

## Antibiogram Pattern of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. Isolates from Broiler Chicken.

S. Khanal<sup>1\*</sup>, M. Kandel<sup>1</sup>, M. P. Shah<sup>2</sup>

<sup>1</sup> Agriculture and Forestry University, Chitwan, Nepal

<sup>2</sup> Army Equine Breeding Center, Chitwan, Nepal

\*Corresponding author: Shrijanaashiya@gmail.com

### ABSTRACT

*This study was conducted on clinical cases of broiler chicken brought at National Avian Disease Investigation Laboratory (NADIL) and Veterinary Teaching Hospital, Agriculture and Forestry University during the period of December, 2018 to April, 2019. The study was aimed to find the antibiogram pattern of Escherichia coli, Salmonella species and Staphylococcus species. A total of 50 ill broiler liver samples were collected and inoculated in Nutrient Agar, XLD agar MacConkey agar, EMB Agar and Mannitol Salt Agar and incubated for 24 hours at 37°C. During microbiological examination, prevalence of E.coli was 36 %, Salmonella species was 2% and Staphylococcus species was 8% where as mixed infection was 40%. Antibiogram profile for E. coli isolates were sensitive to Amikacin (88.89%) followed by Colistin (66.67%), Ciprofloxacin (50%), Levofloxacin (42.10%) and Gentamycin (27.78%) while Ceftriaxone (11.11%) and Tetracycline (11.11%) was recorded as least sensitive, for Salmonella species isolates were highly sensitive to Amikacin (100%) and other remaining antibiotics; Ceftriaxone, Gentamicin, Levofloxacin, Ciprofloxacin, Colistin and Tetracycline were observed to be resistant and for Staphylococcus spp. isolates were sensitive to Amikacin (75%) followed by Gentamicin (25%), Levofloxacin (25%), and Ciprofloxacin (25%) while Tetracycline and Colistin were resistant. In the conclusion, it is strongly recommended to decrease the unethical use of antibiotics to minimize the development of resistance strain of microbes in the future.*

**Keywords:** Broiler, Antibiogram, *E. coli.*, *Salmonella*, *Staphylococcus*

### INTRODUCTION

Poultry farming is flourishing in center of Nepal. Since last two decade poultry production had made tremendous difference from cottage industry to lively agribusiness. The GDP contribution of this sector stands at around 4 percent, and growing up rapidly. In fiscal year 2016/17, the share of the agriculture sector in GDP stood at 29.37 percent, while that of non-agriculture sector was 70.63 percent. The share of the agriculture sector in GDP has been in decreasing trend in the last 15 years. However, the number of commercial poultry farms, dairy cows and buffalo's farms and pigs has grown in recent period with growth in commercialization in the livestock sector (MOAC, 2017).

Chitwan district is the largest egg producer, and with approximately 11 percent of the total broiler population in the country, and has 1923 poultry farms (CBS, 2016). Though, many poultry farmers are facing the problem of poor production, disease and mortality (Conroy *et.al.*, 2005).

*Escherichia coli*, *Salmonella* and *Staphylococcus* species are the major bacteriological pathogens for the mortality of the chicken which causes great economic loss in the farm.

*Escherichia coli* is a Gram-negative, rod shaped, facultative anaerobic bacterium that belongs to the *Enterobacteriaceae* family, which may cause a great hazard to poultry industry causing high mortality, loss of weight and reduction in egg production (Abd El Tawab *et.al.*, 2015). Collibacillosis is one of the most common and serious problem that causes great economic loss to poultry enterprises. *E. coli* are mainly found as intestinal flora of human, mammals and birds and cause disease under the influence of poor managerial practices such as flawed ventilation, overcrowding of birds, extreme temperature and humidity, dehydration, hunger and in many immunosuppressive diseases. Coli septicemia is the most important disease caused by avian pathogenic *Escherichia coli* strains. This organism cause secondary bacterial infection in the upper respiratory tract of birds after primary viral infections such as the Newcastle disease, Infectious Bronchitis disease and *Mycoplasma* infection (Gross, 1991). The respiratory infection caused by avian pathogenic *E. coli* strains, further to the virus infection, is considered to be the initial step for colisepticemia development in birds (Gross, 1991). *E. coli* infection also called as aero sac disease; mostly it occurs among birds of two to twelve weeks of age with high mortality rates of about 20% (Dho-Moulin and Fairbrother, 1999). These bacterial strains are a potential reservoir for antimicrobial resistance genes and play an important role in the ecology of antimicrobial resistance of bacterial populations. The intestinal fecal flora from poultry and other meat producing animals can also transfer antimicrobial resistance to human pathogens via food chain (Rashid *et.al.*, 2017).

*Salmonella* are short bacilli, Gram-negative, aerobic, catalase positive, oxidase negative; they ferment sugars with gas production, produces H<sub>2</sub>S, are non sporogenic and are normally motile with peritrichal flagella, except for *Salmonella pullorum* and *Salmonella gallinarum*, which are non-motile (Plym and Wierup, 2006). These organisms are Gram negative and rod shape which have been divided into over 2700 serotypes based on somatic, flagellar and capsular antigens (Gallegos-Robles *et.al.*, 2003). The pathogen can get transmitted by both horizontal and vertical routes. Avian salmonellosis is divided into two forms; Fowl typhoid and pullorum disease which both have a main economic significance worldwide. Fowl typhoid is attributed to *gSalmonella enterica* subspecies. *Enterica* serovar *gallinarum* biovar *gallinarum* (*S. gallinarum*) can colonize and cause disease in various domestic and wild birds.

*Staphylococci* are spherical, ovoid gram positive cocci, non sporing, non motile, aerobic and normally facultatively arranged anaerobic cocci characteristically arranged in clusters. *Staphylococcus* spp. is an important opportunist that can cause superficial to life-threatening illnesses in a variety of animal species. In poultry, this organism has been implicated in osteomyelitis, synovitis, and cellulitis. The diseases associated with this organism vary in the severity from superficial skin infections to the more life threatening, systemic illnesses. It is not uncommon to isolate *S. aureus* from the yolk sac, liver, and skin of broiler.

In Nepal, Poultry farming has been growing tremendously over few decades. It has significant role to the national economy. It shares 8% of AGDP and 4% of GDP (Karki, 2005). Chitwan district has become the capital city in poultry production. *Salmonella* infection is one of the most significant causes of morbidity and mortality in the world and due to it is common between human

and animals, the human food chain is known as the main source of infection (Mkrtychyan *et.al.*, 2016)

The aim of this study is to estimate the prevalence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. infection in broiler chickens and the antibiogram pattern against the isolated strains. Outbreaks of these bacterial infection remains as a serious economic problem in countries where control measures are not efficient or in those areas where climatic conditions, favor the environmental spread of the microbes. The economic losses are mainly due to morbidity, mortality, reduced growth rate and reduced feed conversion efficiency. Controlling *Escherichia coli*, *Salmonella* species and *Staphylococcus* species in poultry is problematic and it has relied historically on a combination of farm bio-security, water sanitization and the use of antibiotics.

The indiscriminate and injudicious use of antibiotics is an important factor in the emergence of antibiotic-resistant bacteria that subsequently can be transferred to humans through the food chain. In recent years, multidrug-resistant (MDR) phenotypes have been increasingly described among *Escherichia coli*, *Salmonella* species and *Staphylococcus* species all over the world.

## MATERIALS AND METHODS

Study was conducted from December, 2018 to April, 2019 in Chitwan District which is located in the Southwest part of Province 3. The samples were brought from National Avian Disease Investigation Laboratory (NADIL) and Agriculture and Forestry University (AFU), Bharatpur, Chitwan, where the dead birds from different areas of Chitwan were taken for postmortem and disease diagnosis.

A total of 50 swab were taken from liver sample of suspected birds was collected aseptically with the help of sterilized swab. Swab was kept in sterilized vial containing 1ml peptone for the pre-enrichment of the bacteria. The collected swab then it was transported on a cold chain box with ice packs immediately to the microbiology laboratory of AFU.

Further processing was carried out by keeping the peptone water containing sample in incubator for 24 hrs. Then after 24 hour streaking performed directly on MAC agar plate and then it was left for incubation at 37 °C aerobically for 24 hrs. A tentative case diagnosis of avian Collibacillosis was based on the appearance on the Mac Conkey Agar culture plates with colonies resembling *E. coli* (rose pink colonies due to Lactose fermentation). After primary culture a single isolated colony from Mac Conkey agar was sub-cultured into EMB agar plate and incubated at 37 °C aerobically for 24 hrs where it produce colonies with green metallic sheen. However, in Salmonellosis appearance on the Mac Conkey Agar culture plates was colonies resembling convex shape, Small, Circular, translucent, colourless colonies, none Lactose fermenter. After primary culture a single isolated colony from Mac Conkey agar was sub-cultured onto the XLD agar plate and incubated at 37 °C aerobically for 24 hrs where it produce black centered colonies.

Eventually, after enrichment of 24 hour, streaking performed directly on Mannitol salt agar plate also and then it was left for incubation at 37 °C aerobically for 24 hrs. A tentative case diagnosis of *Staphylococcus* species was based on the appearance on the Mannitol salt agar culture plates with colonies resembling *Staphylococcus* spp (phenol red of the media turns to yellow).

Suspected purified colonies was smeared, fixed and stained with Gram's staining for the identification and for further confirmation biochemical test should be done for the identification. Suspected isolates of *Escherichia coli*, *Salmonella* and *Staphylococcus* species was confirmed by performing different biochemical tests like motility, Indole, Methyl Red test, Voges-Proskauer test, Simmon citrate test, Triple sugar iron (TSI) test, Catalase and Oxidase test.

Antimicrobial susceptibility test was done by using the Kirby-Bauer disk diffusion method as described by (Bauer, Kirby, Sherris, andTurck, 1966) following the Clinical and Laboratory Standards Institute, CLSI (CLSI, 2014) guidelines was performed. Before AST, isolated *E. coli*, *Salmonella* and *Staphylococcus* species are again cultured on TSA media. All *E. coli*, *Salmonella* and *Staphylococcus* species isolates were tested for seven different antimicrobial agents on Muller Hinton Agar (HiMedia). Isolates were inoculated in nutrient broth and incubated at 37 °C for 6 hrs. Turbidity of the sample would be adjusted to a 0.5 McFarland standard by dilution. Immediately after dilution, a sterile swab was dipped into the inoculum and streaked over the entire surface of the Mueller Hinton agar three times. Seven suitable antibiotics were selected. They were Amikacin (30 mcg), Ceftriaxone (10 mcg), Gentamicin (10mcg), Levofloxacin (5 mcg), Ciprofloxacin (5 mcg), Colistin (10mcg) and Tetracycline (30 mcg).

## RESULT AND DISCUSSION

A total of 18 samples were found positive of *E. coli*, 1 sample was *Salmonella* and 4 samples were positive for *Staphylococcus* and 20 isolates of mixed infection from 50 broiler liver samples. The overall prevalence of *E.coli* was 36 %, *Salmonella* spp. was 2% and *Staphylococcus* spp. was 8%, whereas mixed infection was found 40% (Table 1).

Table 1: Prevalence of different organism in broiler liver sample

Organism	No. of Isolates	Prevalence Percentage
<i>Escherichia coli</i>	18	36
<i>Salmonella</i> species	1	2
<i>Staphylococcus</i> species	4	8
Mixed infection	20	40
No growth	7	14

Prevalence of was mixed infection was higher followed by *E.coli* similar result was recorded by Rashid *et.al.*, in 2017. This higher prevalence of mixed bacterial infection might be due to the poor management practices at poultry farms as *E. coli*, *Staphylococcus* species and *Salmonella* species is opportunistic pathogen. *E. coli* infection may occur as secondary infection when birds are immune suppressed due to other diseases or environmental stress. Poor managmental practices and general hygienic conditions contribute to higher infection of *E. coli* (Zanella *et.al.*, 2000).

Table 2. Sensitivity pattern of antibiotics against different isolates

Growth of organisms	Antibiotics						
	Amikacin	Colistin	Gentamicin	Levofloxacin	Ciprofloxacin	Ceftriaxone	Tetracycline
<i>Staphylococcus</i>	3	0	1	1	1	14	0
<i>Salmonella</i>	1	0	0	0	0	0	0
<i>E. coli</i>	16	12	5	7	9	2	2

Antibiogram profile for *E. coli* isolates was sensitive to Amikacin was higher i.e. 88.89% followed by Colistin 66.67% while Ceftriaxone and Tetracycline was recorded as least sensitive i.e. 11.11%. Sensitivity to other antibacterials were recorded as; Ciprofloxacin (50%), Levofloxacin (42.10%), Gentamycin (27.78%). Antibiogram profile for *Salmonella* species isolates was highly sensitive to Amikacin (100 %). Other remaining antibiotics; Ceftriaxone, Gentamicin, Levofloxacin, Ciprofloxacin, Colistin and Tetracycline were observed to be resistant for *Salmonella*.

Antibiogram pattern for *Staphylococcus* species was highly sensitive to Amikacin (75%) followed by Gentamicin 25%, Levofloxacin 25%, and Ciprofloxacin 25% Tetracycline and Colistin was resistant. This study showed 36% isolation rate of *E. coli* from clinical cases of broiler liver sample which is lower to 71.2 % isolation rate by P. Shikha (2018). In this study, Amikacin was highly sensitive to *E. coli* i.e. 88.89% which is comparable with the result of T.Khanal (2017) found no resistance to Amikacin. Similarly Subedi *et.al.*, (2018) found 16 % and P. Shikha (2018) found 6.89% resistance to Amikacin which is similar to this study.

## CONCLUSION

This study showed high prevalence of mixed infection followed by *Escherichia coli*, *Staphylococcus* and *Salmonella* infection. Antibiogram profile for *E. coli* isolates were sensitive to Amikacin followed by Colistin, Ciprofloxacin, Levofloxacin and Gentamycin while Ceftriaxone and Tetracycline were recorded as least sensitive, for *Salmonella* spp. isolates were highly sensitive to Amikacin and other remaining antibiotics; Ceftriaxone, Gentamicin, Levofloxacin, Ciprofloxacin, Colistin and Tetracycline were observed to be resistant and for *Staphylococcus* spp. isolates were sensitive to Amikacin followed by Gentamicin, Levofloxacin, and Ciprofloxacin while Tetracycline and Colistin were resistant. Thus, it is strongly recommended to use the antibiotics after antibiotic sensitivity test to minimize the development of resistance strain of microbes in the future and to minimize the economic loss.

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