the high frequency of antibiotic-resistant E. coli in chicken could promote efforts to reduce consumer exposure to this potential pathogen.

Keywords: *E. coli*, chicken meat, antibiogram, multidrug resistance.

INTRODUCTION

Microorganisms with resistance to antimicrobials can affect people at any stage of life and also the animals (livestock) reared in the veterinary and agricultural sectors (ECDC 2015). A study conducted in the United States showed that Escherichia coli (E. coli) isolates from livestock were more resistant than those from human clinical isolates (Tadesse *et al*., 2012). Humans are exposed to antimicrobial-resistant microorganisms and their resistance genes are prevalent in animals, both via the food chain and through widespread release into the environment (Silbergeld *et al*., 2008). Pathogenic bacteria from sources such as livestock can interact with other bacteria, boosting the sharing of genes and genetic components that cause antibiotic resistance. These circumstances can cause non-pathogenic bacteria to develop into resistant reservoirs (Ejaz *et al*., 2021). There is indisputable proof that food derived from a variety of animal sources contains large numbers of resistant bacteria and resistant genes (Marshall *et al*., 2011). A past study reported that chicken meat could be one of the potential causes of infection with multi-drug resistant (MDR) E. coli (Parvin *et al*., 2020). Considering the relatively low production cost and absence of cultural and religious restrictions on its consumption, poultry is one of the most widespread foods; chicken meat and eggs being the most common (Nhung e*t al*., 2017). Antimicrobials are used, not only to treat disease in the poultry industry, but also to promote the growth of broiler chickens (Landoni e*t al*., 2015) (Agunos e*t al*., 2012). In Europe, use of avoparcin in food animals as a growth promoter had been linked with resistance to vancomycin, a last resort antimicrobial in human medicine (Wegener e*t al*., 2012). Therefore, European countries have discontinued use of antimicrobials as growth promoters, but other countries in South America, Africa, and Asia still use it abundantly (Allcock e*t al*., 2017). It has been found that approximately 80% of medically important antimicrobials are used as growth promoters in healthy animals, to fulfill the increasing demand for foods of animal origin (WHO 2017). The overall consumption of antimicrobials in livestock was estimated to have increased by 67% between 2010 and 2030 (Van Boeckel e*t al*., 2015). As a result of consumption and accumulation over time, there is higher chance of the development of multiple pathogens insensitive to medically important antimicrobials (Lees e*t al*., 2021).

The World Health Organization’s (WHO) global action plan on antimicrobial resistance (AMR) emphasizes the “one health” approach, which recognizes the interconnections between humans, animals, and the environment as a single entity, to tackle resistance (Badau e*t al*., 2021). The one health approach provides important insights to plan and control the burden of AMR (Parmley e*t al*., 2012). Systematic reviews on one health approaches have also shown associations between specific interventions targeting reductions in antibiotic use in food-producing animals and decreases in AMR in animals (Scott e*t al*., 2018) (Hoelzer e*t al*., 2017). Knowledge on the burden of AMR and resistance patterns in isolates extracted from food-producing animals is imperative to designing targeted interventions to limit antibiotic use. The use of commensal intestinal E. coli as a marker for the presence of resistance in bacterial flora is a critical component of AMR surveillance programs in both food-producing and wild animals (Wasyl e*t al*., 2013). The chicken gut micro biota constitutes a major source of antibiotic resistance genes that encode several drug efflux pumps, leading to resistance to fluoroquinolones and tetracyclines (Juricova e*t al*., 2017).

In Nepal, the use of antimicrobials has increased in recent times, in order to decrease the morbidity and mortality of chickens (Shrestha., 2022) (Ramdam., 2015). A study conducted in Nepal under the Global Antibiotics Resistance Partnership (GARP) has shown that 46% of veterinary drugs were sold under self-prescription and about 12% on farmer's demand (Ramdam., 2015). Surveillance of animal pathogens commenced in 2011, with a collaboration between the National Public Health Laboratory and various veterinary laboratories. The Ministry of Health and Population, Nepal, attempted to address such issues with National Antibiotics Treatment Guidelines in 2014 (Acharya and Wilson., 2019). There is no clear regulation for control of the use of antimicrobials in animals for human consumption.

Very few studies have assessed AMR in the poultry sector in Nepal. A study conducted on bacteriological quality of poultry meat in Nepal showed that various bacteria, such as E. coli, Staphylococcus, and Klebsiella showed higher resistance to commonly found antibiotics on the market, such as amoxicillin and tetracyclines (Neupane and Kaphle., 2019). While in other study, E. coli was found in 76.1% of poultry meat samples, and resistance to tetracycline was highest (87.7%) and lowest for ceftriaxone (1%) (Acharya., 2022). However, there is no published literature on the pattern of AMR in isolates from chicken meat samples in Nepal. Raw meat samples provide a better insight into the resistance pattern in the microbiota of the chicken and also are not prone to cross-contamination from the environment in the slaughter house.

Majority of Nepalese population consume meat products processed in small shops that store the product at ambient temperature and improper handling, both of which increase microbial growth and contamination.

The present study is designed to investigate the prevalence of antimicrobial drug-resistant bacteria from raw meat sample from Kathmandu, an ancient city and capital city of Nepal.

MATERIALS AND METHODS

Study area

The study area was Kathmandu valley. Sampling was done from different slaughter houses, slaughter places and live bird markets distributed across the market. This study was carried out from July 2022 to December 2022.

Sample size

Sample size was calculated using the statistical software Epi Info 7.2 used in the CDC. The assumptions made were population size of one million broilers in Kathmandu valley; prevalence of AMR to be 50%, and confidence interval of 95%. These yields a sample size were 384. These samples were collected to represent different areas of Kathmandu valley, covering different slaughter houses and live bird markets. Samples were collected following the standard laboratory protocol.

Sample collection protocol

The fresh meat samples were collected together with epidemiological information such as farm size, location of slaughter house, age of birds, and hygienic practices of butcher. Samples were collected to represent large, medium and small sized slaughter places as well as to represent samples from small, medium and large poultry farms. A detailed questionnaire was developed to collect epidemiological information; and the butchers / workers in slaughter places were interviewed during the sample collection.

Laboratory analysis

Sample processing for identification of bacterial pathogens

The samples collected were transported to the laboratory maintaining cold chain using the cool box. The samples were then cultured in selective media (Nutrient, MacConkey and Eosin methyl blue) and then incubated for 24- 48 hours. The media were checked for bacterial growth, and identified by morphology of colony characteristics and appropriate biochemical tests. If there was growth, the identified colony was taken, and antimicrobial susceptibility test was performed according to modified Kirby Bauer disc diffusion method in Mueller Hinton agar. Antibiotic sensitivity testing by the Kirby Bauer’s disc diffusion method was performed for the isolates using commercially available antibiotic discs on Muller–Hinton agar (MHA). Standard suspensions of the isolates were adjusted to 0.5 McFarland Standard. Immediately after standardization a sterile cotton swab was immersed into bacterial suspension; and a lawn culture was performed on the surface of MHA plate. Commercially available antibiotic discs were arranged on the surface of inoculated plates. The plates were incubated at 37^0C for 16-18 hours. After incubation the zone diameter was measured for each antimicrobial agent. Thereby the zone of inhibition was interpreted as sensitivity (S), Intermediate (I) or resistant (R) (Bauer *et al.*, 1997). In this study, the main focus was on common bacteria *E. coli.* The panel of antibiotics was including commonly used antibiotics in animal health and critically endangered antibiotics listed by the World Health Organization.

The sample processing, isolation and identification of bacterial pathogens and antimicrobial susceptibility test was carried out in the pharmacology and microbiology laboratory of Institute of Agriculture and Animal Science, Paklihawa Campus and related government laboratory.

Statistical Analysis

Descriptive analysis and other appropriate statistical procedures were also used to analyse the antimicrobial resistance pattern of bacterial isolates. The data was stored in Excel sheet and Epi Info 7.2 software to analyze the data.

RESULTS

Antimicrobial resistance (AMR) is a global growing issue and several reports suggest that it is an increasing problem of phenomenal proportions, affecting both developed and developing countries.

E. coli prevalence

Total 384 poultry meat samples were obtained from the market. Among the sample obtained, total 189 samples were found contaminated with *E. coli* . The contamination was found to be 49.29 %.

Table 1. Result of sensitivity analysis among *E. coli* Isolates

	Cipro	Chloramphenicol	Gentamicin	Amikacin	Levofloxacin	Colistin
S	64	55	50	92	81	185
I	31	54	60	83	41	0
R	94	80	79	14	66	4
Total Sample	189	189	189	189	188	189

Antibiotic resistance among *E. coli* isolates

Resistance was detected to 6 antibiotics tested. None of the isolates were multi drug resistant. Among the total meat samples obtained, there were 189 samples positives with E. coli. These samples were subjected to antibiotic susceptibility test. Among the antibiotic, Ciprofloxacin had 64 samples were Sensitive, Chloramphenicol had 55 samples sensitive, Gentamicin had 50 samples sensitive, Amikamicin had 92 samples sensitive, Levofloxacin had 81 samples sensitive, Colistin Sulphate had 185 samples sensitive.

Figure 2. *E. Coli* growth in growth media

Table 2. Sensitivity to different drugs

Drugs	Sensitive (f)
Ciprofloxacin	64
Chloramphenicol	55
Gentamicin	50
Amikacin	92
Levofloxacin	81
Colistin	185

Prevalence of resistance among *E. coli* isolates

The prevalence of antibiotic resistance in *E. coli* strains simultaneously isolated from marketed broiler meat. Among the samples, ciprofloxacin had resistance in 94 samples of E.coli, Chloramphenicol had 80 samples resistance, Gentamicin had 79 samples resistance, Amikacin had 14 samples resistance and Colistin Sulpahte had only 4 samples resistance.

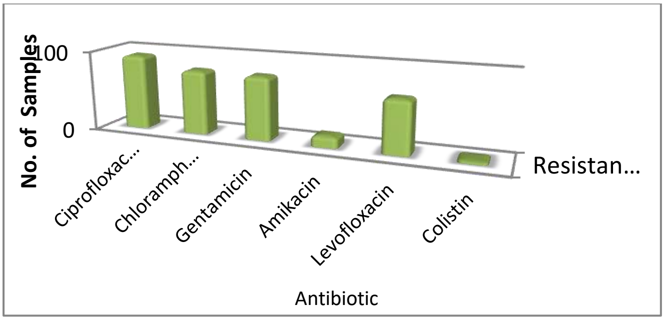

Figure 4. Graph showing resistances of different antibiotics

Ciprofloxacin

Ciprofloxacin is a broad-spectrum antibiotic widely prescribed in clinical and hospital settings. The emergence of antimicrobial resistance against effective antibiotics is a global issue. In the present study among the total 189 positive culture, there were 94 samples resistance, 31 samples were intermediate and 64 were sensitive.

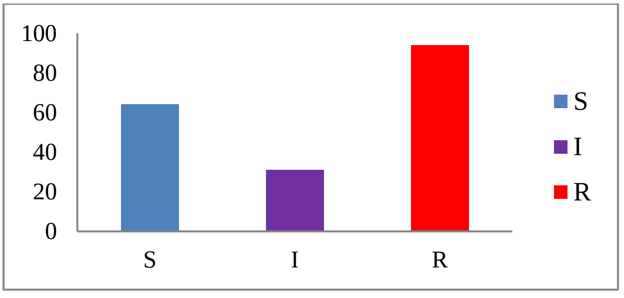

Figure 5. Graph showing the Ciprofloxacin resistance and sensitive pattern

Amikacin

Amikacin is a broad-spectrum antibiotic widely prescribed in clinical and hospital settings. The emergence of antimicrobial resistance against effective antibiotics is a global issue. In the present study among the total 189 positive culture, there were 14 samples resistance, 83 samples were intermediate and 92 were sensitive.

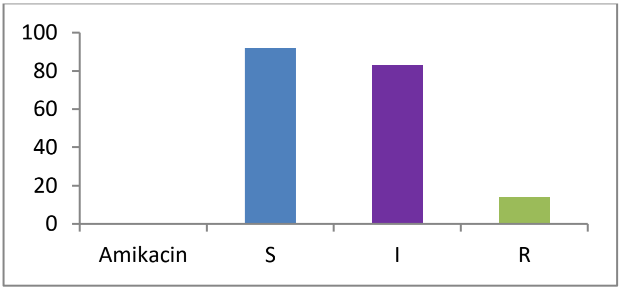

Figure 6. Graph showing sensitivity of Amikacin Anitibiotics

Chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic widely prescribed in clinical and hospital settings. The emergence of antimicrobial resistance against effective antibiotics is a global issue. In the present study among the total 189 positive culture, there were 80 samples resistance, 54 samples were intermediate and 55 were sensitive.

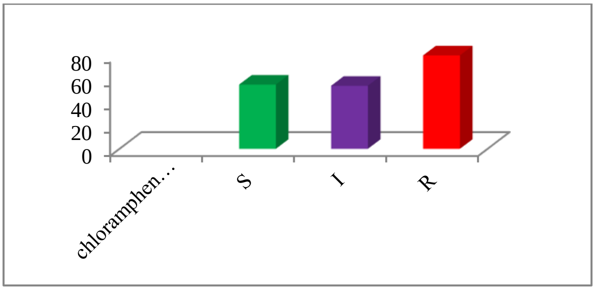

Figure 7. Graph showing sensitivity of Chloramphenicol Anitibiotics

Colistin Sulphate

Colistin Sulphate is a broad-spectrum gut acting antibiotic widely prescribed poultry sector. The emergence of antimicrobial resistance against effective antibiotics is a global issue. In the present study among the total 189 positive culture, there were 4 samples resistance, 0 samples were intermediate and 185 were sensitive.

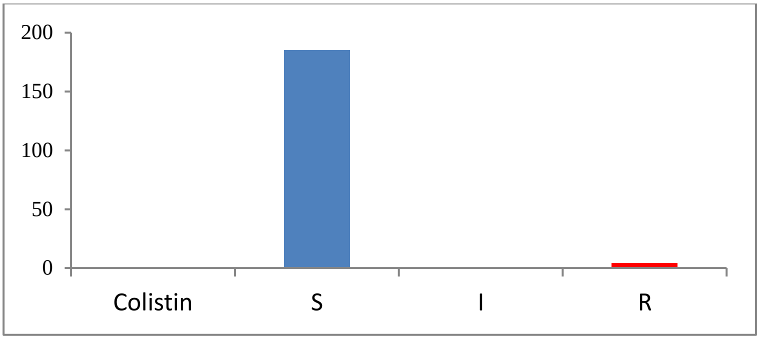

Figure 8: Graph showing sensitivity of Colistin sulphate Anitibiotics

Gentamicin

Gentamicin is a broad-spectrum antibiotic widely prescribed in clinical and hospital settings. The emergence of antimicrobial resistance against effective antibiotics is a global issue. In the present study among the total 189 positive culture, there were 79 samples resistance, 60 samples were intermediate and 50 were sensitive.

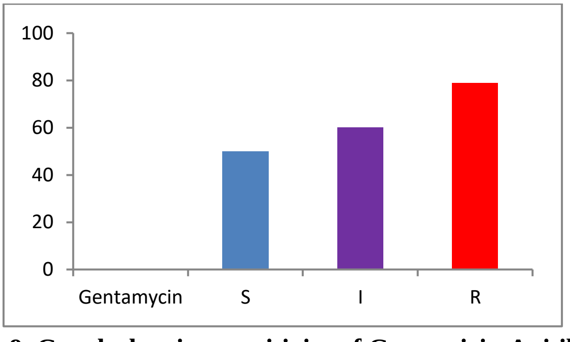

Figure 9. Graph showing sensitivity of Gentamicin Anitibiotics

Levofloxacin

Levofloxacin is a broad-spectrum antibiotic widely prescribed in clinical and hospital settings. The emergence of antimicrobial resistance against effective antibiotics is a global issue. In the present study among the total 189 positive culture, there were 66 samples resistance, 41 samples were intermediate and 81 were sensitive.

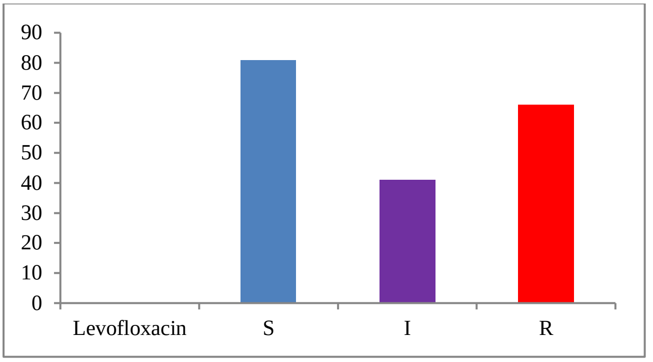

Figure10. Graph showing different drugs and their resistance

DISCUSSION

In the present study, out of 384 samples, 49.21% samples had *E. coli* bacterial growth in different culture medium. A similar but with higher prevalence was reported from Kathmandu, where they found more than 80.0.

In this study, out of 189 isolates of *Escherichia coli* from both chicken meats, 30 % were MDR. A study conducted in chicken breast sample in the United States showed 83.5% prevalence of *Escherichia coli*, of which 38.9% isolates were MDR [Zhao *et al*, 2012]. High number of *Escherichia coli* in retail meats indicates fecal contamination at slaughter or during processing.

The present study provides an assessment of the occurrence of multidrug resistance of *E. coli* from raw chicken meat collected from local market. The results indicated that production methods influenced the frequency of antibiotic-resistant *E. coli* on poultry products available to consumers. Future research to identify the specific practices that cause the high frequency of antibiotic-resistant *E. coli* in chicken could promote efforts to reduce consumer exposure to this potential pathogen.

Nepal being an agriculture-based country, in recent years is known to have increasing production and rise in population dependency on chicken poultry products for meat, egg and manure (Poudel *et al*. 2020). The study shows less than one fourth (14.82%) of the operating poultry farms are registered. The commercial poultry survey 2071/72 has recorded 14% of the registered poultry farm in Kathmandu valley having 16% of trained owner (CBS, 2015).

Multi- drug resistant *E. coli* isolates were found for 10 commonly used and market available antibiotics. Although we did not check the pathogenicity of the isolates, the gene responsible for multi-drug resistance may transfer to consumer via food and results in serious public health hazard as because (Boarlin *et al*., 2005) reported antimicrobial resistance is more frequent in pathogenic than in other porcine E. coli strains, and also shows that the resistance genes found in ETEC isolates are different from those of other porcine *E. coli* isolates and that clear associations exist between specific resistance and virulence genes. Jhonson *et al*., (2007) also reported that the drug resistant human isolates were similar to poultry isolates and thus, concluded that many drug-resistant human fecal *E. coli* isolates may be originated from poultry. This resistance occurs due to possessing of resistant gene found in single and multiple size plasmids in *E. coli* isolates.

The highest prevalence of multi-drug resistant E. coli isolates was obtained from chicken meat 76%. (Adesiyun *et al*., 2007) reported E. coli which was resistant to at least three or more antimicrobial agents. (Álvarez and Fernández *et al*.,2013) reported that 91.7 % E. coli isolates of poultry were multi-drug resistant. Indiscriminate use, improper selection, improper dose, incorrect duration of antibiotics at flock level may be responsible for such a higher occurrence of MDR. (Hassan *et al*., 2013) reported 22.7% MDR *E. coli* isolates from bird samples.

In conclusion, the result of this study provides preliminary data on antimicrobial resistant bacteria from raw chicken meat of Kathmandu valley. From the study, it is evident that meat products are biologically contaminated with various pathogens which have developed various microbial resistance. Extensive researches on this issue that also involve the molecular dynamics should be conducted longitudinally to have a better understanding of the exact scenario throughout the nation and thereby help curb the possible threats. Training and awareness program should be conducted in order to minimize the irrational use of antimicrobials and hence reduce drug resistance evolution via poultry and livestock.

CONCLUSION

Results of antibiograms revealed that *E. coli* isolates were resistant to one or more of the antibiotics tested. Resistance was most frequently observed against Ciprofloxacin (49.73%), Chloramphenicol (42.32%), Gentamicin (41.79%), Levofloxacin (34.92%), Amikacin (7.4%), and Colistin sulphate (2.11%). Fifteen *E. coli* strains showed the multidrug resistance phenotypes and harbored at least three antibiotic resistance genes.

E. coli prevalence

Total 384 poultry meat samples were obtained from the market. Among the sample obtained, total 189 samples were found contaminated with *E. coli*. The contamination was found to be 49.29 %.

Resistance was detected to 6 antibiotics tested. None of the isolates were multi drug resistant. Among the total meat samples obtained, there were 189 samples positives with E.coli. These samples were subjected to antibiotic susceptibility test. Among the antibiotic, Ciprofloxacin had 64 samples were Sensitive, Chloramphenicol had 55 samples sensitive, Gentamicin had 50 samples sensitive, Amikacin had 92 samples sensitive, Levofloxacin had 81 samples sensitive, Colistin Sulphate had 185 samples sensitive.

The prevalence of antibiotic resistance in *E. coli* strains simultaneously isolated from marketed broiler meat. Among the samples, ciprofloxacin had resistance in 94 samples of *E. coli*, Chloramphenicol had 80 samples resistance, Gentamicin had 79 samples resistance, Amikacin had 14 samples resistance, Levofloxacin had 66 samples resistance and Colistin Sulpahte had only 4 samples resistance.

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